Final Program and Abstracts

ASM CONFERENCES

12th ASM Conference on
Candida and Candidiasis

March 26 – 30, 2014
New Orleans, Louisiana

1752 N Street, N.W.
Washington, DC 20036-2904
Phone: 202-737-3600
World Wide Web: www.asm.org

ASM CONFERENCES

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ASM Conferences Mission

To identify emerging or underrepresented topics of broad scientific significance.

To facilitate interactive exchange in meetings of 100 to 500 people.

To encourage student and postdoctoral participation.

To recruit individuals in disciplines not already involved in ASM to ASM membership.

To foster interdisciplinary and international exchange and collaboration with other scientific organizations.

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Acknowledgments

The Conference Organizers and the American Society for Microbiology acknowledge the following for their support of the 12th ASM Conference on Candida and Candidiasis. On behalf of our leadership and members, we thank them for their financial support:

Burroughs Wellcome Fund

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Elsevier – Medical Mycology Case Reviews journal

ISHAM – International Society for Human and Animal Mycology

Scynexis

National Institutes of Health*

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General Information

REGISTRATION AND NAME BADGES

ASM Staff will be available at the registration desk in the Celestin Ballroom Foyer of the Hyatt Regency New Orleans during posted registration hours. Participants may collect name badges and program materials at the registration desk. A name badge is required for entry into all sessions and social events.

GENERAL SESSIONS

All general sessions will be held in the Celestin Ballroom A - D, located on level three of the Hyatt Regency New Orleans.

PARALLEL SESSIONS

As noted in the program schedule there will be three parallel sessions are offered concurrently on both Friday and Saturday afternoons. Parallel sessions will be held in the Strand 10 and 11 rooms, located on level two, and the Celestin Ballroom A - D in the Hyatt Regency New Orleans.

CANDIDA CASE STUDIES LUNCH SESSION

Participants who wish to attend the Candida Case Studies session on Friday should pick up their lunch and bring it to the Celestin Ballroom A - D for this lunch and learn session. The session will convene at 12:30 pm.

POSTER SESSIONS

Poster boards are located Storyville Hall, located on level three of the Hyatt Regency New Orleans.

All Posters will be displayed for informal viewing throughout the conference.

Official presentation will be in three sessions – Poster Session A will be held Thursday, March 27; Poster Session B will be held Friday, March 28; Poster Session C will be held Saturday, March 29.

Please check your assigned number in the abstract index. The same number is used for the presentation and board number. Each poster has a unique number; the letter indicates which session the poster will be officially presented.

SOCIAL EVENTS

Registration includes attendance at the Welcome Reception on Wednesday, Group Lunches on Thursday, Friday, Saturday, and Sunday, and the Conference Dinner Party on Saturday night.

CERTIFICATE OF ATTENDANCE

Certificates of Attendance can be found in the registration packet received at the registration desk.

Note: Certificates of Attendance do not list session information.

CAMERAS AND RECORDINGS POLICY

Audio/video recorders and cameras are not allowed in session rooms or in the poster areas. Taking photographs with any device is prohibited.

CHILD POLICY

Children are not permitted in session rooms, poster sessions, conference meals or social events. Please contact the hotel concierge to arrange for babysitting services in your hotel room.

Travel Grants

STUDENT TRAVEL GRANTS

ASM encourages the participation of graduate students and new postdocs at ASM Conferences. To support the cost of attending the conference, ASM has awarded travel grants of $500 to each of the following individuals:

Darren Abbey
Sara Amorim-Vaz
Jennifer Hogan
Alex Hopke
Stephen Jones
Aline Khayat
Galit Kuznets
Shen-Huan Liang
K Mitchell
Evelyn Nash
Olaniran Olarinde
Melanie Polke
Rebecca Pulver
Neha Rastogi
Lisa Rodrigues
Nuo Sun
Marc Swidergall
Meng Xiao
Jinglin Xie
Li Xiang

Scientific Program

Wednesday, March 26, 2014

7:30 – 8:30 pm
Celestin Ballroom A - D

Opening Session

Welcome Remarks
***Al Brown**; University of Aberdeen, Aberdeen, UNITED KINGDOM*

Candida Evolution and Development
***Alexander Johnson**; University of California, San Francisco, CA*

8:30 – 10:00 pm
Celestin Ballroom Foyer

Welcome Reception

Thursday, March 27, 2014

8:15 – 10:00 am
Celestin Ballroom A - D

Session 1: Fungal and Host Signaling 1
Conveners: Richard Bennett and Leah Cowen

8:15 – 8:45 am

Genomic Architecture of the Evolution of Circuitry Controlling Fungal Drug Resistance and Morphogenesis
***Leah Cowen**; University of Toronto, Toronto, ON, CANADA*

8:45 – 9:00 am

Intra-Species Variation in *Candida albicans* and Consequences for Host Interactions
***Richard Bennett**; Brown University, Providence, RI*

9:00 – 9:15 am

Functional Mapping of the *Candida albicans* Signaling Mucin Msb2
***Marc Swidergall**; Heinrich-Heine-University, Duesseldorf, GERMANY*

9:15 – 9:30 am

Opposing Immunological Imprinting of *Candida albicans* beta-glucan Trained Immunity and Endotoxin Tolerance in Monocytes
***Jessica Quintin**; Radboudumc, Nijmegen, NETHERLANDS*

9:30 – 9:45 am	Identification of 2 Novel Signaling Proteins that Govern the Host Response During in vivo Disseminated and Vaginal Candidiasis ***Vincent Bruno**; Institute for Genome Sciences at the University of Maryland School of Medicine, Baltimore, MD*
9:45 – 10:00 am	A Intracellular Non-Receptor Tyrosine Kinase Mediates the Host Inflammatory Immune Response and Determines Fungal Virulence ***Karl Kuchler**; Medical University, Vienna, AUSTRIA*
10:00 – 10:30 am Celestin Ballroom Foyer	**Coffee Break**
10:30 am – 12:00 pm Celestin Ballroom A - D	**Session 2: Life in the Host** *Conveners: Bernard Hube and Carol Kumamoto*
10:30 – 11:00 am	Adaptation of Candida Species to Macrophages During in vitro Microevolution Experiments ***Sascha Brunke**; Hans-Knoell-Institute, Jena, GERMANY*
11:00 – 11:15 am	Candida/host Interactions: Responding to the Host Environment ***Carol Kumamoto**; Tufts University, Boston, MA*
11:15 – 11:30 am	Small-chain Fatty Acids Inhibit *Candida albicans* in vitro Growth and Murine Gastrointestinal Colonization ***Andrew Koh**; University of Texas Southwestern Medical Center, Dallas, TX*
11:30 – 11:45 am	Passage through the Mammalian Intestine Triggers a Phenotypic Switch that Promotes *Candida albicans* Commensalism ***Kalyan Pande**; University of California, San Francisco, CA*
11:45 am – 12:00 pm	Synergistic Regulation of Hyphal Elongation by Hypoxia, CO_2, and Nutrient Conditions Controls Pathogenicity of *Candida albicans* ***Haoping Liu**; University of California, Irvine, CA*
12:00 – 1:30 pm Storyville Hall	**Lunch**

2:00 – 3:30 pm
Celestin Ballroom A - D

Session 3: Elevator Session
Moderator: Neil Gow

2:00 – 2:05 pm

Dynamic Host-Pathogen Interactions Result in Fungal Epitope Unmasking in vitro and in vivo
***Alex Hopke**; The University of Maine, Orono, ME*

2:05 – 2:10 pm

Rab14 Recruitment to Phagosomes Reduces Macrophage Killing by Live *Candida albicans*
***Judith Bain**; University of Aberdeen, Aberdeen, UNITED KINGDOM*

2:10 – 2:15 pm

Presence of the Multifunctional Protein Als3 on *Candida albicans* Hyphae Depends on the Tup1 Repressor
***Ronny Martin**; Septomics Research Center, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute and Friedrich Schiller University, Jena, GERMANY*

2:15 – 2:20 pm

Investigating the Role of Hsf1 in *Candida albicans* Drug Resistance and Morphogenesis
***Amanda Veri**; University of Toronto, Toronto, ON, CANADA*

2:20 – 2:25 pm

Control of Metabolic Activity by CDK8 Influences Pyocyanin Susceptibility in *Candida albicans*
***Allia Lindsay**; Geisel School of Medicine at Dartmouth, Hanover, NH*

2:25 – 2:30 pm

A Novel Family of Antioxidant Enzymes that Protects the *Candida albicans* Plasma Membrane from Lipid Peroxidation
***Lifang Li**; Stony Brook University, Stony Brook, NY*

2:30 – 2:35 pm

Pipeline for the Analysis of Copy Number and Loss of Heterozygosity Changes in *Candida albicans* Genome Array and Sequence Datasets
***Darren Abbey**; University of Minnesota, Minneapolis, MN*

2:35 – 2:40 pm

Analysis of the *Candida albicans* Transcription Factor Fcr1p Regulon
***Aline Khayat**; Institute for Research in Immunology and Cancer, Montreal, QC, CANADA*

2:40 – 2:45 pm
Ppg1, a PP2A Protein Phosphatase, Controls *Candida albicans* Morphology and Virulence
***Mohammed Albataineh**; UTHSCSA, San Antonio, TX*

2:45 – 2:50 pm
Understanding the Evolution of Post-transcriptional Gene Networks in Fungi
***Jiyoti Verma-Gaur**; Monash University, Clayton, AUSTRALIA*

2:50 – 2:55 pm
Novel Role of a Family of Major Facilitator Transporters in Biofilm Development and Virulence of *Candida albicans*
***Abdul Haseeb Shah**; Jawaharlal Nehru University, New Delhi, INDIA*

2:55 – 3:00 pm
The Endosomal GTPase Vps21p Confers Azole Susceptibility in *Candida albicans*
***Arturo Luna-Tapia**; LSUHSC New Orleans, New Orleans, LA*

3:00 – 3:05 pm
Signaling by a Biofilm Surface Adherence Protein
***Saranna Fanning**; Whitehead Institute for Biomedical Research, MIT, Cambridge, MA*

3:05 – 3:10 pm
Sexual Biofilms of Candida tropicalis, and a Role for a Sexual Agglutinin
***Stephen Jones**; Brown University, Providence, RI*

3:10 – 3:15 pm
Candida albicans-Staphylococcus aureus Intra-abdominal Infection-mediated Lethal Sepsis: Atypical Requirements of Morphogenesis and Virulence
***Evelyn Nash**; LSU Health Sciences Center, New Orleans, LA*

3:15 – 3:20 pm
CgPDR1-dependent Adherence of *Candida glabrata* to Host Cells: Impact of Different Yeast Strain Backgrounds and *CgPDR1* Gain-of-function Mutations
***Luis Vale-Silva**; Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne, SWITZERLAND*

3:20 – 3:25 pm
Candida albicans Eed1 is Involved in the Response to Quorum Sensing
***Melanie Polke**; Hans-Knoell-Institute, Jena, GERMANY*

3:25 – 3:30 pm
Discovery and Characterization of Potential Antifungal Compounds Against *Candida albicans*
***Chelsi Cassilly**; University of Tennessee, Knoxville, TN*

3:30 – 4:00 pm
Celestin Ballroom Foyer
Coffee Break

4:00 – 6:30 pm
Storyville Hall
Poster Session A

Friday, March 28, 2014

8:15 – 10:00 am
Celestin Ballroom A - D
Session 4: Immune Biology
Conveners: Mihai Netea and Salomé LeibundGut-Landmann

8:15 – 8:45 am
Natural Killer Cell-mediated Immunity Against Systemic Candidiasis
***Salomé LeibundGut-Landmann**; ETH Zürich, Zürich, SWITZERLAND*

8:45 – 9:15 am
A Systems Approach to Understand Antifungal Immunity
***Mihai Netea**; Radboud University, Nijmegen, NETHERLANDS*

9:15 – 9:30 am
The Adaptor CARD9 is Required for Adaptive but not Innate Immunity to Oral Mucosal *Candida albicans* Infections
***Sarah Gaffen**; University of Pittsburgh, Pittsburgh, PA*

9:30 – 9:45 am
Dead Macrophages Walking: Much of *Candida albicans* Induced Macrophage Lysis is Actually Due to the Host Programed Cell Death Pathway Pyroptosis
***Melanie Wellington**; University of Rochester, Rochester, NY*

9:45 – 10:00 am
Fungal Chitin Dampens Inflammation through Mannose Receptor Dependent NOD2 and TLR9 Activation
***Neil Gow**; University of Aberdeen, Aberdeen, UNITED KINGDOM*

10:00 – 10:30 am
Celestin Ballroom Foyer
Coffee Break

10:30 am – 12:00 pm Celestin Ballroom A - D	**Session 5: Epidemiology, Evolution and the Genome** *Conveners: Judith Berman and Christina Cuomo*
10:30 – 11:00 am	Transcriptional Consequences of Genomic Variation in Clinical Isolates of *Candida albicans* ***Diego Martinez**; Broad Insitute of MIT and Harvard, Cambridge, MA*
11:00 – 11:15 am	Polyploidy Drives Population Heterogeneity through Random and Stepwise Chromosome Loss ***Meleah Hickman**; University of Minnesota, Minneapolis, MN*
11:15 – 11:30 am	A Resource for Genotype-phenotype Association Studies in *Candida albicans* ***Christophe d'Enfert**; Institut Pasteur, Paris, FRANCE*
11:30 – 11:45 am	Comparative Phenotypic Analysis of Candida Parapsilosis and *Candida albicans* ***Geraldine Butler**; University College Dublin, Dublin, IRELAND*
11:45 am – 12:00 pm	Engineering a Complete Sexual Cycle in *Candida glabrata* ***Jane Usher**; University of Exeter, Exeter, UNITED KINGDOM*
12:00 – 1:30 pm Storyville Hall	**Lunch**
12:30 – 1:50 pm Celestin Ballroom A - D	**Session 6: Clinical Session: Candida Case Reports** *Conveners: Suzanne Noble and Adilia Warris* *Participants may pick up lunch and return to eat in the session room during this special lunch session.*
12:30 – 12:50 pm	Title not available ***Suzanne Noble**; UCSF, San Francisco, CA*
12:50 – 1:10 pm	Title not available ***Adilia Warris**; University of Aberdeen, Aberdeen, UNITED KINGDOM*
1:10 – 1:30 pm	Device-related *Candida albicans* Endocarditis and Persistent Fungemia ***Ryan Shields**; University of Pittsburgh, Pittsburgh, PA*

1:30 – 1:50 pm	Case Report: Culture-negative Intra-abdominal Candidiasis due to *C. glabrata* Leading to Echinocandin-resistant Candidemia ***Neil Clancy**; University of Pittsburgh, Pittsburgh, PA*
2:15 – 3:45 pm Strand 10	**Concurrent Session 7A: Infection Models** *Conveners: Robert Wheeler and Minh Hong Nguyen*
2:15 – 2:30 pm	Title not available ***Minh Hong Nguyen**; University of Pittsburgh, Pittsburgh, PA*
2:30 – 2:45 pm	Host-pathogen Determinants in a Zebrafish Fungal Infection Model ***Robert Wheeler**; University of Maine, Orono, ME*
2:45 – 3:00 pm	Modelling Human *Candida* Infection with the Mouse Commensal *Candida pintolopesii* ***Donna MacCallum**; University of Aberdeen, Aberdeen, UNITED KINGDOM*
3:00 – 3:15 pm	Defining Benign Colonization from Symptomatic Infection during *Candida* vaginitis: A Crucial Role for Fungal Morphogenesis ***Brian Peters**; LSU Health Sciences Center, New Orleans, LA*
3:15 – 3:30 pm	Mini-host and Mouse Infection Models for Probing Virulence of *Candida albicans* Transcription Factors Mutants ***Sara Amorim-Vaz**; University of Lausanne and University Hospital Centre, Lausanne, SWITZERLAND*
3:30 – 3:45 pm	Insight into the Pathogenesis of *Candida glabrata* Using Murine Intra-abdominal Candidiasis (IAC) Model ***Shaoji Cheng**; University of Pittsburgh, Pittsburgh, PA*
2:15 – 3:45 pm Celestin Ballroom A - D	**Concurrent Session 7B: Micronutrients and Metabolism** *Conveners: Duncan Wilson and Michael Lorenz*
2:15 – 2:30 pm	Feeding on the Host: a *Candida albicans* Pore Forming Toxin ***Duncan Wilson**; Hans-Knoell-Institute, Jena, GERMANY*

2:30 – 2:45 pm
Multiple Routes to Environmental Alkalinization via Catabolism of Amino Acids or Dicarboxylic Acids Contribute to Survival and Virulence of *Candida albicans*
***Slavena Vylkova**; University of Texas Health Science Center, Houston, TX*

2:45 – 3:00 pm
A Network of Cell Surface CFEM Proteins Required for Host Heme-iron Acquisition in *Candida albicans*
***Galit Kuznets**; Technion-I.I.T, Haifa, ISRAEL*

3:00 – 3:15 pm
Evolutionary Rewiring of Ubiquitination Targets in *Candida albicans* Promotes Metabolic Flexibility in Host Niches
***Delma Childers**; University of Aberdeen, Aberdeen, UNITED KINGDOM*

3:15 – 3:30 pm
Effects of Metabolic Inhibitors on Ras1 Signaling of *Candida albicans*
***Nora Grahl**; Geisel School of Medicine at Dartmouth, Hanover, NH*

3:30 – 3:45 pm
A Diet High in Coconut Oil Reduces Murine Gastrointestinal Colonization by
Candida albicans
***Kearney Gunsalus**; Tufts University, Boston, MA*

2:15 – 3:45 pm
Strand 11
Concurrent Session 7C: Cool Tools
Conveners: Carol Munro and Gavin Sherlock

2:15 – 2:30 pm
The *Candida albicans* ORFeome Project: Towards a Genome-wide Overexpression Strain Collection
***Adeline Feri**; Institut Pasteur & INRA, Paris, FRANCE*

2:30 – 2:45 pm
Exploring Expression Data at CGD
***Gavin Sherlock**; Stanford University Medical School, Stanford, CA*

2:45 – 3:00 pm
Characterization and Utilization of the *Candida albicans* Haploid Tool Strains
***Guisheng Zeng**; Institute of Molecular and Cell Biology, Singapore, SINGAPORE*

3:00 – 3:15 pm	Complex Haploinsufficiency-based Genetic Interaction Screening in *Candida albicans* ***Damian Krysan**; University of Rochester, Rochester, NY*
3:15 – 3:30 pm	In vivo Imaging of Disseminated Candidiasis in Mice using Luciferase Reporter Strains ***Ilse Jacobsen**; Leibniz Institute for Natural Product Research and Infection Biology, Jena, GERMANY*
3:30 – 3:45 pm	Mapping Protein-protein Interactions with an Expanded Genetic Code ***Silke Grumaz**; Fraunhofer IGB, Stuttgart, GERMANY*
3:45 – 4:30 pm Storyville Hall	**Coffee Break**
4:00 – 6:30 pm Storyville Hall	**Poster Session B**

Saturday, March 29, 2014

8:15 – 10:00 am Celestin Ballroom A - D	**Session 8: Therapies - Vaccines and Antifungals** *Conveners: Scott Filler and David Perlin*
8:15 – 8:45 am	Title not available ***Scott Filler**; UCLA, Torrance, CA*
8:45 – 9:00 am	Transcriptome Profiling of Serial Genetically-Matched Susceptible and FKS-resistant C. glabrata Clinical Isolates ***Christina Jiménez-Ortigosa**; Rutgers University, Newark, NJ*
9:00 – 9:15 am	FKS Mutant *Candida* Infections Occur Among Patients with Prior Echinocandin Exposure and are Associated with Therapeutic Failure of Echinocandin Therapy ***Ryan Shields**; University of Pittsburgh, Pittsburgh, PA*
9:15 – 9:30 am	Anti-Candidiasis Synthetic Glycopeptide Vaccine Acceptable for Human Use ***Hong Xin**; LSUHSC, New Orleans, LA*

9:30 – 9:45 am
Application of the Combined CLSI/EUCAST Breakpoints for Azole and Echinocandin Susceptibilities of *Candida* Isolates in a Tertiary Cancer Center: the Emerging Threat of Multi-drug Resistance
***Dimitrios Farmakiotis**; The University of Texas MD Anderson Cancer Center, Houston, TX*

9:45 – 10:00 am
Crystal Structures of Full-length Wild Type and Mutant *Saccharomyces cerevisiae* Erg11p Give Insight into Triazole and Substrate Binding
***Richard Cannon**; University of Otago, Dunedin, NEW ZEALAND*

10:00 – 10:30 am
Celestin Ballroom Foyer
Coffee Break

10:30 am – 12:00 pm
Celestin Ballroom A - D
Session 9: Morphology and the Cell Cycle
Conveners: Yue Wang and James Konopka

10:30 – 11:00 am
Dissection of the Non-kinase Domain of the Nim1 KinaseGin4 Reveals Novel Regulatory Functions in Cell Cycle Control and Hyphal Growth in *Candida albicans*
***Yue Wang**; Institute of Molecular and Cell Biology, Singapore, SINGAPORE*

11:00 – 11:15 am
Plasma Membrane Architecture Promotes *Candida albicans* Morphogenesis and Virulence
***James Konopka**; Stony Brook University, Stony Brook, NY*

11:15 – 11:30 am
A Transcriptional Circuitry Involving the Master Regulators Ndt80p, Efg1p and Members of the Heat-Shock Factor-type DNA-binding Proteins Controls *Candida albicans* Morphogenesis
***Sadri Znaidi**; Institut Pasteur, Paris, FRANCE*

11:30 – 11:45 am
A New Role for the Class I Chitin Synthase Enzymes During Polarised Growth in *Candida albicans*
***Megan Lenardon**; University of Aberdeen, Aberdeen, UNITED KINGDOM*

11:45 am – 12:00 pm	Rsr1-GTPase Cycling Acts to Maintain Polarized Growth by Promoting the Efficient Clustering of Cdc42 During *Candida albicans* Hyphal Development ***Rebecca Pulver**; University of Minnesota, Minneapolis, MN*
12:00 – 1:30 pm Storyville Hall	**Lunch**
2:00 – 3:30 pm Celestin Ballroom A - D	**Concurrent Session 10A: Cell Biology** *Conveners: Peter Sudbery and Alexandra Brand*
2:00 – 2:15 pm	Directional Polarisation and Surface Exploration by *Candida albicans* Hyphae ***Alexandra Brand**; University of Aberdeen, Aberdeen, UNITED KINGDOM*
2:15 – 2:30 pm	The Role of Kinases in Hyphal Development ***Peter Sudbery**; MBB, Sheffield University, Sheffield, UNITED KINGDOM*
2:30 – 2:45 pm	Opaque to White Switching Occurs Sharply and Bimodally ***Chiraj Dalal**; UCSF, San Francisco, CA*
2:45 – 3:00 pm	Use of Structurally Informed Mutant Proteins to Assess the Contribution of the Peptide-binding Cavity and Amyloid-forming Region to *Candida albicans* Als3 Adhesive Function ***Ernesto Cota**; Imperial College London, London, UNITED KINGDOM*
3:00 – 3:15 pm	Mutational Analysis of Essential Septins Reveals Link Between Septin-mediated Signaling and the cAMP Pathway ***Jill Blankenship**; University of Nebraska at Omaha, Omaha, NE*
3:15 – 3:30 pm	Genotypic and Phenotypic Variability in a Collection of Clinical *Candida glabrata* Strains ***Michael Weig**; University Medical Center Goettingen, Goettingen, GERMANY*
2:00 – 3:30 pm Strand 10	**Concurrent Session 10B: Fungal-Drug Interactions** *Conveners: Dominique Sanglard and David Rogers*

2:00 – 2:15 pm
Drug Resistance in *Candida* Clinical Isolates: a Resource for Unexpected Patterns
***Dominique Sanglard**; University of Lausanne and University Hospital Center, Lausanne, SWITZERLAND*

2:15 – 2:30 pm
The Relative Impact on Azole Antifungal Susceptibility of Specific Mutations in *ERG11* Among Clinical Isolates of *Candida albicans*
***David Rogers**; University of Tennessee Health Science Center, Memphis, TN*

2:30 – 2:45 pm
Chromosome 5 Monosomy of *Candida albicans* Controls Susceptibility to Various Toxic Agents Including Major Antifungals
***Elena Rustchenko**; University of Rochester, Rochester, NY*

2:45 – 3:00 pm
Genetic Interactions Between the Biogenesis of Iron-sulfur Clusters and Fluconazole Tolerance in *Saccharomyces cerevisiae* and *Candida albicans*
***Alessandro Fiori**; VIB Department of Molecular Microbiology/KU Leuven, Leuven, BELGIUM*

3:00 – 3:15 pm
An Inhibitor of Ribosomal Biogenesis has Broad Anti-fungal Activity
***Nuo Sun**; Georgetown University Medical Center, Washington, DC*

3:15 – 3:30 pm
In Vivo Development of Fluconazole Resistance in *Candida parapsilosis* Isolates from a Patient with Persistent Bloodstream Infection and the Discovery of a New *MRR1* Gene Mutation
***Li Zhang**; Peking Union Medical College Hospital, Beijing, CHINA*

2:00 – 3:30 pm
Strand 11
Concurrent Session 10C: Late Breaking Hot Topics
Conveners: Catherine Bachewich and Taiga Miyazaki

2:00 – 2:15 pm
G1/S Transcription Factor Complex Composition and Function in *Candida albicans*
***Catherine Bachewich**; Concordia University, Montreal, QC, CANADA*

2:15 – 2:30 pm	A Single Amino Acid Mutation in IPI1 Confers Multiantifungal Resistance without Affecting Virulence in *Candida glabrata* ***Taiga Miyazaki****; Nagasaki University School of Medicine, Nagasaki, JAPAN*
2:30 – 2:45 pm	Intracellular Survival of *Candida glabrata* in Macrophages ***Lydia Kasper****; Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute (HKI), Jena, GERMANY*
2:45 – 3:00 pm	Super-resolution Imaging of C-type Lectin Spatial Rearrangement During Fungal Particle Recognition ***Aaron Neumann****; University of New Mexico, Albuquerque, NM*
3:00 – 3:15 pm	Probing the Evolutionary Potential of *Candida albicans* with Artificially Activated Transcription Factors ***Joachim Morschhäuser****; Universität Würzburg, Würzburg, GERMANY*
3:15 – 3:30 pm	Iron Binding Negatively Impacts the Fungicidal Activity of Salivary Protein Histatin 5 against *Candida albicans* ***Sumant Puri****; SUNY Buffalo, Buffalo, NY*
3:30 – 4:00 pm Celestin Ballroom Foyer	**Coffee Break**
4:00 – 6:30 pm Storyville Hall	**Poster Session C**
7:00 – 11:00 pm Celestin Ballroom A - D	**Conference Dinner Party**

Sunday, March 30, 2014

8:15 – 10:00 am Celestin Ballroom A - D	**Session 11: Communities - Biofilms and the Microbiome** *Conveners: David Andes and Aaron Mitchell*
8:15 – 8:45 am	Characterization of Extracellular Matrix Mannan Production, Secretion, and Role in *Candida albicans* Biofilm Antifungal Resistance ***Kaitlin Mitchell**; University of Wisconsin, Madison, WI*
8:45 – 9:15 am	Pathogen Response to the Environment of Invasive Infection ***Aaron Mitchell**; Carnegie Mellon University, Pittsburgh, PA*
9:15 – 9:30 am	Appearance of a Novel CTG-clade Specific Histone H3 Variant Regulates a Recently Evolved Transcription Circuit for Biofilm Development in *Candida albicans* ***Laxmi Rai**; Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, INDIA*
9:30 – 9:45 am	Small Molecule Inhibitors of *Candida albicans* Filamentation that Impair Biofilm Formation on Multiple Surfaces ***Paul Kaufman**; University of Massachusetts Medical School, Worcester, MA*
9:45 – 10:00 am	Modulation of *Candida albicans* Virulence by Oral Commensal Bacteria ***Anna Dongari-Bagtzoglou**; University of Connecticut, Farmington, CT*
10:00 – 10:30 am Celestin Ballroom Foyer	**Coffee Break**
10:30 am – 12:00 pm Celestin Ballroom A - D	**Session 12: Fungal and Host Signaling 2** *Conveners: Jesus Pla and Gordon Brown*
10:30 – 11:00 am	C-type Lectins and Anti-Candida Immunity ***Gordon Brown**; University of Aberdeen, Aberdeen, UNITED KINGDOM*
11:00 – 11:15 am	Is MAPK Signalling in *Candida albicans* Involved in the Colonization of Mouse Gut? ***Jesus Pla**; Universidad Complutense de Madrid, Madrid, SPAIN*

11:15 – 11:30 am	*Candida albicans* SOD5 Represents a New Family of Extracellular Copper Only Superoxide Dismutase Enzymes Required for Protection from Host Derived Oxidative Stress ***Julie Gleason****; Johns Hopkins Bloomberg School of Public Health, Baltimore, MD*
11:30 – 11:45 am	*Candida albicans* Ece1 is a Novel Pore-forming Toxin Critical for Activating the Epithelial Cell 'Danger' Response ***David Moyes****; The Dental Institute, King's College, London, London, UNITED KINGDOM*
11:45 am – 12:00 pm	Blocking Two-component Signalling Enhances *Candida albicans* Virulence and Stimulates a Novel Mechanism to Survive SAPK Hyper-activation ***Janet Quinn****; Newcastle University, Newcastle upon Tyne, UNITED KINGDOM*
12:00 – 2:00 pm Celestin Ballroom A - D	**Closing Lunch and Summary Discussion**

Speaker Abstracts

OS:01

CANDIDA EVOLUTION AND DEVELOPMENT

A. Johnson;
Microbiology and Immunology, University of California, San Francisco, CA.

S1:1

GENOMIC ARCHITECTURE OF THE EVOLUTION OF CIRCUITRY CONTROLLING FUNGAL DRUG RESISTANCE AND MORPHOGENESIS

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S1:2

INTRA-SPECIES VARIATION IN *C. ALBICANS* AND CONSEQUENCES FOR HOST INTERACTIONS

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Candida albicans is the primary fungal pathogen in humans, yet clinical isolates vary widely in their both in vitro and in vivo properties. Here, we performed extensive phenotypic and genotypic analyses on a diverse set of 21 clinical *C. albicans* isolates. This set contains strains of different clades and mating types, different types of infection, and exhibit marked differences in the murine model of systemic virulence (1). Isolates were examined for a variety of in vitro properties including growth rate, filamentation, biofilm formation, and resistance to stress. Of these properties, only growth rates in rich medium at 30°C correlated with systemic virulence. One clinical isolate, P94015, which was a natural MTLa/a strain, was chosen for closer inspection as this isolate showed an altered phenotypic state, an inability to filament, and the lowest virulence of any of the strains in the collection. Genome sequencing of P94015 revealed that it contained a homozygous, premature stop codon in the EFG1 transcription factor. We show that this allele represents a recessive, loss of function allele of EFG1. Restoration of full-length EFG1 into P94015 restored the normal phenotypic state, enhanced filamentous growth, and increased virulence in a systemic model of infection. However, the EFG1-complemented P94015 strain actually showed decreased fitness in a commensal gastrointestinal model of colonization. Together, these results reveal the extent of intra-species variation in *C. albicans*, and demonstrate that natural mutations exist that can alter the balance between commensal and pathogenic lifestyles.

References:
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S1:3

FUNCTIONAL MAPPING OF THE *CANDIDA ALBICANS* SIGNALING MUCIN MSB2

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Signaling mucins regulate various signal transduction pathways in eukaryotes. In the fungal pathogen *C. albicans*, the mucin Msb2 controls protein kinase signaling to allow adaption to environmental stresses and to regulate cellular morphogenesis. In human host cells, surface recognition of *C. albicans* triggers cell signaling cascades, which elicit gene expression for a number of growth factors, chemokines,

cytokines and antimicrobial peptides (AMPs). Msb2 is a highly glycosylated plasma membrane protein, which is efficiently cleaved during growth, resulting in shedding of the extracellular part into the growth medium. Interestingly, the shed extracellular domain of Msb2 effectively protects fungal and bacterial cells from the action of AMP, which kill pathogens and attract immune cells to the site of infection [1]. In addition, secreted Msb2 inactivates the lipopeptide antibiotic daptomycin [2], which is of special importance in current anti-infectious therapy because it serves as reserve antibiotic for the treatment of multiresistant Gram-positive bacteria. Besides its cross-kingdom protective function, Msb2 is a key signaling element in response to defects in the cell wall of *C. albicans* that occur under immune attack or by treatment with antifungals. A systematic deletion analysis of Msb2 variants revealed different regions, which have a function in Msb2-mediated protection and signal transduction in *C. albicans*. Specifically, sequences surrounding the single transmembrane region are essential for sensing and regulation of different target genes during cell wall stress. 1. Szafranski-Schneider E, Swidergall M, Cottier F, Tielker D, Roman E, *et al*. (2012) Msb2 shedding protects *Candida albicans* against antimicrobial peptides. PLoS Pathog 8: e1002501. 2. Swidergall M, Ernst AM, Ernst JF (2013) *Candida albicans* mucin Msb2 is a broad-range protectant against antimicrobial peptides. Antimicrob Agents Chemother 57: 3917-3922.

S1:4

OPPOSING IMMUNOLOGICAL IMPRINTING OF *CANDIDA ALBICANS* BETA-GLUCAN TRAINED IMMUNITY AND ENDOTOXIN TOLERANCE IN MONOCYTES.

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Non-specific protective effects against reinfection have been described following infection with *Candida albicans*. Mice defective in functional T and B lymphocytes were protected against reinfection with *C. albicans* in a monocyte-dependent manner. *C. albicans* and beta-glucans induced functional reprogramming of monocytes or “trained immunity”, leading to enhanced cytokine production in vivo and in vitro. The training required the beta-glucan receptor dectin-1 and the non-canonical Raf-1 pathway and was associated with epigenetic reprogramming through genome-wide changes in histone trimethylation at H3K4. *Candida albicans* is primarily a human commensal of mucocutaneous surfaces. Interestingly, other commensal organisms such as Escherichia coli induce tolerance towards secondary challenges. Immunological imprinting of either tolerance or trained immunity after an infection or vaccination determines the functional fate of monocytes and macrophages, and the susceptibility of the host to secondary infections. We further investigated the epigenomic alterations that occur during these processes using in vitro training and tolerance of primary human peripheral blood monocytes with beta-glucans and LPS respectively. Genome-wide analysis of H3K4me1, H3K27ac and H3K4me3 patterns revealed multiple molecular pathways, including cAMP-dependent signalling. These potential novel mechanisms implicated in trained immunity induction were functionally validated both in vitro and in vivo. Altogether, these genome-wide approach of the innate immune memory further document the impact of microbial molecular patterns on the epigenome.

S1:5

IDENTIFICATION OF 2 NOVEL SIGNALING PROTEINS THAT GOVERN THE HOST RESPONSE DURING IN VIVO DISSEMINATED AND VAGINAL CANDIDIASIS.

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The ability to develop novel, desperately needed antifungal treatments requires a detailed knowledge of how the host and fungal pathogens respond to one another during infection. To address this need, we used RNA-seq to simultaneously characterize the transcriptomes of both *Candida albicans* and host cells during *in vitro* infection. We analyzed the interactions of *C. albicans* SC5314 and WO-1 with endothelial cells (HUVECs) and oral epithelial cells (OKF6-TERT2) at 1.5, 5, and 8 h post infection. Network analysis of the differentially expressed host genes confirmed that *C. albicans* infection activated multiple signaling pathways that are known to be involved in the response to *C. albicans* infection, including the ERK, Jnk, TNF, and EGF/ERBB2. This analysis also predicted the activation of two additional signaling pathways that have not previously been associated with the response to fungal infection, platelet derived growth factor BB (PDGF BB) and neural precursor cell expressed developmentally down-regulated protein 9 (NEDD9). We observed that *C. albicans als3*, *egf1*, *och1* mutants that have reduced host cell invasion also had decreased capacity to induce the expression of PDGF BB and NEDD9 target genes during *in vitro* infection. Furthermore, siRNA knockdown of either the PDGF receptor (PDGFR) or NEDD9 significantly reduced *C. albicans* invasion of both epithelial and endothelial cells. These results are consistent with a model in which *C. albicans* activates PDGFR and NEDD9 to facilitate invasion into host cells. To extend our *in vitro* observations, we performed RNA-seq analysis of the kidneys from uninfected mice and from mice with hematogenously disseminated candidiasis. In addition, we performed RNA-seq on vaginal samples collected longitudinally from women during health (absence of vaginal symptoms) and during clinically diagnosed episodes of vulvovaginal candidiasis. Network analysis of the differentially expressed genes predicted the activation of the PDGF and NEDD9 pathways in both the mouse model of disseminated candidiasis and in women with vulvovaginal candidiasis. Taken together, these results suggest that PDGFR and NEDD9 govern the interaction between *C. albicans* and host cells during systemic and mucosal infections.

S1:6

A INTRACELLULAR NON-RECEPTOR TYROSINE KINASE MEDIATES THE HOST INFLAMMATORY IMMUNE RESPONSE AND DETERMINES FUNGAL VIRULENCE

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The human fungal pathogen *Candida albicans* represents the 4th-most frequent cause of hospital-acquired infections with a mortality rate of about 40%. Cells of the innate immune system rapidly sense and respond to fungal pathogens, orchestrating the host response. The subsequent activation of pattern recognition receptor signaling pathways in immune cells regulates the immune response by cytokine production and many other innate effector functions directed against the pathogen. One group of signaling molecules involved in this process is the Tec kinase family. Members of the Tec kinase family constitute the second-largest group of non-receptor tyrosine kinases,

which are preferentially expressed in cells of the haematopoietic system. Deficiencies in Tec family kinases cause several immunological defects in humans and mice, including defects in B cell and T cell function. Notably, Tec kinase family members are also expressed in innate immune cells including monocytes, macrophages and dendritic cells. However, very little or nothing is known about their signaling function in these immune cells and a possible role in fungal-host interactions. We show here for the first time an essential role for a Tec family kinase in driving the inflammatory immune response in primary macrophages invaded by *Candida albicans*. We have identified all upstream components generating the pathogenic signal, as well as the downstream components required for inflammasome activation during the *Candida albicans* response. Remarkably, lack of this Tec family kinase attenuates fungal virulence, as we demonstrate that challenging kinase knock-out mice with *Candida albicans* dramatically improves survival to invasive candidiasis. Our data demonstrate a novel role for a non-receptor tyrosine kinase in the downregulation of the inflammatory response and in fungal pathogenesis. Moreover, our data strongly suggest a therapeutic relevance of host signaling kinases for antifungal drug development.

S2:1

ADAPTATION OF CANDIDA SPECIES TO MACROPHAGES DURING IN VITRO MICROEVOLUTION EXPERIMENTS

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Macrophages represent one of the first lines of host defence against invading microorganisms. Different pathogenic *Candida* species have evolved distinct strategies to cope with attack by these immune cells. Following phagocytosis, *Candida albicans* can readily escape macrophages, via hypha formation, whilst *C. glabrata* can survive and replicate intracellularly.

We have demonstrated that continuous exposure of *C. glabrata* to macrophages results in a striking morphological transition: following six months co-incubation, this normally unicellular yeast formed pseudohyphal-like structures. The "evolved" *C. glabrata* morphology was able to escape from and damage macrophages more effectively than the parental strain and exhibited higher virulence in infection models. Using whole genome sequencing and functional analysis, we were able to trace this novel morphogenic form to a single amino acid substitution in the catalytic domain of a chitin synthase.

Based on these results, we reasoned that the harsh environment of the macrophage phagosome exerts sufficient pressure to select for fungal escape mechanisms. Therefore, we have now performed a similar micro-evolution experiment using a *C. albicans* mutant lacking two key regulators of morphogenesis: Efg1 and Cph1. This yeast-locked strain is unable to form hyphae upon macrophage phagocytosis and cannot escape from these immune cells. Following several months of co-incubation of *efg1*/*cph1* cells with macrophages, this *C. albicans* mutant regained the ability to form hyphae and escape from the phagocytes via piercing. Moreover, these "evolved" *efg1*/*cph1* cells were able to undergo the morphological transition, and expressed hyphae-associated genes, under *in vitro* hypha-inducing conditions. The evolved strain also exhibited elevated virulence *in vivo*. Therefore *C. albicans* is able to bypass Efg1 and Cph1 and form hyphae in the absence of these key regulators of morphogenesis.

Together, our studies demonstrate a remarkably high degree of morphological flexibility, even over evolutionary short time-spans, and show that fungal filamentation provides an effective mechanism for distantly related yeasts to escape the harsh environment imposed by immune cells.

S2:2

TITLE NOT AVAILABLE CANDIDA/HOST INTERACTIONS: RESPONDING TO THE HOST ENVIRONMENT

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The opportunistic fungal pathogen *Candida albicans* commonly coexists with a host as a gut commensal. However, if the host becomes immunocompromised, the organism can cause life-threatening disease. The interactions between host, *C. albicans* and the co-colonizing microbiota that maintain the benign state of *C. albicans* colonization are incompletely understood. We hypothesized that specific *C. albicans* genes regulate colonization and maintain it at an optimal level. Studies showed that the 2 basic helix-loop-helix transcription factors Efg1p and Efh1p play key roles in regulating colonization. In experimental murine colonization, expression of *EFH1* at relatively high levels is associated with lower colonization while deletion of *EFH1* results in enhanced persistence of *C. albicans* in the GI tract. Deletion of *EFG1* results in transient high level colonization. Longer term, however, a strain lacking Efg1p is less fit than a WT strain, resulting in out-competition by the WT strain. These patterns of colonization may occur because Efg1p affects expression of metabolic genes and stress response genes during colonization. To provide further insight into the regulation of this important phase of the *C. albicans* lifestyle, continued analysis of gene expression during colonization is on-going.

S2:3

SMALL-CHAIN FATTY ACIDS INHIBIT *CANDIDA ALBICANS* IN VITRO GROWTH AND MURINE GASTROINTESTINAL COLONIZATION

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Background: Adult mice are resistant to *C. albicans* gastrointestinal (GI) colonization. The mechanisms of murine *C. albicans* colonization resistance (CACR) are unknown. We hypothesized that the small-chain fatty acids (SCFAs) that specific commensal bacteria produce might directly inhibit *C. albicans* growth and/or reduce GI colonization. **Methods:** C3H/HeN mice were GI colonized with *C. albicans* strains SC5314 after oral antibiotic pretreatment [1]. Bacterial gDNA was extracted from murine fecal samples for 16S rRNA sequencing and bacterial Phylum/Class specific qPCR. SCFAs -- acetic acid (AA), butyric acid (BA), and propionic acid (PA) -- were added to YPD to assess the effect on *C. albicans* growth (measured by OD600) in aerobic and anaerobic conditions. SCFAs were also administered to mice colonized with SC5314 via the drinking water to assess its effect on GI colonization. Transcriptome analysis (RNASeq on the Illumina HiSeq2000) was performed on RNA extracted from *C. albicans* grown aerobically in YPD at 30°C +/- 50 mM acetic acid. RNA-Seq data analysis was performed using DESeq software. **Results:** Penicillin treatment induced *C. albicans* colonization susceptibility (CACS) whereas streptomycin treatment resulted in continued CACR. 16S rRNA and bacterial group qPCR analysis of fecal contents showed a significant decrease in Firmicutes (most notably SCFA-producing Clostridia) and Bacteroidetes in mice that were CACS compared to CACR mice. Bacterial add-back experiments in *C. albicans* GI colonized mice (both antibiotic-treated mice and germ-free mice) showed that the SCFA-producing bacteria, Blautia producta (Firmicute) and Bacteroides thetaiotamicron (Bacteroidetes) were most effective in promoting CACR. AA, BA, and PA (at physiologically relevant concentrations) inhibited *C. albicans* growth in YPD under aerobic and anaerobic conditions and also reduced *C. albicans* GI colonization in mice. RNASeq analysis of *C. albicans* exposed to AA showed significantly decreased expression of genes involved in carbohydrate transport and metabolism: most notably the transcrip-

tion factor TYE7. TYE7 has been shown to be critical for regulating carbohydrate metabolism [2] and has recently been shown to be essential for murine GI colonization [3]. **Conclusion:** Bacteria are critical for maintaining CACR in mice. Genetically-distinct bacterial species (i.e. B. producta and B. theta) can promote CACR, and this effect may be mediated by the SCFAs that these bacteria produce. SCFAs inhibit in vitro and in vivo growth of *C. albicans*. AA induces down-regulation of *C. albicans* carbohydrate metabolism genes, including the transcription factor TYE7. Thus, CACR may be dependent on the gut metabolite (SCFAs) milieu rather than specific gut microbiota populations. 1. PLoS Pathog. 2008; 4(2):e35. 2. PLoS Pathog. 2009;5(10):e1000612 3. PLoS Biol. 2013;11(3):e1001510

S2:4

PASSAGE THROUGH THE MAMMALIAN INTESTINE TRIGGERS A PHENOTYPIC SWITCH THAT PROMOTES *CANDIDA ALBICANS* COMMENSALISM

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Candida albicans persistently colonizes the gastrointestinal tract of most healthy humans. However, it also causes debilitating infections in patients with immune deficits or antibiotic-associated remodeling of the normal gut microbiota. Research on *Candida* pathogenesis has been conducted primarily with virulence models, and much less is known about the mechanisms of fungal persistence in the commensal milieu. We report that exposure of *C. albicans* to the mammalian gut triggers a developmental switch, driven by the Wor1 transcription factor, to a commensal cell type[1]. Wor1 expression was previously observed only in cells that are homozygous at their mating locus, where it controls a white-opaque phenotypic switch important for mating. We show that passage of wild-type cells that are heterozygous at their mating locus (a/alpha cells) through the mouse gastrointestinal (GI) tract triggers *WOR1* expression. Mutants lacking *WOR1* were rapidly depleted from the murine GI tract when competed with wild-type cells. In contrast, a/alpha strains engineered to constitutively express *WOR1* (*WOR1*^{OE}) outcompeted wild-type cells in the GI tract. Upon passage through the mouse GI tract, these *WOR1*^{OE} cells underwent a novel phenotypic switch (termed GUT for gastrointestinally induced transition) over time. GUT cells formed darker, flatter colonies and looked elongated under the microscope. Although GUT cells showed some morphological characteristics of traditional 'opaque' cells, these cells were not competent for mating. GUT cells also showed virulence defects when compared with 'white' cells. Moreover, transcriptional analysis showed that GUT cells express a transcriptome that is optimized for the digestive tract. Our results suggest that GUT cells differ morphologically and functionally from previously defined cell types. The white-GUT switch illuminates how a microorganism can use distinct genetic programs to transition between invasive pathogenesis and commensalism. [1] Pande, K, Chen, C, Noble, SM (2013) Passage through the mammalian intestine triggers a phenotypic switch that promotes *Candida albicans* commensalism. *Nature Genetics* 45-1088-1091

S2:5

SYNERGISTIC REGULATION OF HYPHAL ELONGATION BY HYPOXIA, CO_2, AND NUTRIENT CONDITIONS CONTROLS PATHOGENICITY OF *CANDIDA ALBICANS*

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Candida albicans reversibly switches between yeast and hyphal morphologies, with hyphae being associated with virulence. Hyphal initiation and maintenance depend on host environment sensing. Hyphal maintenance *in vitro* requires chromatin remodeling of hypha-specific gene promoters, although disrupting chromatin-remodeling does not disrupt *C. albicans* hyphal elongation and virulence during invasive infection. We find that the combination of hypoxia and high CO_2, but neither condition alone, maintains hyphal elongation, even in mutants lacking the nutrient-responsive chromatin-remodeling pathway. Ume6, the transcriptional activator of hypha-specific genes, is stabilized via regulation by Ofd1, a prolyl hydroxylase family member inhibited by hypoxia, and by an uncharacterized pathway that senses high CO_2. Virulence and hyphal elongation in *vivo* are attenuated only when the parallelly acting Ume6 stabilization and chromatin-remodeling pathways are both blocked. The evolution of redundant signaling pathways allowing *C. albicans* to adapt to varied host environments may explain this commensal's success as a pathogen.

S3:1

DYNAMIC HOST-PATHOGEN INTERACTIONS RESULT IN FUNGAL EPITOPE UNMASKING IN VITRO AND IN VIVO.

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The ability of a host to recognize pathogens is the first step in their clearance. The cell wall of *Candida albicans* has many components that the host can recognize, including the β-glucan polysaccharide recognized by the host receptor Dectin-1. While β-glucan is typically masked, it becomes unmasked during disseminated infection. Exposure levels of this epitope during infection may be important, as Dectin-1 mediates protection against at least some strains of *C. albicans* and *in vivo* alterations in the organization and composition of the *Candida* cell wall influences the host response. To understand β-glucan unmasking during infection, we looked to identify the mechanisms whereby host-pathogen interactions alter fungal epitope exposure. We reasoned that β-glucan unmasking could result from direct stripping of the cell wall, by an active mechanism independent of immune attack, or by an active fungal response to immune attack. To examine this, we tested if immune cells can cause β-glucan exposure *in vitro*. We biotinylated cell wall proteins and labeled with fluorescent streptavidin, then incubated with neutrophils. Timelapses show that streptavidin fluorescence is lost rapidly at sites of neutrophil attack, suggesting that there is immune-mediated damage to the cell wall. Staining with soluble Dectin-1-Fc and Calcofluor White revealed that areas where streptavidin was lost had β-glucan exposure and increased chitin deposition. This suggests that neutrophils can cause β-glucan exposure, and to determine if neutrophils are required for unmasking *in vivo*, we examined β-glucan exposure on *C. albicans* in mice with neutropenia. Depletion of neutrophils largely prevented β-glucan exposure *in vivo*. These lines of evidence suggest that neutrophilic immune attack is necessary for β-glucan unmasking. We then tested if immune-mediated unmasking requires an active fungal response. We found that UV-inactivated *Candida* did not respond to attack with increased chitin deposition or β-glucan exposure, suggesting that these are active processes. Taken together, the data suggests that β-glucan unmasking and chitin deposition result from an active fungal response to immune attack. Current work is focused on the mechanisms that direct this interaction. On the host side, we are testing the importance of neutrophil proteases and reactive oxygen species. We are also exploring the mechanisms required on the fungal side for unmasking in response to attack. Overall, this work helps elucidate host-pathogen interactions in disseminated candidiasis, including insight into immune mechanisms of attack and how *C. albicans* responds. Given the importance of the cell wall as a drug target, understanding how

this fungus maintains integrity during immune attack may identify novel therapeutic targets to aid the treatment of candidiasis.

S3:2

RAB14 RECRUITMENT TO PHAGOSOMES REDUCES MACROPHAGE KILLING BY LIVE *C. ALBICANS*

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Candida albicans virulence is in part due to its ability to switch from yeast to hyphal form and physically rupture and escape from macrophages after phagocytosis {1}. This escape is likely to occur before phagosomes have fully matured into a microbicidal organelle. This makes *C. albicans* a good model organism to study the role of the small GTPase Rab14 in phagosome maturation. Rab14 has been found to recruit to bacterial phagosomes{2}, but its role in fungal phagosome maturation is not fully understood. Here, we demonstrate an important role for Rab14 in protecting macrophages against killing by live *C. albicans*. Macrophages were transfected to express GFP-Rab14, GFP-dominant negative constructs ($Rab14^{S25N}$ and $Rab14^{N124I}$) or siRNA to mediate Rab14 knockdown and then infected with live or UV killed *C. albicans* and studied using confocal and sophisticated live cell imaging and image analysis tools {3}. We showed that phagosomes containing live *C. albicans* became Rab14 positive following phagocytosis. Interestingly, we observed differential recruitment of Rab14 to phagosomes depending on *C. albicans* morphology. Phagosomes containing yeast *C. albicans* retained Rab14 GFP for 5 minutes while hyphal phagosomes retained Rab14 for approximately 12 minutes. Depletion of endogenous Rab14 in J774.1 macrophages did not affect macrophage migration towards live fungi, the rate of engulfment or phagosome acidification of UV killed and live *C. albicans* phagosomes when compared to controls. We also found that a knockdown of Rab14 expression did not influence either the time of Rab5-GFP localisation to phagosomes following uptake or the duration of Rab5-GFP on phagosomes containing *C. albicans*. Importantly, we found that silencing Rab14 disrupts LAMP1 colocalisation, cathepsin activation and Rab7 recruitment to live *C. albicans* phagosomes. Rab14 protein knockdown was associated with a significant increase in macrophage killing by *C. albicans* when compared to controls. Together, these data indicate that in macrophages, Rab14 is mainly localized to early endosomes upstream of Rab7 and plays an important role in the initiation of late stages of phagosome maturation. Our data points to a model where Rab14 may be important in protecting macrophages against killing by hyphal *C. albicans.* 1. McKenzie CGJ, Koser U, Lewis LE, Bain JM, Mora-Montes HM, Barker RN, et al. Contribution of *Candida albicans* cell wall components to recognition by and escape from murine macrophages. Infect Immun 2010;78(4):1650-1658. 2. Kyei, G.B., Vergne, I., Chua, J., Roberts, E., Harris, J., Junutula, J.R. and Deretic, V. (2006). Rab14 is critical for maintenance of Mycobacterium tuberculosis phagosome maturation arrest. *Embo Journal* **25,** 5250-5259. 3. Lewis LE, Bain JM, Lowes C, Gow NAR, Erwig L-. *Candida albicans* infection inhibits macrophage cell division and proliferation. Fungal Genetics and Biology 2012;49(9):679-680.

S3:3

PRESENCE OF THE MULTIFUNCTIONAL PROTEIN ALS3 ON *CANDIDA ALBICANS* HYPHAE DEPENDS ON THE TUP1 REPRESSOR

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The *Candida albicans ALS3* gene encodes a protein with multiple functions in adhesion and invasion as well as iron acquisition *in vitro* and *in vivo*. *ALS3* belongs to a small set of genes whose expression are increased during the yeast to hyphae transition regardless of the environmental stimulus. These core filamentation response genes are characterized by large promoter regions and are heavily regulated by several transcriptional activators and repressors. In this work, we have analyzed the expression dynamics of *ALS3* in the absence of the repressors Tup1 and / or Nrg1. In response to a hyphal growth trigger, the amount of *ALS3* mRNAs increased by at least 30 fold in wild type as measured by qRT PCR. In contrast to this, it remained stable at an intermediate level in *tup1*Δ, well above of comparable wild type yeast cells, but significantly lower than in wild type hyphae. These expression dynamics were not found in *nrg1*Δ where *ALS3* expression was higher than in wild type yeast cells, but reached the height of wild type hyphae under filament- inducing conditions. The absence of this filament- specific transcriptional shift correlated with very low Als3 protein signal on the surface of *tup1*Δ filaments, regardless of the environmental condition. The mutant was consequently neither able to bind ferritin nor to grow in media with that protein complex as the exclusive iron source. In contast, *nrg1*Δ was able to accomplish growth under this condition, indicating a full functionable Als3 level. A *tup1*Δ / *nrg1*Δ double mutant showed *ALS3* expression dynamics which were similar to that of *tup1*Δ alone and consequently, the double mutant failed to use ferritin as sole iron source. In line with these findings, *nrg1*Δ, but neither *tup1*Δ nor *tup1*Δ / *nrg1*Δ, was still able to invade and damage human host cells in an *in vitro* approach. This was supported by a remaining virulence of *nrg1*Δ in an intraperitoneal mouse infection model. These results indicate that the presence of the Tup1 repressor is required to reach high *ALS3* expression levels as found in wild type hyphae. Additionally, we could show that there seems to be a threshold of mRNA amounts which must be surpassed to generate enough Als3 protein for its full function in iron acquisition. Our data revealed also functional differences between the repressors Tup1 and Nrg1 during the regulation of at least one core filamentation response gene.

S3:4

INVESTIGATING THE ROLE OF HSF1 IN *CANDIDA ALBICANS* DRUG RESISTANCE AND MORPHOGENESIS

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Invasive fungal infections caused by *Candida albicans* are among the leading causes of death in immunocompromised individuals. Treatment is challenging due to the emergence of resistance to the limited number of antifungal drugs in clinical use. A key regulator of *C. albicans* drug resistance and morphogenesis is Hsp90, an essential molecular chaperone that stabilizes diverse client proteins. Inhibition of Hsp90 also regulates the *C. albicans* morphological transition from yeast to filamentous

growth, which is a key virulence trait. Hsp90 inhibition induces filamentation and abrogates drug resistance, rendering resistant pathogens susceptible to treatment in multiple infection models. It has recently been found that the transcription factor Hsf1, which regulates the expression of HSP90 and ~3% of the yeast genome, is essential for *C. albicans* virulence. Here, we focus on elucidating the role of Hsf1 in two key virulence traits: drug resistance and morphogenesis. We have found that depletion of Hsf1 has similar effects on drug resistance and morphogenesis as Hsp90 inhibition. Genetic depletion of Hsf1 reduces resistance to echinocandins, which target the cell wall, and induces filamentation, even when HSP90 expression is driven by a constitutive promoter, independent of Hsf1. These phenotypes are also observed upon induction of HSF1 expression suggesting that dramatic changes in HSF1 expression are sufficient to induce filamentation and increase echinocandin susceptibility, independently of HSP90 expression. To determine if these effects on drug resistance and morphogenesis are a result of compromised Hsp90 function, we monitored Hsp90's capacity to enable activation of the client protein Hog1 by monitoring the levels of activated, phosphorylated Hog1. We determined that Hsp90 function in enabling activation of Hog1 is compromised upon Hsf1 depletion. We hypothesize that alterations in the expression of Hsf1 affects drug resistance and morphogenesis by modulating the expression of a co-chaperone or other critical regulator of Hsp90, leading to compromised chaperone function. We are currently taking an unbiased approach to determine Hsf1-dependent genes by performing microarray analysis under Hsf1 induction and depletion conditions. To identify Hsf1-dependent genes through which it controls morphogenesis and drug resistance, we will engineer strains with constitutive expression of candidate effectors to determine those that block the impact of Hsf1 alterations on drug resistance and morphogenesis. This work illuminates a novel role for Hsf1 in regulating drug resistance and morphogenesis of *C. albicans*. Elucidating the circuitry through which Hsf1 regulates drug resistance and morphogenesis will suggest new targets for treating fungal infections.

S3:5

CONTROL OF METABOLIC ACTIVITY BY CDK8 INFLUENCES PYOCYANIN SUSCEPTIBILITY IN *C. ALBICANS*

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A key virulence factor of *Candida albicans* is the ability to form biofilms that are composed of adhesin-expressing yeast and hyphal cells which are together encased in an extracellular matrix. Biofilms, which are structurally and metabolically similar to wrinkled colonies, can readily form on indwelling biomedical devices and cause increased resistance to antifungals. Pyocyanin (PYO), a redox-active molecule produced by *Pseudomonas aeruginosa*, inhibits filamentation and interferes with respiratory activity in *C. albicans*. Here, we used wrinkled colonies to further characterize the effects of PYO on *C. albicans* morphology and metabolism. We screened a mutant collection that revealed the *ssn3* and *ssn8* mutants had increased resistance to the inhibitory effects of PYO (PYO^R). Ssn3 and Ssn8 are components of Mediator, a highly conserved eukaryotic complex that can serve as both a transcriptional co-activator and co-repressor. The identification of Mediator mutants in our screen is consistent with the key role of transcription in modulating the transition to biofilm or wrinkled colony formation. These proteins are specifically components of the heterotetrameric CDK8 module of Mediator which is primarily implicated in transcriptional repression, and subsequent studies revealed all CDK8 mutants—*ssn3*, *ssn8*, *srb8* and *srb9*—had identical PYO^R phenotypes. These mutants were also found to be hyperalkalinizers, which is indicative of increased amino

acid oxidation. Consequently, we hypothesized that mutation of the CDK8 complex resulted in basal level differences in metabolism, and that these differences were even more striking in the presence of PYO. To test this hypothesis, we completed a metabolomics study using the WT, *ssn3*Δ/Δ and *ssn3*Δ/Δ-*SSN3* strains grown under wrinkling-inducing conditions in the presence and absence of PYO. Mutation of *Ssn3* resulted in perturbation of multiple metabolic pathways, and the effect of PYO on these pathways varied tremendously between the WT and *ssn3*Δ/Δ strains. More direct studies revealed that a compromised CDK8 complex caused increased glycolysis and oxidative metabolism, but not fermentation. Furthermore, all CDK8 mutants had higher levels of intracellular ATP compared to the WT and, in contrast to the WT, these levels were not decreased in the presence of PYO. In summary, our results support the hypothesis that the CDK8 component of Mediator regulates aspects of metabolism in ways that alter the response of *C. albicans* to PYO.

S3:6

A NOVEL FAMILY OF ANTIOXIDANT ENZYMES THAT PROTECTS THE *CANDIDA ALBICANS* PLASMA MEMBRANE FROM LIPID PEROXIDATION

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The plasma membrane (PM) of *Candida albicans* is rich in polyunsaturated fatty acids (PUFAs), which increase the fluidity of the PM and are thought to help this fungal pathogen to adapt to different environments in the host. However, PUFAs also make the PM more susceptible to oxidative stress, as they more readily undergo lipid peroxidation, which can in turn lead to other kinds of oxidative damage to proteins and nucleic acids. Although protecting against lipid peroxidation is extremely important, very little work has been done to define the mechanisms, because most previous studies have focused on antioxidant enzymes that are either secreted or cytosolic rather than in the PM. Based on recent studies in mammalian cells and *S. cerevisiae*, it is likely that ubiquinone, also known as Coenzyme Q, acts as the major antioxidant in the PM. Ubiquinone is a long chain quinone molecule that is best known for its role in the mitochondrial electron transport chain, but it is also abundant in PM of eukaryotic cells. Previous studies have shown that cells must use special mechanisms to safely reduce oxidized ubiquinone to ubiquinol so that it can be reused. One electron reduction of ubiquinone produces a semi-quinone intermediate, which is highly deleterious as it generates reactive oxygen species. Cells try to avoid this by using enzymes that carry out a two-electron reduction of ubiquinone. Our search for candidate proteins identified four flavodoxin-like proteins (FLPs) Pst1, Pst2, Pst3 and Ycp4 that displayed strong sequence similarity to NAD(P)H quinone oxidoreductases in *E. coli* and we found that they localized to the PM. Our data show that mutation of these genes causes increased lipid peroxidation and decreased viability after cells are exposed to oxidized PUFAs. Co-incubation with antioxidant Vitamin E reverses the deleterious effect of PUFAs. Thus, our studies identify a novel mechanism by which fungal cells protect the PM from oxidative stress. Virulence assays are currently being conducted in mice and will be presented at the meeting.

S3:7

PIPELINE FOR THE ANALYSIS OF COPY NUMBER AND LOSS OF HETEROZYGOSITY CHANGES IN *C. ALBICANS* GENOME ARRAY AND SEQUENCE DATASETS.

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Candida albicans is an opportunistically pathogenic yeast known for a robust tolerance of even dramatic changes to its genome, such as large-scale copy number changes and the reduction of genetic information caused by loss of heterozygosity events. These changes

can strongly impact the ability of *C. albicans* to survive in the various and changing environments of the human host, including the formation of problematic invasiveness and drug resistance changes in clinical settings. In an experimental context these changes can form concurrently to intended alterations, resulting in secondary phenotypes that confound the intended analyses. Understanding what changes have happened is an important first step in discerning the impact of large-scale genome structural changes or in identifying causes of major unexpected variation in experimental results. To this end, we have developed a generalized genome data analysis pipeline, incorporating freely available and novel in-house tools, to analyze SNP/CGH microarray, whole genome sequence, and double-digest RADseq datasets with a common interface and visualization scheme. The pipeline uses whole-genome ploidy estimates, from flow cytometry or other sources, and local copy number ratios, calculated from the high-density array or sequence datasets, to generate copy number estimates across the genome. The pipeline incorporates known hapmap features for the *C. albicans* reference (SC5314) extended with whole genome sequence data and can also display changing SNP distributions in clinical strains without pre-determined haplotype information. Combining these features, the pipeline rapidly condenses very large datasets into figures showing copy number and SNP allele changes across each chromosome of a genome using simple to interpret colors. Inferences about observed large-scale genomic alterations can then be made to further the analysis of drug resistance, growth alteration, or other phenotypic changes in *C. albicans*.

S3:8

ANALYSIS OF THE *CANDIDA ALBICANS* TRANSCRIPTION FACTOR FCR1P REGULON

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Candida albicans is a dimorphic human fungal pathogen that causes life-threatening infections in immunocompromised individuals. The *C. albicans* genome contains 82 genes encoding transcription factors (TFs) of the zinc cluster family. Some of these TFs have been shown to regulate cellular processes such as sugar metabolism, filamentation or drug resistance, yet a majority remains to be characterized. We previously identified Fcr1p, a zinc cluster TF whose deletion increases cell tolerance to multiple drugs. It was also shown in a large-scale screen that an fcr1 heterozygous transposon mutant was hyperfilamentous. These findings indicate that Fcr1p functions as a negative regulator but its transcriptional targets are still unknown. We used genomic approaches to characterize the Fcr1p regulon. To identify where Fcr1p binds in the genome, we tagged it with an HA epitope and performed a ChIP-Chip analysis under standard growth conditions (YPD, log phase). We identified 144 genes bound by Fcr1p (binding ratio $\geq$ 1.5-fold, $p \leq 0.01$), including several genes involved in ammonium, amino acid and oligopeptide transport (MEP1, CAN1, CAN2, CAN3, GAP2, GAP6, OPT1, OPT4, OPT9), nitrogen metabolism (GLT1, GLN1, GDH3, DUR1) and transcriptional regulation of nitrogen utilization (GAT1, STP3). Strikingly, Fcr1p was found to bind predominantly within the open reading frame of its targets, suggesting that it may bind DNA indirectly, through association with the transcriptional or chromatin machinery. To identify the genes whose expression is regulated by Fcr1p, we constructed an fcr1Δ/Δ mutant in the SC5314 background using the SAT1 flipper strategy and performed expression profiling under the same growth conditions. We found 57 downregulated and 30 upregulated genes in the mutant compared to the wild-type (ratio $\geq$ 1.5-fold, $p \leq 0.01$), indicating that Fcr1p can function as an activator or a repressor of gene expression. Among the most significantly downregulated processes are genes pertaining to nitrogen utilization (CAN1, AAP1) while upregulated processes include genes involved in filamentous growth

and biological adhesion (EFH1, HWP1, ALS3, SAP5). Finally, only a minority (6 %) of the modulated genes were bound by Fcr1p, mainly those involved in amino acid transport, suggesting that a majority of the modulated genes are indirect targets of Fcr1p. Taken together, our results indicate that Fcr1p regulates its target genes in a complex manner, acting as a positive regulator of nitrogen metabolism and as a negative regulator of filamentation and cell adhesion. This later proposition was validated by the demonstration that FCR1 overexpression abrogates filamentation. In addition, Fcr1p seems to mediate its function mainly indirectly, possibly through intermediate effectors, a hypothesis that would position this TF upstream of other important regulators.

S3:9

PPG1, A PP2A PROTEIN PHOSPHATASE, CONTROLS *CANDIDA ALBICANS* MORPHOLOGY AND VIRULENCE

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Candida albicans, the most prevalent human fungal pathogen, is a major cause of hospital-acquired bloodstream infections in the U.S. *C. albicans* infections, which occur frequently in immunocompromised individuals, have become more difficult to treat due to a limited number of antifungal drugs and increased frequency of drug-resistant isolates. *C. albicans* virulence requires the ability to undergo a reversible transition from single ovoid budding yeast cells to elongated cells attached end-to-end known as filaments. This transition allows for efficient tissue invasion, immune evasion and dissemination. While many filament-specific target genes have been identified, considerably less is known about *C. albicans* filamentous growth signaling pathways and regulatory circuits. We have demonstrated that a serine/threonine protein phosphatase type 2A (PP2A), Ppg1, is important for *C. albicans* morphogenesis under a variety of both solid and liquid filament-inducing conditions. Ppg1 also controls down-regulation of *NRG1*, which encodes a key transcriptional repressor of filament-specific target genes, in response to growth in serum at 37°C. We have recently generated a Ppg1 catalytic mutant using site-directed mutagenesis. This mutant shows a filamentation defect equivalent to that of the *ppg1*Δ/Δ strain, demonstrating that Ppg1 phosphatase activity is specifically important for *C. albicans* morphogenesis. The *ppg1*Δ/Δ mutant was also found to be highly attenuated for virulence in a mouse model of systemic candidiasis. Finally, we have demonstrated that a strain expressing high constitutive levels of *PPG1* is defective for filamentation under a variety solid inducing conditions. We are currently investigating the relationship between Ppg1 and several known *C. albicans* filamentous growth signaling pathways. Overall, our data suggest that a PP2A protein phosphatase plays an important role in controlling both morphology and virulence of *C. albicans*. These studies are significant because gaining a better understanding of regulatory circuits that control *C. albicans* filamentous growth and virulence will provide information that may eventually lead to the development of novel and more effective antifungal strategies to treat candidiasis.

S3:10

UNDERSTANDING THE EVOLUTION OF POST-TRANSCRIPTIONAL GENE NETWORKS IN FUNGI

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Evolutionary rewiring presents a fascinating insight into how related gene networks can develop distinct functions in closely related

species. Most studies so far have focused on transcription, and comparatively little is known about the evolution of post-transcriptional networks, and what this means for biological outcomes within the organism. The model yeast Saccharomyces cerevisiae and the fungal pathogen *Candida albicans* are evolutionarily related, but live in different environments and have had to adapt to specific conditions in their respective niches. It has been proposed that changes to gene expression control have contributed to phenotypic diversification. We are focusing on posttranscriptional regulation of mitochondrial biogenesis by the RNA binding protein Puf3, which binds to a large number of mRNAs encoding mitochondrial proteins, and targets them to the surface of mitochondria for localised translation. Our data suggests that rewiring has occurred at multiple stages of the evolution of gene expression related to the Puf3 network between S. cerevisiae and *C. albicans*, particularly in regards to the regulation by carbon source. The ability of *C. albicans* to respond properly to changing nutrient conditions is key for successful host infection, and our studies aim to address the role of Puf3-dependent posttranscriptional gene network in this process.

S3:11

NOVEL ROLE OF A FAMILY OF MAJOR FACILITATOR TRANSPORTERS IN BIOFILM DEVELOPMENT AND VIRULENCE OF *CANDIDA ALBICANS*

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We had earlier demonstrated that *Candida albicans* cells were susceptible to malachite green (MG) and its effect was independent of known antifungal targets. Transcriptional profiling in response to MG treatment revealed that out of total responsive genes, QDR1 (Quinidine Drug Resistance) was among the most induced gene. QDR family of genes encode transporters belonging to Major Facilitator Superfamily (MFS) of proteins. Out of 95 members of MFS super family, Candida genome has three QDR genes annotated in CGD (http://www.candidagenome.org) as QDR1 (orf19.508), QDR2 (orf19.6992) and QDR3 (orf19.136). Present study explores the role of QDR genes. We show that QDR transporters do not play any role in drug resistance. In addition, deletion mutants of QDR1, QDR2 and QDR3 show no impact on susceptibilities towards azoles, polyenes, echinocandins, polyamines, quinolines as well as cell wall inhibitors and many other stresses. However, the deletion of QDR genes individually or collectively led to defects in biofilm architecture and thickness. Interestingly, qdr1, 2 and 3 deletion mutants displayed attenuated virulence but maximum effect was observed in case of qdr2Δ, qdr3Δ and qdr1/2/3Δ strains. Notably, the attenuated virulence and biofilm defects could be reversed in QDR revertants. The transcription profiling of QDR deletion mutants and its validation confirmed differential expression of many biofilm and virulence related genes in deletion mutants which were down regulated minimum by 2 folds in comparison to WT Candida. Furthermore, lipidomic analysis of QDR deletion mutants suggest remodelling of membrane lipids which may affect cell signalling leading to defect in biofilm and attenuation in virulence. Taken together, our results suggest that QDR genes of Candida differ from its known roles in Saccharomyces cerevisiae and these genes have interesting implications in biofilm formation, virulence and in maintaining membrane lipid homeostasis in Candida cells.

S3:12

THE ENDOSOMAL GTPASE VPS21P CONFERS AZOLE SUSCEPTIBILITY IN *CANDIDA ALBICANS*

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Development of resistance to the most commonly used antifungals, the azoles, is an increasing clinical problem. In *Candida albicans* known mechanisms of azole resistance include increased expression of the target enzyme Erg11p and of drug efflux pumps such as Cdr1p and Mdr1p. However, the resistance of many clinical isolates is not accounted by established mechanisms. The azole antifungals block ergosterol biosynthesis through inhibition of lanosterol 14α-demethylase (Erg11p), and result in the accumulation of 'toxic' intermediate sterol species, which are thought to compromise the function of the plasma membrane. However, the consequences of azole treatment and resulting ergosterol depletion upon the function and integrity of *intracellular* membranes is not well characterized. Using a high-throughput screening assay, we recently identified two azoles as causing vacuolar defects in *C. albicans*. The purpose of this study was to investigate the impact of azole treatment upon the integrity of the *C. albicans* vacuole, and determine if vacuolar function can influence azole susceptibility. Specifically, we hypothesized that mutants with vacuolar defects may have altered azole susceptibility. We therefore tested the azole susceptibility of several mutants with defects in distinct vacuolar trafficking pathways, using the CLSI broth microdilution method. Strikingly, mutants lacking the endosomal Rab GTPase Vps21p had significantly reduced susceptibility to several azole antifungals. The azole resistance of the *vps21Δ/Δ* mutant was not dependent upon the Cdr1p or Mdr1p efflux pumps, since *vps21Δ/Δcdr1Δ/Δ* and *vps21Δ/Δmdr1Δ/Δ* double mutants remained resistant. However, inhibition of calcineurin signaling using cyclosporin A, abolished the azole resistance of the *vps21Δ/Δ* mutant. Finally, ergosterol depletion caused by azole treatment has been linked to inhibition of the vacuolar proton pump (V-ATPase), and thus reduced vacuolar acidification. Unexpectedly, we found that quinacrine accumulation increased following fluconazole treatment, suggesting increased vacuolar acidification. Furthermore, in contrast to the equivalent *Saccharomyces* mutant, our *C. albicans vps21Δ/Δ* mutant had increased vacuolar acidification compared to isogenic control strain. We are currently testing if increased V-ATPase activity contributes to the azole resistance of the *vps21Δ/Δ* mutant. Thus perturbation of endosomal trafficking through the Rab GTPase Vps21p may represent a novel, calcineurin dependent mechanism of azole resistance.

S3:13

SIGNALING BY A BIOFILM SURFACE ADHERENCE PROTEIN

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The cell wall is the fundamental sensing and responding interface between a pathogenic cell and its microenvironment. It is particularly critical for *C. albicans*, playing roles in protection, morphogenesis and adherence. Cell wall proteins (CWPs), specifically the adhesins, are important for biofilm formation promoting adherence to a substrate during initial biofilm establishment. Als1 is one such critical adhesin. We show Als1 is a key signalling component to a genetic program regulating biofilm formation circuitry. Als1 signals to the transcription factor Brg1 initiating a signature 'biofilm program' and is essential for biofilm

formation specifically in the denture stomatitis model. Transient expression of Als1 or Brg1 is sufficient to switch on the biofilm program and form a biofilm. Importantly, we demonstrate that CWPs may signal to diverse regulatory pathways promoting biofilm formation in differing niche environments. This may extend to other eukaryotes. Just as the cell wall is a dynamic structure, CWP gene expression may be dynamic and imperative in signalling in key *C. albicans* pathways and processes.

S3:14

SEXUAL BIOFILMS OF CANDIDA TROPICALIS, AND A ROLE FOR A SEXUAL AGGLUTININ

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Candida tropicalis is an opportunistic human pathogen that was recently shown to undergo the white-opaque phenotypic switch, similar to that in *Candida albicans*. In both species, opaque cells respond to mating pheromones by forming mating projections and completing conjugation, while in *C. albicans*, white cells respond to pheromones secreted by opaque cells by forming 'sexual' biofilms. Here, we demonstrate that, in contrast to *C. albicans*, it is C. tropicalis opaque cells that form biofilms when responding to sexual pheromones, while white cells do not. These sexual biofilms are dependent on pheromone receptors Ste2 and Ste3, yet pheromone signaling alone does not result in robust biofilm formation, suggesting that cell-cell contact between mating partners plays an important role in this process. The structure of sexual biofilms produced by C. tropicalis was studied using confocal and scanning electron microscopy. This analysis revealed that biofilms were composed of several layers, with the outermost layer consisting of filamentous cells and extracellular matrix, while yeast-form cells dominated the basal layer. Transcriptional profiling showed that many genes necessary for mating were upregulated in sexual biofilms, including genes involved in conjugation. In particular, FGR23, which encodes an ortholog of the S. cerevisiae sexual agglutinin Aga1, was upregulated in sexual biofilms, and deletion of this gene significantly reduced sexual biofilm formation and also reduced the frequency of sexual mating. Taken together, this work indicates C. tropicalis opaque cells are competent for sexual biofilm formation, and that biofilms exhibit a complex structure consisting of stratified layers of cells and extracellular matrix. In addition, a conserved sexual agglutinin is revealed to be important for mediating both mating and biofilm responses in this species.

S3:15

CANDIDA ALBICANS-STAPHYLOCOCCUS AUREUS INTRA-ABDOMINAL INFECTION-MEDIATED LETHAL SEPSIS: ATYPICAL REQUIREMENTS OF MORPHOGENESIS AND VIRULENCE

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Background: Intra-abdominal polymicrobial infections cause significant morbidity and mortality. An experimental mouse model of *C. albicans/S. aureus* intra-abdominal infection resulted in ~60% mortality within 48 h post-inoculation concomitant with amplified local inflammatory responses, while monomicrobial infections were avirulent. The purpose of this study was to characterize early local and systemic responses during co-infection, determine the role of *C. albicans* morphogenesis in lethality, a trait involved in virulence and interaction with *S. aureus,* and define which fungal/bacterial interactions induce motility. **Methods**: Outbred mice were inoculated i.p. with *S. aureus* ($8x10^7$) and/or *C. albicans* ($7x10^6$-$3.5x10^7$) and followed for survival over 5 days. Peritoneal lavage fluid, spleen, brain, and serum was collected at defined time points post-inoculation to quantify microbial burden, cytokine levels, and cellular infiltrate (peritoneal). A similar survival design included yeast- and hyphae-locked *C. albicans* mutants, and

other yeast species that cannot form hyphae (*C. glabrata* and *Saccharomyces cereviseae)*. **Results**: Local and systemic pro-inflammatory cytokines were significantly elevated during co-infection at early time points (4-12h). Conversely, microbial burden in the organs and peritoneal lavage was similar between mono- and co-infected animals through 24 h, as was peritoneal neutrophil infiltration. After optimizing the model for 100% mortality in 48 h with 5x higher *C. albicans* and the same number of *S. aureus*, co-infection using the *C. albicans* morphogenesis mutants showed similar mortality, dissemination, and local and systemic inflammation as the isogenic control. Conversely, co-infections with *C. glabrata* or *S. cerevisiae* could not replicate the synergistic lethality. Finally, co-infection with the yeast-locked *C. albicans* inoculated i.v. and *S. aureus* inoculated i.p. also failed to induce mortality. **Conclusions**: These results suggest a unique intra-abdominal interaction between the host and *C. albicans/S. aureus* that results in strong inflammatory responses, dissemination and lethal sepsis, which is unique to *C. albicans and* not dependent on morphogenesis.

S3:16

CGPDR1-DEPENDENT ADHERENCE OF *CANDIDA* GLABRATA TO HOST CELLS: IMPACT OF DIFFERENT YEAST STRAIN BACKGROUNDS AND *CGPDR1* GAIN-OF-FUNCTION MUTATIONS

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There are increasing numbers of multidrug-resistant *Candida glabrata* isolates recovered from patients presenting with disseminated infections non-responsive to therapy [1]. *C. glabrata* frequently develops secondary resistance to azole drugs, the most commonly used drugs to manage candidiasis, usually due to increased drug efflux. The mechanism typically consists on *CgPDR1*-mediated upregulation of ATP-binding cassette (ABC) multidrug transporter genes. Our laboratory reported a collection of matched pairs of *C. glabrata* clinical isolates including over 50 gain-of-function (GOF) mutations leading to *CgPDR1* hyperactivity and azole drug resistance. Surprisingly, the tested GOF mutants in an isogenic strain background (clinical isolate DSY562) were found to display increased fitness *in vivo* and virulence in comparison to the azole-susceptible wild type strains [2]. Follow-up work using *ex vivo* co-cultures of GFP and RFP-expressing yeast cells revealed a decreased adherence and phagocytosis of three tested GOF mutants (including mutations L280F, R376W and T588A) by murine bone marrow-derived macrophages (BMDMs) in competition with the wild type [3]. In turn, adherence assays to human and murine epithelial cell lines revealed an opposite trend, with GOF mutants displaying increased adherence [3]. In this work we report further studies to extend our previous findings. Reduced adherence of the GOF mutants to macrophages was closely reproduced using murine monocytic macrophage cell line RAW264.7 and human acute monocytic leukemia THP-1 cell line-derived macrophages. Increased adherence to epithelial was also observed with Chinese hamster CHO-Lec2 cells, human Caco-2 and HeLa cells and mouse CMT-93 cells. However, when testing additional CgPDR1 GOF mutations (Y584C, P822L, D1082G and E1083Q) we found that they do not all conserve the previously reported differences in adherence to mammalian cells. Furthermore, expressing the CgPDR1 GOFs in other *C. glabrata* strain backgrounds, including CBS138, BG2 and two additional clinical isolates from our collection, also yielded variable results. Our previous work suggested a dual contribution of *CgPDR1* hyperactivity to the increased virulence of *C. glabrata*: an optimized adherence to and colonization of epithelial tissues, as well as the evasion from the host's innate immune response. The data presented here show that these effects

are not inherent to *CgPDR1* hyperactivity, but rather dependent on specific CgPDR1 GOFs and strain backgrounds. Further studies are underway in our laboratory to understand the molecular and genetic mechanisms behind the *CgPDR1*-mediated regulation of *C. glabrata*-host interactions. References: [1] Pfaller MA, (2012) Am J Med, 125, S3-13 [2] Ferrari S *et al.*, (2009) PLOS Pathog 5, e1000268 [3] Vale-Silva LA *et al.*, (2013) Infect Immun, 81, 1709-1720

S3:17

CANDIDA ALBICANS EED1 IS INVOLVED IN THE RESPONSE TO QUORUM SENSING

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A complex network of regulatory factors controls the morphology of *Candida albicans* and various factors are involved in yeast-to-hypha transition. The Eed1 protein is a regulatory factor of filamentation in *C. albicans* and especially important for the maintenance of hyphal development and growth. Mutants lacking the *EED1* gene retain the ability to initialize hyphal growth by the formation of germ tubes, but do not form elongated true hyphae but pseudohyphae before switching back into an elongated yeast cell-like growth form. The exact molecular role of Eed1 in hyphal elongation is still unclear. We found that filamentation of an *eed1*Δ mutant strongly depends on cell density which suggested a link between *EED1* and quorum sensing. This has been confirmed by filamentation assays, where we observed that *eed1*Δ mutants were significantly more sensitive to the inhibitory effect of farnesol in comparison to the wild type. Hypothesizing that the link between *EED1* and quorum sensing is mediated by farnesol, *eed1*Δ mutants lacking different regions of the *EED1* gene were challenged with farnesol in vitro under various filament-inducing conditions. The results show that the N-terminal region of *EED1* is mainly responsible for farnesol hypersensitivity. To test if the association between *EED1* and the farnesol pathway occurs via cAMP signaling, we tested the effect of dB-cAMP on preventing germ tube inhibition by farnesol but did not observe a significant effect of dB-cAMP on the sensitivity of the *eed1*Δ mutant. Additional assays such as determination of intracellular cAMP levels are thus needed to provide further insights into this hypothesis. Similar to the results obtained for farnesol hypersensitivity, analysis of *eed1*Δ mutants lacking different regions of the *EED1* gene showed that the N-terminal region of Eed1 is also essential for the hyphal maintenance for cells grown in liquid medium and surface contact. In contrast, the whole protein seems to be necessary for filamentation during agar embedded growth. This suggests that during *EED1*-mediated hyphal elongation, Eed1 may interact with different partners depending on the stimulating condition. Taken together our data indicate that *EED1* is involved in *Candida albicans* response to farnesol, although the mechanisms still have to be revealed. Therefore a His-tagged Eed1 version has been constructed to analyze protein localization. This construct, as well as recombinant Eed1 C- and N-terminal peptides, are currently investigated for interaction with DNA and proteins in gel-shift and pull-down assays, respectively. These experiments will provide valuable information about the possible role of *EED1* in *C. albicans* tolerance to farnesol and contribute to the elucidation of its molecular function in morphogenesis.

S3:18

DISCOVERY AND CHARACTERIZATION OF POTENTIAL ANTIFUNGAL COMPOUNDS AGAINST *CANDIDA ALBICANS*

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Candida albicans, a commensal organism living in the gut of many humans, can cause infections in immunocompromised individuals. These infections include vaginal, oral, and systemic infections, the latter being life-threatening. The three main types of antifungals commonly used to treat systemic *C. albicans* infections are azoles, polyenes, and echinocandins. In past years, drug toxicity, as well as a rise in drug resistant strains of *C. albicans*, have lessened the efficacy of these drugs and created a need for new antifungal drugs. The fungal phosphatidylserine (PS) synthase (Cho1p) is a good drug target in *C. albicans* because Cho1p is 1) required for virulence in *C. albicans*, 2) conserved among fungi, and 3) absent within the mammalian phospholipid biosynthesis pathways. Identifying chemical compounds that inhibit Cho1p is the first step in finding a new, more effective antifungal drug. In order to identify potential Cho1p inhibitors, we developed a novel, high-throughput drug screen based on the compound Papuamide A. Pap-A, a cyclic depsipeptide, binds specifically to PS on the membrane of wildtype (WT) *C. albicans* where it causes lysis and death of the cells. Since the avirulent cho1ΔΔ mutant does not produce PS, Pap-A cannot bind to the membrane, and these cells survive. Thus, the cho1ΔΔ displays the phenotype for a compound that inhibits the Cho1p enzyme: survival in the presence of Pap-A. We screened over 5,500 compounds with Pap-A and identified the compound SB-224289 as a potential inhibitor of fungal PS synthesis. Further assays confirmed that SB-224289 confers Pap-A resistance to *C. albicans* at a 50μM concentration. Current studies to elucidate the mechanism of action of SB-224289 are underway, including TLC, LC-MS/MS, PS synthase assays, and fluorescence microscopy.

S4:1

NATURAL KILLER CELL-MEDIATED IMMUNITY AGAINST SYSTEMIC CANDIDIASIS

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Protection from systemic candidiasis depends critically on innate immunity. Acting as key players in antifungal defense, neutrophils rapidly accumulate in the kidney in response to infection. However, the candidacidal activity of neutrophils is not constitutive but rather depends on specific activation events. We have recently identified natural killer (NK) cells as essential mediators of neutrophil activation during systemic candidiasis. Here, I will discuss new data on NK cell-mediated control of antifungal immunity and on the regulation of this process.

S4:2

A SYSTEMS APPROACH TO UNDERSTAND ANTIFUNGAL IMMUNITY

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Understanding the physiological and molecular mechanisms of antifungal immunity is a crucial step for designing successful adjuvant immunotherapy for fungal infections. The advance in "omics" technology provides novel opportunities for an integrated approach to understanding host defense. The powerful combination of genomics, transcriptomics and metagenomics approaches with classical immunological methodology will be presented as a powerful tool that permits the discovery of novel aspects of antifungal immunity.

S4:3

THE ADAPTOR CARD9 IS REQUIRED FOR ADAPTIVE BUT NOT INNATE IMMUNITY TO ORAL MUCOSAL *CANDIDA ALBICANS* INFECTIONS

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Oropharyngeal candidiasis (OPC, oral thrush) is an opportunistic infection caused by the commensal fungus *Candida albicans*. OPC is common in individuals with HIV/AIDS, infants, patients on chemotherapy and individuals with congenital immune defects. Immunity to OPC is strongly dependent on the IL-23/IL-17R cytokine axis, as mice and humans with defects in IL-17R signaling (IL17F, ACT1, IL-17RA) or in genes that direct Th17 differentiation (STAT3, STAT1, CARD9) are highly prone to mucocutaneous candidiasis. Conventional CD4+ Th17 cells are induced in response to *C. albicans* via signals from C-type lectin receptors, which signal through the adaptor CARD9 leading to production of Th17-inducing cytokines such as IL-6, IL-1β and IL-23. Recent data indicates that IL-17 can also be made by numerous innate cell subsets. These innate 'Type 17' cells resemble conventional Th17 cells, but they can be activated without need for prior antigen exposure. Because *C. albicans* is not a commensal organism in rodents and therefore mice are naïve to this fungus, we had the opportunity to compare the role of CARD9 in innate versus adaptive responses using an OPC infection model. As expected, CARD9-/- mice failed to mount an adaptive Th17 response following oral Candida infection. Surprisingly, however, CARD9-/- mice had preserved innate IL-17-dependent responses to Candida, and were almost fully resistant to OPC. In contrast, MyD88-/- mice were highly susceptible to OPC, implicating TLRs and the IL-1R pathway. Thus, CARD9 is important primarily for adaptive immunity to *C. albicans*, whereas alternate MyD88-dependent pattern recognition receptors are needed for innate responses. Funding: NIH DE022550, DE023815

S4:4

DEAD MACROPHAGES WALKING: MUCH OF *CANDIDA ALBICANS* INDUCED MACROPHAGE LYSIS IS ACTUALLY DUE TO THE HOST PROGRAMED CELL DEATH PATHWAY PYROPTOSIS

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Macrophages are crucial components of the host response to *C. albicans* infection. Although macrophages have anti-*Candida* activity, the fungus is also able to destroy the macrophage. The most common model for the mechanism of *C. albicans*-induced macrophage killing is that hypha formation leads to physical rupture of the macrophage. Surprisingly, we found that hypha formation is not sufficient to induce macrophage lysis. Deletion of the *C. albicans* transcription factors Ahr1 or Upc2, which have no effect on hyphae formation within the macrophage, dramatically reduces *C. albicans* induced lysis in J774 and murine bone marrow derived macrophages (BMDM) as determined by LDH release assay. Macrophage lysis in response to *ahr1Δ/Δ* or *upc2Δ/Δ* mutants was 6 or 4 fold lower than that induced by WT *C. albicans*, respectively. The ability of *C. albicans* mutants to induce macrophage lysis strongly correlates with their ability to trigger IL-1β release, a process dependent on the NLRP3 inflammasome in macrophages. Cell lysis that occurs in tandem with IL-1β release are the hallmarks of the caspase-1 dependent programmed cell death pathway pyroptosis, which can also be mediated by the NLRP3 inflammasome. Pyroptosis has been well-characterized as part of the host response to intracellular bacterial pathogens such as *Salmonella* and *Legionella*, but has never been reported to be induced by a fungal

pathogen. Consistent with our hypothesis that *C. albicans* induces pyroptosis, we found that BMDM isolated from mice lacking components of the NLRP3 inflammasome (*Casp1*, *Asc*, and *Nlrp3*) showed dramatically reduced *C. albicans*-induced macrophage lysis whereas BMDM lacking the NLRC4 inflammasome were similar to wild-type. When normalized to lysis in WT BMDM, lysis in BMDM from knockout mice was: *casp1 Δ/Δ*: 12%; *asc Δ/Δ:* 21%; *nlrp3 Δ/Δ:* 7%; *nlrp4 Δ/Δ:* 83%. In BMDM, capase-1-dependent *C. albicans*-induced macrophage lysis accounted for 60% (MOI 2:1) and 80% of cell lysis (MOI 1:2) during the first 12 hours of infection. Taken together our observations are consistent with a model in which many *C. albicans* infected macrophages undergo NLRP3 inflammasome-dependent programmed cell lysis (pyroptosis) well before hyphae cause physical disruption of the macrophage cell structure. These data as well as additional studies directed at understanding the role of pyroptosis and other cell death pathways in the *C. albicans*-macrophage interaction will be presented.

S4:5

FUNGAL CHITIN DAMPENS INFLAMMATION THROUGH MANNOSE RECEPTOR DEPENDENT NOD2 AND TLR9 ACTIVATION

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Chitin is an essential polysaccharide of the walls of all fungal pathogens and the exoskeleton and eggs of invertebrate parasites. We identify NOD2, TLR9 and the mannose receptor as three essential fungal chitin-recognition receptors of innate immune cells and show that the activation of NOD2/TLR9 receptors by low concentrations of small chitin particles leads to selective secretion of the anti-inflammatory cytokine IL-10. In mice, intraperitoneal injection of pure chitin abrogated the inflammation induced by co-administration of bacterial LPS. Chitin may therefore contribute to the resolution of the immune response once the fungal pathogen has been defeated. NOD2 and TLR9 polymorphisms are associated with susceptibility to inflammatory conditions such as Crohn's disease, allergy and asthma. Chitin recognition is therefore critical for immune homeostasis and is likely to have a significant role in infectious and allergic disease. Lenardon, et al. (2010). Chitin synthesis and fungal pathogenesis. *Current Opinion in Microbiology* 13, 416-423. Mora-Montes, et al. (2011). Recognition and blocking of innate immunity cells by *Candida albicans* chitin. *Infection and Immunity* 79, 1961-1970. Lee, et al (2012). Elevated cell wall chitin in *Candida albicans* confers echinocandin resistance *in vivo. Antimicrobial Agents and Chemotherapy.* 56, 208-217.

S5:1

TRANSCRIPTIONAL CONSEQUENCES OF GENOMIC VARIATION IN CLINICAL ISOLATES OF *C. ALBICANS*

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Large-scale genomic alterations, such as chromosomal duplication and loss of heterozygosity, are common in clinical isolates of *Candida albicans*, and may contribute to survival in the host. To gain insight into the connection between genomic changes, virulence, and host response, we investigated 21 clinical isolates that vary widely in virulence in a murine model$_{1}$. We analyzed these isolates by performing extensive phenotypic tests, whole genome sequencing and transcriptional analysis. The 21 isolates provided a broad sampling of the *C. albicans* population, including clades I, II, III, SA, and E. These isolates displayed large-scale genomic variation in one out of every three strains. Copy number changes in-

cluded a hemizygous region in one strain, two strains with large segmental duplications, and four strains with trisomies of the four smallest chromosomes. In all cases, the average transcription rate was significantly correlated with ploidy level, although individual genes were variably affected. A GO term analysis of differentially expressed genes indicated that there was no apparent large-scale response to stress as seen with chromosomal aneuploidies in other organisms. Loss of heterozygosity (LOH) was highly variable across strains, with the percentage of LOH ranging from 11% to 52% of the genome. The strain with the highest LOH was azole resistant and harbored point mutations known to confer azole resistance, found within homozygous regions of the genomes of these isolates. It is possible that the growth advantage under drug pressure provided by these mutations provided selective pressure for LOH events in these regions. Further analysis of individual mutations may establish direct connections between genotype and phenotypes important for growth in a mammalian host.

[1] Wu, W., Lockhart, S. R., Pujol, C., Srikantha, T. & Soll, D. R. Heterozygosity of genes on the sex chromosome regulates *Candida albicans* virulence. Mol Microbiol 64, 1587-1604 (2007).

S5:2

POLYPLOIDY DRIVES POPULATION HETEROGENEITY THROUGH RANDOM AND STEPWISE CHROMOSOME LOSS

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Candida albicans, often described as an imperfect yeast and obligate diploid, has a large repertoire of mechanisms to generate genetic and phenotypic diversity despite the lack of a "true" meiosis in its lifecycle. Shifts in ploidy via the parasexual cycle facilitate recombination, aneuploidy and homozygosis of whole chromosomes and fuel rapid adaptation. Here we show how the polyploid state potentiates ploidy variation and drives population heterogeneity. Tetraploidy is intrinsically stressful to *C. albicans* cells and the rate of loss of a heterozygous marker (LOH) increases by over an order of magnitude relative to diploid cells. LOH rates in tetraploids also vary on the degree of selection_and the number of LOH events being selected against. Furthermore, LOH selection on tetraploid cells enriches for the rare cells that lose chromosomes and thus have initiated the chromosome loss phase of the parasexual cycle. Analysis of isolates that underwent LOH revealed a broad range of karyotypes including some with a combination of di-, tri and tetra-somic chromosomes. This suggests that chromosome loss is not necessarily 'concerted' and that few, if any, chromosomes are lost coordinately. Rather, the chromosome loss process appears to be random, because viable non-diploid progeny with extra copies of many combinations of the eight *C. albicans* chromosomes were recovered. Using a combination of high-throughput technologies, sequencing, and experimental evolution, we analyzed the ploidy trajectories of several hundred tetraploid-derived isolates and their fitness consequence. Despite the relatively frequent occurrence of viable, non-diploid states, these states are highly unstable under standard laboratory conditions and resolve their genomes to a stable euploid (diploid, triploid or tetraploid) state very rapidly. Euploid cells are most frequently diploid, however stable triploid and tetraploid isolates are a consistent minority. Furthermore, transient ~haploid populations were detected in a small number of isolates. Under standard rich media laboratory conditions, non-diploid cells are considerably less fit than heterozygous diploid strains. However, under less favorable growth conditions, non-diploids often grow as well or better than their heterozygous diploid parents. Together our results indicate that tetraploid progenitors produce progeny cells with a high degree of genomic diversity within a single population, and as such, are an excellent source of genetic variation upon which selection can act.

S5:3

A RESOURCE FOR GENOTYPE-PHENOTYPE ASSOCIATION STUDIES IN *CANDIDA ALBICANS*

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Candida albicans is responsible for the majority of life-threatening fungal infections occurring in hospitalized patients and is also the most frequent fungal commensal of humans. Population studies have revealed that the *C. albicans* population includes at least 18 phylogenetic groups (or clades). Each clade comprises strains that have evolved independently from those in other clades, possibly through past association to a geographic locale. Specific phenotypes can distinguish isolates from a given clade from those in other clades and yet, the relationships between *C. albicans* natural genetic diversity and phenotypic diversity have not been explored in depth. As a starting point to develop genotype-phenotype association studies in *C. albicans*, we have now sequenced the diploid genomes of 137 *C. albicans* isolates (100 bp paired end reads, >50X average sequencing depth) carefully selected from a large collection of commensal/clinical isolates previously used to characterize the population structure of the species. These included isolates from the 12 major *C. albicans* clades chosen to represent strains from healthy carriers, and superficial and invasive infections. Here, we will present results of the analysis of these 137 *C. albicans* genomes focusing on single nucleotide polymorphisms, their distribution across the genomes and their impact on proteins. Notably, our results indicate that sequencing of this panel of isolates was sufficient to uncover most *C. albicans* single nucleotide polymorphisms which often show clade-specificity. Moreover, while loss-of-heterozygosity (LOH) events were pervasive across the sequenced isolates, few hot spots of LOH were identified suggesting that most LOH events resulted from independent double-strand break events experienced by individual isolates. The 137 *C. albicans* isolates are currently being characterized for a variety of phenotypes - growth, biofilm formation, antifungal resistance, virulence - and will represent a unique resource for genotype-phenotype association studies in the future.

S5:4

COMPARATIVE PHENOTYPIC ANALYSIS OF CANDIDA PARAPSILOSIS AND *CANDIDA ALBICANS*

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Candida albicans and Candida parapsilosis are both members of the CUG clade, and it is generally assumed that they share common biological properties. However, the two organisms have variable virulence characteristics, and different phenotypes. For example, only *C. albicans* can grow in true hyphal forms. To enable a detailed comparison of the two species, we generated double allele deletions of >100 genes in C. parapsilosis including transcription factors and protein kinases, and compared the phenotypes to the corresponding deletions of *C. albicans*. Two independent C. parapsilosis deletions were constructed for each target gene. Growth in > 40 conditions was tested, including carbon source, temperature, and the presence of antifungal drugs. We found that many phenotypes are shared in the two species, such as the role of Upc2 as a regulator of azole resistance. However, other

characteristics are unique to one species. For example, Cph2 plays a role in the hypoxic response in C. parapsilosis and not in *C. albicans*, and SEF1, a regulator of iron uptake in *C. albicans*, is required for utilization of different carbon sources only in C. parapsilosis. HAP genes have a more pronounced role in determining copper resistance in *C. albicans* than in C. parapsilosis. Some of the biggest differences relate to the regulation of biofilm development. We identified 7 transcription factors that are required for biofilm development in C. parapsilosis. Only three (Efg1, Bcr1 and Ace2) are shared with *C. albicans*. In contrast, 2-4 transcription factors required for biofilm formation in *C. albicans* do not have the same role in C. parapsilosis. We also compared the transcription profile of *C. albicans* and C. parapsilosis biofilms. Our analysis suggests the processes shared between the two species are predominantly metabolic, whereas *C. albicans*-specific genes are associated with hyphal growth. Overall, whereas there is a significant overlap in gene function between *C. albicans* and C. parapsilosis, there are also considerable differences. We are currently exploring novel methods of generating large numbers of gene deletions in C. parapsilosis, which will be used in future analysis.

S5:5

ENGINEERING A COMPLETE SEXUAL CYCLE IN *CANDIDA GLABRATA*.

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To date no sexual cycle has been described in the pathogenic yeast *Candida glabrata*. This is curious as the population structure contains evidence of recombination, the genome contains mating-type loci that can switch at sites of infection and orthologues of the majority of genes involved in fungal sexual reproduction and meiosis, including many of those missing in other Candida species. The lack of a complete sexual cycle in some hemiascomycete yeasts is surprising as they contain many of the required genes. We naively hypothesized that the functions of the encoded proteins may have changed, as has been previously described for regulators of ribosome biosynthesis in *C. albicans*, resulting in an inability to complete a sexual cycle. To test this hypothesis we cloned each of 33 *C. glabrata* genes, functionally annotated to the GO terms mating or meiosis, and attempted to systematically complement the mating/meiosis defects of the orthologous S. cerevisiae mutants, as we have previously done for ste11, ste12 and ste20 mutants. Here we show that a complete sexual cycle can be engineered in *C. glabrata*. IME1, encoding the principle regulator of meiosis and IME2, encoding a meiosis specific kinase from *C. glabrata* are unable to complement the mating defects of the orthologous S. cerevisiae null mutants. The replacement of the native *C. glabrata* IME1 and IME2 with their orthologous from S. cerevisiae results in *C. glabrata* cells that can mate, form stable diploids and undergo sporulation, to give tetrad-like structures containing viable haploid progeny. These progeny when sequenced show evidence of having undergone recombination. Our results demonstrate that in addition to the loss of orthologous mating and/or meiosis proteins seen in some fungi, those retained may have had their function reassigned, thereby resulting in a reliance on asexual reproduction. This is the first report of an apparently asexual ascomycetous yeast that has been engineered to successfully complete a sexual cycle. This will not only allow forward genetic analysis in *C. glabrata*, but offers an approach that may be successful in revealing hidden mating pathways in other species such as *Candida albicans* in which stable haploids have recently been described. This data also highlights key evolutionary changes between pathogenic and non-pathogenic fungi.

S6:3

DEVICE-RELATED *CANDIDA ALBICANS* ENDOCARDITIS AND PERSISTENT FUNGEMIA

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A 62 year old man with atrial fibrillation requiring an implantable defibrillator (AICD) presented to the hospital in April with shortness of breath and pneumonia. During his admission, he had a single positive blood culture growing *C. albicans* and was successfully treated with fluconazole. Two months later, he returned with similar symptoms. Blood cultures grew *C. albicans* for 6 days despite fluconazole. He was treated with caspofungin for 4 weeks and blood cultures became negative. At this time, the source of recurrent candidemia was unclear. Ten days after completing caspofungin therapy, he returned with fevers. His blood cultures were positive for 9 days despite fluconazole. A trans-esophageal echocardiogram revealed 4 lesions on AICD wire and a vegetation on the tricuspid valve, findings consistent with endocarditis. The AICD wire was removed, the patient placed on caspofungin, and blood cultures sterilized. Over the next two years, he had multiple episodes of recurrent *C. albicans* bloodstream infections requiring continued therapy with fluconazole and caspofungin. He deferred valvular replacement. Caspofungin and fluconazole minimum inhibitory concentrations (MICs) did not change over time despite continuous antifungal therapy (caspofungin (MIC) = 0.06 mcg/mL, fluconazole MIC = 0.5 mcg/mL). Gene sequencing did not reveal an FKS mutation. Endocarditis is a biofilm-associated infectious disease. Treatment requires antifungal agents in conjunction with valve replacement to remove the infected focus. In the present case, *C. albicans* demonstrated the ability to adhere to both a foreign body (AICD wire) and native tissue (heart valve). Biofilm is comprised of *C. albicans* embedded in aggregates of platelets, fibrin, and extracellular matrix. In order to form biofilm, *C. albicans* must first adhere to biomaterial surface or disrupted host tissue (in this case, valve injury induced by AICD); this process is mediated by both non-specific factors such as cell surface hydrophobicity and electrostatic forces, and Candida-specific factors such as adhesins. The process involves germ tube formation early, followed by more mature biofilm production consisting of yeasts, pseudohyphae and hyphae. Indeed, hyphae play an important role in biofilm development by providing structural integrity to the biofilm architecture. The patient's persistent and recurrent candidemia is a typical outcome of endocarditis when surgical debridement and/or removal of the vegetation (biofilm) cannot be performed. To date, the mechanism for recalcitrant infection despite antifungal therapy is not fully understood, but thought to be related to the high density of Candida cells within the biofilm, decreased growth rate and nutrition limitation, and/or expression of efflux pumps. Antifungal MICs determined against planktonic cells may not reflect activity against sessile cells.

S6:4

CASE REPORT: CULTURE-NEGATIVE INTRA-ABDOMINAL CANDIDIASIS DUE TO *C. GLABRATA* LEADING TO ECHINOCANDIN-RESISTANT CANDIDEMIA.

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A 64 year old man underwent partial liver-biliary resection for a non-malignant tumor. His post-operative course was complicated by high leukocyte (WBC) counts. No infection was found, but he was empirically treated with broad-spectrum antibiotics. His WBC normalized. Pus was noted from an abdominal drain 2 weeks later. CT scan revealed an abscess at the small intestine-biliary anastomosis. Abscess cultures revealed *E. coli* and vancomycin-resistant *Enterococcus* (VRE). Serum β-D-glucan (BDG) was 220 pmol/mL (positive >80). He was treated with drainage,

antibiotics and micafungin for 3 wks. Thereafter, the abscess re-accumulated; VRE was re-cultured. BDG was 280 pmol/mL. He was treated with drainage and the same antimicrobials for 4 wks. His course was complicated by liver failure, and he underwent liver transplant. Intra-operative cultures were negative, but antibiotics and micafungin were continued. He was unstable post-transplant. Abdominal drain and blood cultures were negative for 4 days, before revealing *C. glabrata* (micafungin MIC: 2 µg/mL (resistant >0.125); *FKS1* D632H mutation). Micafungin was switched to ABLC. Follow-up cultures were negative, but care was withdrawn due to multi-organ failure. This case highlights the clinical importance of intra-abdominal candidiasis (IAC), poor sensitivity of cultures, role of non-culture diagnostics, and emergence of echinocandin (EC) resistance. IAC is 2nd to candidemia as a manifestation of invasive candidiasis. It stems from infected abdominal catheters or gastrointestinal leaks (as in this case). In the abdominal cavity, *C. albicans* relies upon processes like adhesion and alkaline pH, osmolar and oxidative stress responses. *RIM101* is a major regulator of survival in abscesses, partly through *SAP5* activation. TNF-α and lymphotoxin-α play key roles in eliminating *C. albicans* from abscesses by enhancing neutrophil killing and Th1 responses. Despite drainage and antifungal treatment, mortality rates resemble candidemia. Blood/abdominal cultures are ~20/50% sensitive. BDG has sensitivity/specificity of ~60/75%. Used widely in a typical ICU, BDG has poor positive but excellent negative predictive value (PPV/NPV: ~6/98%). In a high-risk setting like this, PPV/NPV is ~58/79%. Therefore, BDG here was highly suggestive of IAC despite negative cultures, and merited antifungal treatment. ECs are agents of choice against candidemia, but efficacy within abscesses is unknown. EC resistance is mediated by *FKS* mutations, which are found in 10% of *C. glabrata* bloodstream isolates (BSI) at some centers. Resistance occurs in ~3% of *C. albicans* BSI and is very rare in other spp. *FKS* mutations are seen with several wks-mos of prior EC exposure. Recent data suggest that abdominal sites are hidden reservoirs of EC resistance, likely due to prolonged, subinhibitory exposures. An earlier switch to ABLC in this case may have been warranted.

S7A:1

TITLE NOT AVAILABLE

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S7A:2

HOST-PATHOGEN DETERMINANTS IN A ZEBRAFISH FUNGAL INFECTION MODEL

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Current models of systemic candidiasis are limited in their application to questions of early host-pathogen interaction. We recently described a larval zebrafish model of candidiasis that provides a transparent and manipulable model for high-resolution noninvasive visualization of the innate immune-fungal interaction. Using this model we showed that interaction of *C. albicans* with phagocytes in vivo is different from that described in vitro, with macrophages able to control fungal growth only in vivo. In this published work, we also demonstrated a novel role of NADPH oxidase in control of filamentous *C. albicans* growth. We have now characterized the cellular consequences of defective NADPH oxidase activity and defined a new role of two separate NADPH oxidases in early phagocyte response to *C. albicans*. In addition, we have identified key prognostic elements of early immune response. Finally, our data suggest that *C. albicans* also regulates ROS-dependent phagocyte recruitment to the infection site. These recent experiments implicate NADPH oxidases in early immune recruitment to the *C. albicans* infection site, a previously unappreciated function of these important enzyme complexes. We continue to utilize the larval zebrafish to

approach other long-standing questions of fungal-host interaction. In one project we are modeling the more common but less dangerous types of candidiasis using an epithelial infection model that mimics key aspects of in vitro mammalian epithelial responses to *C. albicans*. Here, we have found differential immune responses depending on fungal burden by monitoring fungal infection concurrently with epithelial activation, inflammatory gene expression, and phagocyte recruitment. In another project, we have implicated fungal dimorphism in the ability of *C. albicans* to disseminate from one tissue to another. Current work focuses on following the movement of fungal morphotype-locked mutants and labeled phagocytes to determine the role of phagocytes in dissemination.

S7A:3

MODELLING HUMAN *CANDIDA* INFECTION WITH THE MOUSE COMMENSAL *CANDIDA PINTOLOPESII*

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Animal models have been hugely important in mycology, allowing investigation of virulence, assessing antifungal agents and dissecting host immune responses. For *Candida albicans* infections the mouse remains the most commonly used model host, although minihosts, e.g. *Galleria mellonella* and zebrafish, are increasingly being utilised. However, *Candida* species associated with human infection are not naturally occurring in mice, and manipulation of the host can be required to establish commensalism or some infections, potentially altering the natural interaction between host and fungus. To overcome this issue, we have investigated use of the murine commensal, and potential opportunistic pathogen, *Candida pintolopesii* to model human *Candida* systemic infection. In this study we investigate the ability of *C. pintolopesii* to establish infection in the minihost *G. mellonella*, and then characterise the ability of this fungus to establish infection when administered intravenously or orally in immunosuppressed or immunocompetent BALB/c mice. *C. pintolopesii* was capable of killing *G. mellonella,* although high doses (approx. 1e6 cells) were required to obtain 100% killing. In a mouse intravenous challenge model, *C. pintolopesii* was unable to establish progressive infection in immunocompetent mice, but progressive infections could be established in immunosuppressed mice. High fungal burdens were found in all organs assayed at time of culling. Gastrointestinal colonisation could be established without any prior antibiotic therapy, and could even be established via coprophagia. Colonisation was maintained over 24 days, but no evidence for dissemination was found. These preliminary findings suggest that this mouse commensal may be a good model for investigating establishment of systemic infection from commensal fungi.

S7A:4

DEFINING BENIGN COLONIZATION FROM SYMPTOMATIC INFECTION DURING CANDIDA VAGINITIS: A CRUCIAL ROLE FOR FUNGAL MORPHOGENESIS

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Vulvovaginal candidiasis, caused primarily by *Candida albicans*, presents significant health issues for women of childbearing age worldwide. Long believed to be a disease resulting from defects in adaptive immunity, a recent paradigm has emerged which places focus on the role of the host innate inflammatory response in promoting vaginitis symptomatology. Despite this recent shift in the philosophy of disease immunopathogenesis, relatively little is understood about fungal virulence factors that contribute to the immunopathological response. As a polymorphic fungus, the ability of *C. albicans* to switch between yeast and hyphal morphologies is considered

its central virulence attribute. Therefore, we hypothesized that the transition from ovoid yeast to filamentous hypha was associated with the hallmark inflammatory response observed during *Candida* vaginitis. In order to define symptomatic infection, an established estrogen-dependent mouse model of vaginitis was used in which C57Bl/6 mice were intravaginally inoculated with *C. albicans* (wild-type, DAY185) or PBS control and inflammatory parameters (PMNs, S100A8, interleukin-1b (IL-1b), lactate dehydrogenase) and fungal burden were kinetically monitored by standard techniques (selective staining, immunoassays, enzymatic assay, microbiological plating) at several time points (d1, 3, 7) post-inoculation. Findings indicated that while fungal burden remained constant throughout the observation period, inflammatory parameters increased only from d3 onward. In order to determine the significance of fungal morphogenesis during vaginitis, two complementary approaches were utilized. First, mice were intravaginally inoculated with *C. albicans* strains deleted for key transcriptional regulators (*bcr1*Δ/Δ, *efg1*Δ/Δ, *cph1*Δ/Δ, *efg1*Δ/Δ/*cph1*Δ/Δ and their reconstituted strains) controlling the yeast-to-hypha switch, revealing a crucial role for morphogenetic signaling via the Efg1, and to a lesser extent, the Bcr1 pathways in contributing to vaginitis immunopathology. Importantly, failure to elicit immunopathology was not dependent on fungal burden, as strains deficient in morphogenetic pathways colonized comparable to wild-type levels. Second, overexpression of transcription factors *NRG1* and *UME6*, to maintain yeast and hyphal morphologies respectively, confirmed the importance of morphogenesis in generating innate immune responses *in vivo*. These results highlight the yeast-to-hypha switch and associated morphogenetic response as important virulence components for the immunopathogenesis of *Candida* vaginitis, with implications for transition from asymptomatic colonization to symptomatic infection. Importantly, inhibition of fungal morphogenesis may serve as a novel therapeutic target to eliminate the immunopathological triggers associated with vaginitis symptomatology.

S7A:5

MINI-HOST AND MOUSE INFECTION MODELS FOR PROBING VIRULENCE OF *CANDIDA ALBICANS* TRANSCRIPTION FACTORS MUTANTS

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Mammalian models such as mice are essential to understand the virulence of organisms such as *Candida albicans.* However for financial, time and ethical reasons, alternative models, also called mini-hosts, are being developed to evaluate fungal virulence and drug response. In this study we aimed to identify *C. albicans* transcription factors (TF) involved in virulence in two different models: the mouse systemic infection model and the mini-host *Galleria mellonella* model measuring mouse kidneys or entire larvae fungal burden. This large-scale analysis will thus address the reliability of the *G. mellonella* model. For this purpose, a collection of 234 barcoded TF mutants were first screened by pools of 8 strains in mice using qPCR detection and quantification of the barcodes in kidneys. Among 49 TF mutants with a fungal burden significantly different from a wild type strain, 22 showed a higher score (hyper-colonizers) and 27 a lower score (hypo-colonizers). Eight out of these 49 TF (*SEF1, RIM101, NOT5, RFX1, HAP43, SPT3, CTF1* and *ZCF13*) had already been described as being involved in virulence. Next, 63 strains were tested individually in *G. mellonella* including 41 mutants with a significant score in the mice screening but not yet annotated, 5 undetected mutants and 17 mutants not tested for technical reasons in mice pool infections. From these 63 mutants, 3 confirmed a significant decrease of colonization in *G. mellonella* (BCY130, 199, 152), 4 confirmed their hyper-colonizer

phenotypes (BCY454, 458, 247, 197). One previously undetected mutant (BCY431) and 4 not previously tested (BCY401, 178, 313, 340) were hypo-colonizers. Following these two screenings, a significant discrepancy was observed between the two models of infection. This could be due either to a pool effect in the first screen or to intrinsic differences between the two models. To discriminate between the two hypotheses, 20 out of the 63 *G. mellonella* tested mutants were injected individually in the mouse tail vein. In 50% of the cases, the same colonization phenotype was measured in mouse and *Galleria* infections. In 35% of the cases, mutants had a phenotype in mice but not in the mini-host. *Galleria* was less sensitive to detect changes in virulence, since 35% of hypo / hyper-colonizer mutants in mice were considered as wild type in this model. This diminished sensitivity could be attributed to a high biological variability inherent to the *Galleria* model. In conclusion, only 15% of the mutants showed an opposite phenotype between the two models. This first large-scale analysis supports the reliability of the *G. mellonella* model in comparison to the gold-standard mouse systemic infection model. Our combined approach allowed the selection of 6 hypo- or hyper-colonizers mutants (BCY130, 199, 431, 454, 150, 152) in both models and their characterization is underway.

S7A:6

INSIGHT INTO THE PATHOGENESIS OF *CANDIDA GLABRATA* USING MURINE INTRA-ABDOMINAL CANDIDIASIS (IAC) MODEL

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Background: The study of the molecular pathogenesis of *C. glabrata* (Cg) infections has been limited by the lack of an animal model that mimics disease in humans. **Methods:** We developed a mouse model of Cg intra-abdominal candidiasis (IAC). **Results:** No mice died over 28 days (d) following intra-peritoneal (IP) infection with $1x10^7$ or $1x10^8$ CFU of Cg strain BG2 in the presence or absence of sterile stool (SS), or with $5x10^8$ CFU in the absence of SS. Mice infected with $5x10^8$ + SS began to die on d2; mortality was 100% on d8. Following infections of $1x10^7$ + SS, peak burdens in peritoneal fluid (PF) were observed at 1d, and decreased progressively before clearing by d28. BG2 alone resulted in lower burdens, and was cleared by d7. Neutrophil (PMN) infiltration in PF peaked at 1d, and was greater in presence of SS ($p \leq 0.009$). Monocytic rather than PMN predominance was noted starting on d3. Abscesses (IAA) containing BG2 were only evident in presence of SS, peaking on d7 and persisting through d28. All subsequent experiments were performed with SS, which mimics GI perforation. We performed survival ($5x10^8$) and tissue burden ($1x10^7$) studies with Cg clinical strains recovered from patients with various types of invasive candidiasis. 3 strains killed mice rapidly like BG2, while 1 strain (# 356) was significantly attenuated ($p<0.001$). A virulent clinical strain (# 356) caused greater IAA burdens than #346 beginning at d7 ($p \leq 0.02$). Next, we compared BG2 with a phospholipase B2 *plb1-2* null mutant. The latter was not attenuated in causing death, but did result in more rapid clearance of IAA and lower intra-IAA burdens beginning on d14 ($p \leq 0.04$; results reversed by *PLB1-2* re-insertion). Of note, BG2 and *plb1-2* caused similar tissue burdens in mouse kidneys during hematogenous disseminated candidiasis (DC). Compared to *C. albicans* (Ca) SC5314, Cg BG2 at the same inoculum ($1x10^7$) caused significantly less PMN infiltration in PF, displayed tropism for the pancreas, and formed IAA that contained higher organism burdens and persisted longer. We previously showed that Ca*ALS1* was among the genes mostly strongly expressed during IAC. Over-expression of Ca*ALS1* in BG2 significantly increased intra-IAA Cg burdens at late, but not early time points. The over-expression strain was

more resistant to phagocytosis and killing by PMNs *in vitro*. **Conclusions:** A mouse model of Cg IAC mimics progression from peritonitis to IAA, as seen in humans, and is sensitive for discriminating the relative virulence of strains. Cg differs from Ca during IAC by eliciting a dampened PMN response in the abdominal cavity, and achieving long-term, high-level persistence within IAA. Candidal factors like Plb2 and Als1 do not contribute to virulence through tissue penetration or adherence (as hypothesized), but rather by facilitating IAA persistence (perhaps though resistance to PMNs). The model of IAC advances the field by overcoming the shortcomings of the DC model.

S7B:1

FEEDING ON THE HOST: A *CANDIDA ALBICANS* PORE FORMING TOXIN

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It is assumed that invading hyphae allow *Candida albicans* access to nutrients from within host cells. However, we have previously shown that, during epithelial invasion, hyphae do not puncture directly into the host cell cytoplasm, instead residing in a compartment surrounded by what appears to be intact host membrane. We term this compartment the invasion pocket. This raises the question: how does *C. albicans* gain access to intracellular host-derived nutrients?
We now show that a single peptide fragment, resulting from the proteolysis of Ece1 (Ece1-III), is able to cause permeabilisation of host cell membranes. A deletion mutant, lacking *ECE1*, although fully capable of hypha formation and host cell invasion, was unable to damage human oral, gastrointestinal or vaginal epithelial cells. Furthermore, a synthetic version of Ece1-III efficiently permeabilised human epithelial cells, macrophages and erythrocytes, resulting in host cell lysis. Therefore, as Ece1-III is essential and sufficient for host damage, this peptide represents a bona fide *C. albicans* virulence factor.
The mechanism of Ece1-III driven host cell lysis is via pore formation in the host cell membrane. These pores are heterogeneous in size, reaching up to at least 30 nm in diameter, and sufficiently large to allow the efflux of proteins, including lactate dehydrogenase and haemoglobin. Ece1-III physically interacts with several membrane-associated phospholipid species and pore formation was found to be phosphatidylserine-dependent. This is the first example of a pore forming peptide toxin from a fungal pathogen.

S7B:2

MULTIPLE ROUTES TO ENVIRONMENTAL ALKALINIZATION VIA CATABOLISM OF AMINO ACIDS OR DICARBOXYLIC ACIDS CONTRIBUTE TO SURVIVAL AND VIRULENCE OF *C. ALBICANS*

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When phagocytosed by macrophages, *Candida albicans* switches from the yeast to hyphal form, allowing it to escape by rupturing the immune cell. While a variety of factors induce this switch in vitro, including neutral pH, it has not been clear what triggers morphogenesis within the macrophage. Based on both *in vitro* and *in vivo* data, we have recently proposed that wild-type *C. albicans* cells neutralize the phagolysosome through the excretion of ammonia derived from catabolism of amino acids as a carbon source, which transcriptional profiling data indicates are an abundant nutrient in this environment. To probe the significance of this phenomenon in phagocytosed cells, we have used as a tool mutants lacking *STP2,* a transcription factor that regulates expression of amino acid permeases, which are unable to neutralize media in which amino acids are the primary carbon source. In contrast to

wild-type cells, phagocytosed *stp2*Δ mutant cells occupied acidic phagosomes, had reduced hyphal formation, and reduced survival within macrophages; phenotypes that could be suppressed by pharmacological neutralization of the phagosome. These defects are reflected in an attenuation of virulence in a mouse model of disseminated candidiasis. Thus, *C. albicans* has adapted its metabolism to take advantage of available nutrients in a manner that modulates the innate immune response. Yet, despite the clear in vitro phenotypes, the effects on virulence or macrophage function were only partial. To address this we considered whether other potential nutrients could promote environmental alkalinization. To our surprise, *C. albicans* both grew avidly and rapidly raised the pH on media containing either pyruvate or [[Unsupported Character - Symbol Font ]]-ketoglutarate ([[Unsupported Character - Symbol Font ]]KG), compounds structurally related to serine and glutamate, respectively, but without amine groups to produce ammonia. Consistent with this, [[Unsupported Character - Symbol Font ]]KG-grown cells do not excrete ammonia. Moreover, mutants that impair alkalinization on amino acids, such as *stp2*Δ, *ato5*Δ, and *dur1,2*Δ, do not affect the pH changes on these dicarboxylic compounds, emphasizing that this is a unique physiological process. We identified two genes, *CPH1* and *CWT1*, required for alkalinization on αKG. We suggest that the flexible metabolism of *C. albicans* gives it multiple routes to generating a neutral environment within host niches and that this contributes to its pathogenic potential.

S7B:3

A NETWORK OF CELL SURFACE CFEM PROTEINS REQUIRED FOR HOST HEME-IRON ACQUISITION IN *C. ALBICANS*

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Candida albicans, a normally harmless commensal organism, can cause life-threatening systemic infections among immunocompromised patients. The human host invests substantial efforts into withdrawing iron from potential pathogens. To overcome the extreme iron limitation in the host, *C. albicans*, similar to many pathogenic bacteria, has evolved several mechanisms, including a pathway for heme-iron scavenging, enabling access to hemoglobin, the largest iron pool in the human body. Rbt5 is an extracellular GPI-anchored protein of the CFEM family (defined by 8 identically-spaced cysteines) that was identified as a *C. albicans* hemin- and hemoglobin receptor. Genetic analysis suggested a pathway in which hemoglobin or hemin is bound by extracellular receptors, then internalized into the endosome, where it is stripped of its iron by the acidic pH, after which the iron is transferred to the cytoplasm. However the initial part of the pathway, including the mechanism of heme transfer across the cell wall to the endosome, remained less well understood. Here we genetically and biochemically analyzed an additional CFEM protein of *C. albicans*, Pga7. We found that Pga7, in spite of being much less abundant than Rbt5, is more important than Rbt5 for heme-iron utilization: deletion of *PGA7* resulted in a more profound defect in heme-iron utilization than deletion of *RBT5*. The *pga7*$^{-/-}$ mutant was less virulent in a mouse model of systemic infection, supporting the importance of heme-iron utilization for pathogenicity of *C.albicans*. Although Pga7 and Rbt5 are both predicted to be GPI-anchored extracellular proteins, our data indicated that they have distinct cell wall attachments, and discrete localization within the cell envelope, with Rbt5 being more exposed on the outer cell wall than Pga7. Both proteins were found to bind to a heme column *in vitro*, but isothermal calorimetry with the recombinant proteins revealed that, while Rbt5 has a K_D of about 10^{-5}M for heme, Pga7 had a significantly higher affinity ($K_D \approx 10^{-7}$M). Finally, we found that heme can be efficiently transferred between recombinant Rbt5 and Pga7 *in vitro*. Taken

together, these results evoke a mechanism involving vectorial transfer of heme across the fungal cell wall by sequential binding to different CFEM proteins, followed by internalization into the cell.

S7B:4

EVOLUTIONARY REWIRING OF UBIQUITINATION TARGETS IN *CANDIDA ALBICANS* PROMOTES METABOLIC FLEXIBILITY IN HOST NICHES.

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Candida albicans is a major fungal pathogen of humans that encounters nutritionally diverse host niches during infection. While *C. albicans* is considered a Crabtree-negative yeast (continues respiration in the presence of glucose), exposure to glucose down-regulates key metabolic transcripts involved in the utilization of alternative carbon sources similar to the Crabtree-positive yeast, *Saccharomyces cerevisiae*. We have recently shown that *C. albicans* has undergone significant evolutionary rewiring in the posttranslational regulation of gluconeogenic and glyoxylate cycle enzymes whereby glucose exposure down-regulates these key metabolic transcripts, but the encoded enzymes are retained. The presence of these enzymes allows simultaneous assimilation of alternative carbon sources (i.e. fatty acids and sugars). In contrast, in *S. cerevisiae*, simultaneous carbon assimilation is prevented by catabolite inactivation. We have previously shown that certain enzymes, such as isocitrate lyase (CaIcl1), lack critical ubiquitination sites compared to *S. cerevisiae* Icl1 (ScIcl1) that mediate this catabolite inactivation. Interestingly, the components of the Glucose-Induced Degradation (GID) complex, which is responsible for targeting these enyzmes for degradation in *S. cerevisiae*, are conserved in *C. albicans* and appear to be functional, as evidenced by the rapid glucose-signalled degradation of ScIcl1 when expressed in *C. albicans*. Thus, while aspects of carbon assimilation are conserved in *C. albicans* compared to *S. cerevisiae*, there has been significant rewiring of ubiquitination targets in *C. albicans*. Interestingly, 59% of *S. cerevisiae* clinical isolates examined *in vitro* display Crabtree negative traits and are able to utilize lactate in the presence of glucose. Taken together, the data suggest that metabolic pathways have undergone post-transcriptional rewiring to promote flexibility in carbon utilization, which may be important for the survival of fungi *in vivo*. The goal of our study is to determine the impact of this metabolic flexibility on *C. albicans* pathogenesis using a murine model of systemic candidiasis. We expect that metabolic flexibility will play an important role for *Candida* species fitness and virulence in the dynamic micro-niches of the host.

S7B:5

EFFECTS OF METABOLIC INHIBITORS ON RAS1 SIGNALING OF *CANDIDA ALBICANS*

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In *Candida albicans*, the morphological switch between yeast and hyphal forms is important for virulence and biofilm formation. *C. albicans* hyphal growth and biofilm formation requires a pathway controlled by the highly conserved Ras G-protein, Ras1. Our recent studies of microbe-microbe interactions suggest that bacterial phenazines along with the phenazine-like compound methylene blue inhibit the morphological switch to hyphal growth by *C. albicans* by inhibiting activation of the key regulator Ras1. Our data indicate that the inhibition of respiration caused by these compounds results in decreased Ras1 activation. We are very interested in uncovering the links

between metabolism and Ras1 signaling. Inhibition of Ras1 activation is independent of low pH, reactive oxygen species, and growth inhibition. However, we found that Ira2, the Ras1 GTPase activating protein, is essential for this effect indicating a specific regulatory pathway. We performed a metabolomics analysis of cells grown with different metabolic inhibitors, and found that specific phosphoplipids and sterols are altered in treated cells, and we are relating these changes to decreased Ras1 activation. Of additional clinical relevance, phenazines cause an alteration in the cidal effects of antifungals in multiple strains. methylene blue inhibition of *C. albicans* hyphal growth is interesting as it is well tolerated by humans and has been previously used as an antimalarial drug, and we are examining its effects on *C. albicans* in vivo.

S7B:6

A DIET HIGH IN COCONUT OIL REDUCES MURINE GASTROINTESTINAL COLONIZATION BY *C. ALBICANS*

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C. albicans infections arise from pre-existing sites of colonization, such as the gastrointestinal (GI) tract, where *C. albicans* is a normal resident. Reducing *C. albicans* colonization in patients at risk of developing Candida infections is therefore of great clinical importance. However, administration of prophylactic anti-fungal drugs carries the danger of selecting for drug-resistant organisms. Thus, a non-drug strategy to reduce GI colonization would have great therapeutic potential. It is well established that diet can alter the gastrointestinal flora; we therefore hypothesized that *C. albicans* colonization of the GI tract could be altered by diet.We measured the effect of diet on *C. albicans* GI colonization in mice and found that different types of dietary fats can either promote or suppress *C. albicans* colonization. Importantly, we also found that pre-existing *C. albicans* colonization can be reduced by a change in diet. Compared to a standard murine control diet, *C. albicans* colonization is higher in mice fed a diet rich in beef tallow or soybean oil, and lower in mice fed a diet rich in coconut oil. Because coconut oil is composed primarily of medium-chain fatty acids, while beef tallow and soybean oil contain long-chain fatty acids, we hypothesized that metabolism in colonizing *C. albicans* cells would differ depending on the host's diet. We used qPCR to measure the expression of lipid metabolism genes by *C. albicans* resident in the GI tracts of mice fed the experimental diets and identified a set of genes that were differentially regulated in response to host diet. We found that, in mice fed beef tallow, *C. albicans* genes involved in fatty acid beta-oxidation and the glyoxylate cycle were upregulated, suggesting that the long-chain fatty acids in beef tallow promote alternative carbon utilization. Intriguingly, we found that coconut oil in the diet resulted in reduced colonization even in the presence of beef tallow: mice fed a mix of beef tallow and coconut oil exhibited the same low colonization observed in mice fed the coconut oil-only diet. In addition, genes involved in beta-oxidation and the glyoxylate cycle were not upregulated when the mice were fed a diet that contained both beef tallow and coconut oil. Medium-chain triglycerides are known to improve intestinal absorption of long-chain fatty acids, and therefore, we hypothesized that the addition of coconut oil to the diet helped to remove long-chain fatty acids from the environment surrounding the *C. albicans* cells. These data suggest that dietary supplementation with medium-chain triglycerides may represent a viable therapeutic alternative to the use of prophylactic anti-fungal drugs as a means to prevent life-threatening Candida infections by reducing GI colonization.

S7C:1

THE *CANDIDA ALBICANS* ORFEOME PROJECT: TOWARDS A GENOME-WIDE OVEREXPRESSION STRAIN COLLECTION

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C. albicans has 6214 predicted ORFs; however 74% of them remain uncharacterised. To elucidate the function of genes and advance our understanding of *C. albicans* pathogenicity, gene deletions or knock-outs have been widely used. However, this approach has some limitations due to the diploid nature of *C. albicans* and the essentiality of some genes. In addition, functional compensation has been observed with paralogous genes and multigene families in some null mutants, which could impact on the exhibition of substantial phenotypes. Therefore, an alternative strategy, which has been successfully applied to other species, is to create over-expression strain collections and screen for gain of functions or suppression of mutant phenotypes. The *C. albicans* ORFeome project is creating three new resources for the Candida community, including (1) a *C. albicans* ORFeome library by cloning and sequencing every predicted ORF into a Gateway vector in E. coli, (2) a library of bar-coded *C. albicans* over-expression vectors by placing each ORF under the control of a reverse tetracycline promoter in a *C. albicans* integrative plasmid based on CIp10, and (3) a library of *C. albicans* over-expression strains by integrating the overexpression vectors generated in (2) into the RPS1 chromosomal locus. In addition, a compendium of 49 expression plasmids has been established that combines different constitutive or inducible promoters (ACT1, TDH3, TET, PCK1), N-terminal or C-terminal tags (3xHA, GFP, TAP-tag) and transformation markers (URA3, NAT1). Data about this resource are available at http://www.candidaorfeome.eu. All three libraries will be available to the community upon completion. Here we will report on the progress of the ORFeome project and applications of a first set of overexpression strains to the study of different aspects of *C. albicans* biology such as morphogenesis, biofilm formation, cell wall biogenesis or genome dynamics. Our results exemplify how the resources developed in the framework of the *C. albicans* ORFeome project will advance the research field of genome-wide over-expression screens, identification of antifungal drug targets, the development of libraries for protein localization, protein complex identification, and other applications, which will also enrich our understanding of *C. albicans* pathogenicity. The *C. albicans* ORFeome project is funded by the Wellcome Trust.

S7C:2

EXPLORING EXPRESSION DATA AT CGD

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The Candida community is using high throughput technologies to investigate Candida biology at an increasing rate, such that almost every week a new paper is published with an associated high throughput dataset. For the most part, these datasets are submitted to national repositories, such as GEO or ArrayExpress, but even then, the datasets are not available in a format that can be easily browsed to make biological sense of the data. Furthermore, many of the older datasets are available often as tab-delimited files or excel spreadsheets either as journal supplements, or on lab websites, often in a multitude of different formats. To address these issues, CGD is systematically collecting all available high throughput datasets associated with Candida publications, curating them, and archiving

them on our download site. In addition, for expression datasets, we are converting each dataset into a common format, and clustering those data, such that the datasets can be easily browsed on the web, within the CGD site. In this way, the datasets will be readily accessible, and the biology contained therein will be readily discoverable, often yielding testable hypotheses that start out with the very simply question 'what genes are co-regulated with my favorite gene'. CGD aims to be a one stop shop for all Candida relevant datasets, collecting them in a single place, in a consistent format, so that the data can be best taken advantage of by the community.

S7C:3

CHARACTERIZATION AND UTILIZATION OF THE *CANDIDA ALBICANS* HAPLOID TOOL STRAINS

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The recent discovery of haploids in *Candida albicans* and construction of a set of stable haploid tool strains with multiple auxotrophic markers promise to greatly speed up the molecular and genetic study of *C. albicans* biology and pathogenicity. Although such haploids have essentially the same characteristics as the diploids, their genomes are somewhat unstable and spontaneously duplicate (auto-diploidization) at different frequencies. To facilitate the utilization of these haploid tool strains, we further analyze their genome stability during routine genetic and molecular manipulations. All the haploid tool strains are highly stable upon refreshing from -80 C glycerol stock. During the transformation for targeted gene deletion, the strain (GZY803) containing a single auxotrophy (ura3) consistently shows a correct targeting rate of ~90-100% and an auto-diploidization rate of ~10-20%. In contrast, the rates of auto-diploidization and mis-targeting are a little higher, although varied, in other haploid tool strains (GZY815, GZY822, and GZY823) with multiple auxotrophic markers (ura3, arg4, his1, and lys2). Nevertheless, successful deletion of a target gene can be readily achieved in each of the haploid tool strains. Moreover, typical cell biology and biochemical works, such as visualization of GFP/mCherry-tagged proteins and detection of HA/Myc-tagged protein, have been demonstrated to be applicable in these haploid tool strains.
We have used GZY803 to construct a mini-library containing deletion mutants of ~40 genes encoding GTPase and their regulators. The mutants were screened for various biological defects such as temperature sensitivity, defective hyphal formation on Spider and Lee's plates, sensitivity to DNA damaging agents (HU) and different cell wall stresses (CFW, SDS, and Congo Red). The screens have revealed new regulators of several cellular processes including morphogenesis, membrane trafficking, and cell wall integrity, demonstrating that the haploid strains are useful tools for genetic and molecular study of gene functions in *C. albicans*. More detailed characterization of these mutants is now in progress.

S7C:4

COMPLEX HAPLOINSUFFICIENCY-BASED GENETIC INTERACTION SCREENING IN *C. ALBICANS*

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Large-scale genetic analysis of *C. albicans* has been focused mainly on the evaluation of libraries of single gene mutants (heterozygotes or homozygotes). From these studies, it is clear that many biologically and medically important processes in *C. albicans* are modulated by intricate genetic networks. Our laboratories have been interested in using large-scale genetic interaction screening as an approach to understanding how multiple pathways converge to regulate the biology of *C. albicans*. To do so, we have developed strategies based on

complex haploinsufficiency, the phenomenon by which strains with heterozygous mutations at two loci generate a more severe phenotype than strains with single mutations. We have developed two technical approaches to complex haploinsufficiency based screening. First, we have carried out transposon mutagenesis of a parental heterozygote of interest (query strain). Most recently, we have screened a library of mutants derived from a heterozygote the **r**egulation of **A**ce2 and **m**orphogenesis (RAM) kinase Cbk1 (*cbk1*Δ/*CBK1*) designed to identify Ace2 independent functions of this pathway. We have identified a collection of 121 mutants showing complex haploinsufficiency (confirmed by *CBK1* complementation). In addition, we have carried out transcriptional profiling of a *cbk1*Δ/Δ mutant using RNA-seq as a means to further evaluate the results of the complex haploinsufficiency screen. Second, we are constructing an ordered library of transcription factor deletion cassettes derived from strains originally created by the Johnson laboratory as a more convenient, targeted resource for moderate scale genetic interaction screens. The results of these screens and progress towards developing this community resource will be presented.

S7C:5

IN VIVO IMAGING OF DISSEMINATED CANDIDIASIS IN MICE USING LUCIFERASE REPORTER STRAINS

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To study pathogenesis of disseminated candidiasis and therapeutic approaches the mouse model of intravenous infection is commonly used. In this model, disseminated candidiasis manifests primarily in the kidneys and reduction of renal fungal burden is used as a marker for treatment efficacy. Determination of fungal burden by quantification of colony forming units or PCR requires sacrificing the animal to obtain organ samples. Thus, these methods provide only insight into the stage of infection at a chosen time point but do not allow continuous monitoring of disease development. Non-invasive imaging techniques, such as bioluminescence imaging, allow real-time monitoring of infection in mice and have been used successfully for mucosal candidiasis and subcutaneous biofilm formation. However, suitable reporter systems that facilitate imaging of disseminated candidasis in deep-seated organs have not been described so far. We developed a real-time non-invasive bioluminescence imaging technique which allows visualization of disseminated infection with *Candida albicans* in internal organs such as the kidney. Using *C. albicans* reporter strains expressing a synthetic optimized luciferase, we could detect less than 5 x 103 Candida cells per kidney and additionally identified individual animals with foci of infection in the brain and translocation of *C. albicans* to the urinary bladder. Quantification of renal bioluminescence correlated well with determination of fungal burden as colony forming units (Spearman correlation 0.92, $p < 0.0001$), suggesting that this method is suitable to monitor disease progression and efficacy of therapeutic intervention. As prove of principle, we monitored persistence of *C. albicans* during consecutive treatment with caspofungin and fluconazole: While therapy significantly improved clinical outcome and mediated clearance of *C. albicans* from kidneys as expected, we made the unexpected discovery that *C. albicans* cells can persist and withstand antifungal therapy in cryptic niches. Our data imply that these niches might provide a reservoir for de novo colonization of a host by *C. albicans* after discontinuation of antifungal therapy and may contribute to recurrence of Candida infection. In summary, non-inva-

sive bioluminescence imaging can be used to quantitatively monitor systemic infection with *C. albicans* in mice, providing temporal and spatial information on disease development in individual animals.

S7C:6

MAPPING PROTEIN-PROTEIN INTERACTIONS WITH AN EXPANDED GENETIC CODE

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In previous work, we expanded the genetic code of *Candida albicans* with the synthetic photocrosslinker amino acid p-azido-L-phenylalanine. During translation, the synthetic amino acid is site-specifically incorporated into proteins in response to an amber codon via an orthogonal tRNA and tRNA synthetase. Due to the highly reactive nature of the photoactivated synthetic amino acid, protein interactions can be covalently linked following UV irradiation. Consequently, this methodology is particularly suited to unravel unknown in vivo protein-protein interactions and due to its high resolution it can be applied to map amino acids involved in protein-protein or protein-ligand interactions. As proof of principle, we analyzed the transcriptional repressor Tup1p in this study. The recently resolved crystal structure denotes the N-terminal tetramerization domain of Tup1p as an alpha helix. Four monomers form a coiled coil dimer of dimers, and nonpolar knob residues are involved in the inter- and intradimerization. By inserting amber codons at the respective amino acid position, we generated a set of C-terminally tagged mutant Tup1p variants which contain the synthetic amino acid p-azido-L-phenylalanine at defined positions of one of the two coiled-coil domains or the residues predicted to stabilize the interdimerization. After in vivo crosslinking, protein extracts were analyzed by western blotting. Apart from the monomeric form of Tup1p, higher molecular weight complexes were detected for all Tup1p variants, where an amino acid in the d and g position of the heptad repeat sequence was replaced by p-azido-L-phenylalanine. Furthermore, variants which contained p-azido-L-phenylalanine at positions of the interdimerization stabilizing residues also yielded higher molecular weight complexes, whereas no higher molecular weight complexes could be observed for any other residue of the heptad repeat sequence. These results are in complete accordance with the crystal structure, underscoring the exceptional specificity of this method. In conclusion, we could experimentally verify the residues necessary for tetramerization identified for the Tup1p N-terminal domain crystal structure by mapping in vivo intermolecular interactions with highest resolution. We propose that this methodology might also be valuable for mapping in vivo protein-protein interactions of other virulence factors.

S8:1

TITLE NOT AVAILABLE

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S8:2

TRANSCRIPTOME PROFILING OF SERIAL GENETICALLY-MATCHED SUSCEPTIBLE AND FKS-RESISTANT *C. GLABRATA* CLINICAL ISOLATES

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Background and Objectives: *Candida glabrata* is an important opportunistic pathogen and is the second most common cause of candidiasis after *C. albicans* in North America. Echinocandin antifungal drugs are first-line therapy for *C. glabrata* infections but drug resistance is a clinical confounding factor that is on the rise. Reduced susceptibility to echinocandins has been associated with amino acid substitutions in highly conserved hot-spot

regions of the Fksp subunits of glucan synthase. To better understand underlying cellular factors contributing to the emergence of echinocandin resistance in *C. glabrata*, we applied high-throughput sequencing technologies to sequence the transcriptome of an echinocandin resistant clinical isolate harboring a point amino acid substitution in the FKS2 gene (S663P) and its isogenic drug sensitive counterpart. **Methods:** Total RNA was extracted from cells grown overnight at 37º C in YPD rich medium using the RNeasy kit (Qiagen), with DNAse treatment. RNA sequencing (RNA-seq) was carried out using the high-throughput Illumina HiSeq2000 sequencing technology by BGI Americas. Reference sequence C_glabrata_CBS138 was used for bioinformatics analysis. **Results and Conclusions:** RNA-seq preliminary data revealed differential expression of 234 genes (175 genes were up-regulated and 59 down-regulated) including genes previously identified as involved in drug tolerance, genes not previously associated to a resistant phenotype, and novel transcripts whose function as a gene is uncharacterized. Preliminary studies indicate that genes for DNA repair are down-regulated, which may contribute to enhanced selection for resistance. These results provide new perspectives for our understanding of the genetic mechanisms that lead to the acquisition of drug resistance in *C. glabrata*, with potential for future improvements of therapeutic strategies.

S8:3

FKS MUTANT CANDIDA INFECTIONS OCCUR AMONG PATIENTS WITH PRIOR ECHINOCANDIN EXPOSURE AND ARE ASSOCIATED WITH THERAPEUTIC FAILURE OF ECHINOCANDIN THERAPY

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Background: Echinocandin (EC) resistance among Candida spp. is emerging and attributed to FKS mutations. The objectives of this study were to define the incidence of FKS mutations among patients (pts) with invasive candidiasis (IC) in the presence or absence of prior EC exposure, and to identify associations between FKS mutations and responses to EC therapy. **Methods:** Isolates were collected from pts with bloodstream or intra-abdominal (IA) infections. Caspofungin (CSP) minimum inhibitory concentrations (MICs) were determined by broth microdilution. Prior EC exposure was defined as ≥ 3 days of CSP before IC, and breakthrough (BT) as receipt of CSP at onset of IC. Hot spots 1 and 2 of FKS1 (all spp) and FKS2 (*C. glabrata* [Cg] only) were amplified by PCR. **Results:** 256 isolates representing unique episodes of IC were tested; 54% (139), 20% (52), 17% (44), 4% (11), and 4% (10) were Cg, *C. albicans* (Ca), C. parapsilosis (Cp), C. tropicalis (Ct), and C. krusei (Ck), respectively. 56% (144/256) of isolates (90 Cg, 27 Ca, 16 Cp, 8 Ck, 3 Ct) were collected from patients with IC (83 blood, 29 IA sites) and no prior CSP exposure; none of these isolates harbored FKS mutations. Among the remaining 112 isolates (49 Cg, 25 Ca, 28 Cp, 8 Ct, 2 Ck) from pts with prior CSP exposure (median 21.5 days, range 3 - 438), 27% (30) were BT (11 Cg, 9 Cp, 7 Ca, 2 Ct, 1 Ck) and 13% (15) FKS mutants (12 Cg [4 FKS1, 8 FKS2], 3 Ca). BT IC was more common among IA than bloodstream isolates (45%, 13/29 vs 20%, 17/83; p=0.015). Spp-specific rates of FKS mutations were 24% and 12% for Cg and Ca, respectively. 73% (8) and 14% (1) of BT Cg and Ca were mutants, respectively. FKS mutations were absent among other spp, notably including Cp (median CSP exposure of 28 d). Median CSP MICs were higher (2 vs 0.5; p=0.0002) and duration of prior CSP exposure longer (68 vs 20d; p=0.002) in FKS mutants versus wild-type (WT). 21% (6) and 11% (9) of isolates from IA and blood were mutants, respectively (p=0.21). 83% (5/6) of BT Cg from pts with IA IC were FKS mutants. 61% (157) of IC cases were treated with an EC; 41% (64) failed therapy, including 93% (13/14) of pts with IC

due to FKS mutants compared to WT (36%; p<0.0001). Spp-specific failure rates were 51% (19/37), 50% (3/6), 40% (11/27), 38% (3/8), and 35% (28/79) for Cp, Ck, Ca, Ct, and Cg, respectively. Failure was more common among pts with IA versus bloodstream infection (71%, 20/28 vs 34%, 44/129; p= 0.0005) and pts with prior CSP exposure (58%, 46/80 vs 23%, 18/77; p<0.0001). Median CSP MICs did not differ among pts who failed or responded to EC therapy (p=0.11). **Conclusions:** FKS mutations are associated with therapeutic failure of EC therapy and occur most commonly in Cg and Ca collected from pts with prior EC exposure. Pts with IA candidiasis are at a high risk for BT infection during EC therapy, infection due to FKS mutant strains, and failure of EC treatment.

S8:4

ANTI-CANDIDIASIS SYNTHETIC GLYCOPEPTIDE VACCINE ACCEPTABLE FOR HUMAN USE

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Background: Our research on pathogenesis of experimental disseminated candidiasis led to the discovery that antibodies specific for *Candida albicans* cell surface β-1, 2-mannotriose [β-$(Man)_3$] protect mice against the disease. Use of a 14 mer peptide Fba, which derived from the *N*-terminal portion of the *C. albicans* cell surface protein fructose-bisphosphate aldolase, as the carrier for the glycan has resulted in a novel synthetic glycopeptide vaccine β-$(Man)_3$-Fba. This conjugate uniquely induces protective antibody responses against both the glycan and peptide carrier parts of the vaccine in mouse models of human disseminated candidiasis. We have isolated monoclonal antibodies (MAbs) specific for the Fba peptide. Use of these MAbs in flow cytometric and confocal microscopic analyses provide evidence that the peptide is expressed on the fungal cell surface during growth as yeast and hyphal forms. In addition, Fba specific MAbs (IgM & IgG1) given passively to naïve mice protect the animals against disseminated candidiasis. **Current work:** To move toward a synthetic vaccine against disseminated candidiasis acceptable for human use, we have modified the β-$(Man)_3$-Fba conjugate by coupling it to tetanus toxoid (TT) in order to improve immunogenicity and allow for use of adjuvant suitable for human use. Our data show that addition of tetanus toxoid to glycopeptide conjugate resulted in a vaccine that induces dual antibody-dependent protective immunity without the need for adjuvant. Moreover, even the glycan part of the vaccine, β-$(Man)_3$ is expressed by several *Candida* species of medical significance in addition to *C. albicans*, the peptide part of the vaccine, Fba peptide is only expressed at 85-98% homology by other *Candida spp*. To maintain the protective duality of the glycopeptide vaccine against other *Candida spp.*, we further improve the composition of the vaccine by defining peptides that are universally produced by medically important *Candida* species in addition to *C. albicans.* Three universal peptide candidates can induce antibody production and protection against disseminated candidiasis in mice. **Conclusion:** These findings represent unique steps forward in the development of a fungal vaccine for human use. The new glycopeptides vaccine, which comprised of the universal peptide epitope and the key β-$(Man)_3$ epitope, could provide broader protection against a variety of medically important *Candida spp.*

S8:5

APPLICATION OF THE COMBINED CLSI/EUCAST BREAKPOINTS FOR AZOLE AND ECHINOCANDIN SUSCEPTIBILITIES OF CANDIDA ISOLATES IN A TERTIARY CANCER CENTER: THE EMERGING THREAT OF MULTI-DRUG RESISTANCE

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Background: Increasing rates of resistance to antifungals pose an emerging threat to patients with multiple risk factors for severe *Candida* infections, such as those with advanced malignancies. Recently, the CLSI and EUCAST developed common in-vitro susceptibility breakpoints, based on the integration of epidemiological, genomic and clinical data. We investigated the rate of cross-resistance between azoles and echinocandins, and identified predictors of echinocandin non-susceptibility, by retrospectively applying the updated CLSI/EUCAST definitions in a cohort of patients with cancer and candidemia or deep-seated candidiasis. **Methods:** We reviewed demographic, clinical and basic laboratory data of patients with positive cultures for *Candida spp*. from the blood or other sterile sites, who were on micafungin monotherapy, over an 103 month-period. We applied the updated CLSI/EUCAST definitions to identify fluconazole, voriconazole and caspofungin-resistant isolates. **Results:** Seventy-three patients (38 [51.4%] with hematologic malignancies) were studied. Most cases (58, 78.3%) were caused by non-*albicans Candida spp*. 18 isolates (24.6%) were fluconazole-resistant. 4/7 (57.1%) non-*krusei* fluconazole resistant isolates would be considered susceptible to fluconazole, based on previous breakpoints. 6/7 (85.7%) were also voriconazole-resistant and 4 of those 6 (66.6%) would be classified as susceptible, based on previous definitions. Nine isolates (12.3%) were non-susceptible to caspofungin and 4 (44.4%) would be considered susceptible, based on the previous cut-off (≤ 2 mcg/mL). 6/73 (8%) isolates were resistant to both fluconazole and caspofungin ("multi-drug resistant", Figure 1). Among all isolates with recent (within one month) echinocandin exposure (n=27), 3/18 (16.7%) fluconazole-susceptible isolates were non-susceptible to caspofungin, as opposed to 5/9 (55.6%) isolates that were fluconazole-resistant. Overall, fluconazole resistance was associated with caspofungin non-susceptibility, independent of recent echinocandin exposure (Table 1: adjusted OR 8.4, 95% C.I. 1.05-46.24, *P*=.014). **Conclusions:** In a contemporary group of cancer patients with serious *Candida* infections, we observed a worrisome trend for (multi)azole and echinocandin cross-resistance, which might be independent of antifungal use, and indicative of a propensity for multi-drug non-susceptibility. The interpretation of our results is limited by the small number of resistant isolates and retrospective data collection. Prospective, larger-scale clinical registries, with molecular data on mutations that confer non-susceptibility are needed.

S8:6

CRYSTAL STRUCTURES OF FULL-LENGTH WILD TYPE AND MUTANT *SACCHAROMYCES CEREVISIAE* ERG11P GIVE INSIGHT INTO TRIAZOLE AND SUBSTRATE BINDING

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Lanosterol 14α-demethylase (Erg11p), encoded by *ERG11*, is a cytochrome P450

monospanning membrane protein that is the target of the azole antifungal drugs. One mechanism of fungal azole resistance is point mutations in Erg11p that reduce affinity for azole drugs. It is therefore important to understand how Erg11p binds its substrate, product and potential inhibitors. To date, homology models of fungal Erg11ps have been based on the crystal structures of CYP51s without the N-terminal transmembrane domain. The objective of this study was to obtain high-resolution X-ray crystal structures of full-length *S. cerevisiae* Erg11p with, and without, substrates or inhibitors bound, and compare these structures with those for Erg11p containing single mutations reported in the literature to confer azole resistance. *S. cerevisiae ERG11* was cloned with a C-terminal 6xHis tag at the *PDR5* locus in *S. cerevisiae* ADΔ. Erg11p was solubilised from membrane preparations with N-decyl-β-D-maltoside, purified by Ni-NTA agarose affinity chromatography and Superdex 200 size exclusion chromatography, and its structure determined by X-ray crystallography. The empty structure of ScErg11p, mutant ScErg11p, and five ligand complexes (with the substrate lanosterol, the pseudosubstrate estriol, and the triazole inhibitors itraconazole, fluconazole and voriconazole) were determined at resolutions ranging from 1.9 to 2.8 Å. The structures revealed two N-terminal helices oriented at ~60° to each other which not only tether ScErg11p to the membrane but also orient the partially embedded enzyme relative to the bilayer. The ScErg11p structures showed lanosterol in the active site, a substrate channel linked to the lipid bilayer, and a proposed product exit channel. The crystal structures also indicated how triazole antifungals interact with ScErg11p to block catalysis and identified possible interactions that confer triazole resistance. The full-length ScErg11p structures exhibited less conformational heterogeneity than other N-terminal truncated eukaryotic cytochrome P450 structures, suggesting that the transmembrane domain itself, or associated hydrophobic molecules such as lipids or reaction product, may decrease conformational heterogeneity. The Y132F mutation in CaErg11p has been reported to confer azole resistance on clinical isolates. Yeast cells with the corresponding mutation (Y140F) introduced into ScErg11p became resistant to short-tailed, but not long-tailed, azoles. The crystal structure of Y140F ScErg11p showed normal binding of itraconazole to the mutated enzyme. These results may enable the development of improved molecular models to facilitate drug design that targets fungal CYP51s or other eukaryotic cytochrome P450s, and provide a practical basis for the design of therapeutics with off-target effects minimized.

S9:1

DISSECTION OF THE NON-KINASE DOMAIN OF THE NIM1 KINASEGIN4 REVEALS NOVEL REGULATORY FUNCTIONS IN CELL CYCLE CONTROL AND HYPHAL GROWTHIN *CANDIDA ALBICANS*

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The Nim1-family kinases play important roles in the regulation of cell cycle progression, polar growth and septin organization in fungi. In *Candida albicans*, the kinase Gin4 belongs to this family. Deletion of *GIN4* causes severe morphological and cytokinetic defects and failure in septin ring assembly in yeast cells, leading to formation of chains of greatly elongated and swollen cells. *GIN4* also plays a key role in the regulation of hyphal growth. It phosphorylates and primes the septin Cdc11 for further phosphorylation by CDKs in the yeast-to-hyphae transition. Gin4 also regulates the phosphorylation of another septin Sep7 and influences septin ring dynamics. Most previous studies have been focused on the role of the kinase domain of Gin4. However, we found that mutant *GIN4* alleles with the kinase domain inactivated or deleted exhibited markedly weaker phenotypes than did the *gin4Δ/Δ* mutant, indicating that some Gin4 functions are associated with the nonkinase

domains. In this study we performed molecular dissection of the nonkinase region of Gin4 and uncovered at least three distinct functional domains. The C-terminal 200-amino acids contain a phospholipid-binding domain (LBD). When expressed alone, it localizes to the plasma membrane. Recombinant LBD binds a number of phospholipids *in vitro* in a manner dependent on multiple basic residues. Deleting the LBD causes similar defects as deleting the entire gene, and ectopic expression of the LBD in *GIN4* OFF cells can partially support septin ring formation. Interestingly, constitutive expression of the LBD in a mutant depleted of the phosphoinositide-4-kinase Stt4 can bypass the need for Stt4 in hyphal formation, suggesting that LBD's interaction with lipids has an important role in hyphal growth. Another domain identified is the septin-binding domain (SBD) from aa 751 to 1050. The SBD is required for Gin4 to localize to the bud neck, and the SBD fragment can localize to the bud neck in cells with a functional septin ring in manners similar to WT Gin4. Co-IP experiments revealed that the bud-neck localization of Gin4 is mediated via direct interaction of the SBD with the septins. Deletion of the SBD results in Gin4 mis-localization to the cytoplasm and a significant loss of its cellular function. An unexpected discovery is a nucleolus localization domain (NAD) that mediates localization to a specific area on the periphery of the nucleolus. This domain is located between aa 451 and 750, a region closely downstream of the kinase domain and containing multiple perfect CDK phosphorylation motifs. Although Gin4 has never been observed to localize to the nucleus, both GFP-tagged NAD and a Gin4 mutant lacking the LBD was found as one or two distinct spots in the nucleus. The spots were found to localize to the periphery of the nucleolus co-labelled with Nop1-mCherry. Moreover, cells expressing a Gin4 mutant lacking the NAD exhibited frequent premature spindle elongation in the mother compartment and the formation of binucleate cells, suggesting defects in spindle alignment and/or the spindle position checkpoint (SPC). Although the underlying molecular mechanisms remain to be determined, the specific localization of the NAD in the nucleolus inspires exciting possibilities. Cdc14, the effector of the mitotic exit network (MEN), is sequestered in the nucleolus throughout most of the cell cycle and is only released when the MEN is activated. Our observations suggest that Gin4 might play a previously unknown role in the nucleolus to regulate the MEN. Alternatively, the regulation may occur at the bud neck where Cdc14 transiently co-localizes with Gin4 at the end of the cell cycle. In support of both models, our preliminary results have detected co-IP of Cdc14 with the NAD and full length Gin4. Taken together, our findings suggest that Gin4 may have a previously unappreciated role in coupling cell cycle progression to mitotic exit in addition to its roles in septin ring assembly and polar growth *in C. albicans*.

S9:2

PLASMA MEMBRANE ARCHITECTURE PROMOTES *C. ALBICANS* MORPHOGENESIS AND VIRULENCE

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The *C. albicans* plasma membrane is not just an essential barrier; it also mediates diverse processes that are important for virulence, including secretion of virulence factors, endocytosis, cell wall synthesis, nutrient import, and invasive growth. The architecture of the plasma membrane is important to organize these diverse activities into appropriate compartments. One recently described type of domain called the MCC/eisosomes corresponds to furrows in the plasma membrane that are about 300 nm long and 50 nm deep. The MCC portion of these domains includes integral membrane proteins, and the eisosome portion is a complex of peripheral membrane proteins that lie underneath the MCC. The MCC protein Sur7 is needed for proper architecture of the plasma membrane.

A sur7Δ mutant mislocalized plasma membrane proteins and displayed broad defects in cell wall synthesis, morphogenesis, resistance to copper, and virulence. Mutants lacking another MCC protein, Nce102, displayed a unique phenotype in that they were defective in undergoing invasive hyphal growth into low concentrations of agar, but could invade well into higher agar concentrations. This phenotype was likely due to a defect in actin organization. In support of this, the invasive growth defect of a bni1Δ mutant that mislocalized actin due to lack of the Bni1 formin was also reversed at high agar concentrations. Current studies are focused on assessing the role of the paralogous eisosome proteins, Pil1 and Lsp1, which were shown in S. cerevisiae to promote formation of eisosomes and the membrane furrows. Interestingly, a pil1Δ lsp1Δ mutant that failed to form MCC/eisosomes showed a broad range of defects similar to the sur7Δ mutant. Since eisosomes still assemble in the sur7Δ mutant, these results indicate Sur7 is required for proper eisosome function. We also observed that the phenotypes for the sur7Δ mutant were often slightly stronger than for the pil1Δ lsp1Δ mutant, indicating that Sur7 also has functions that are independent of the eisosome. Altogether, these results demonstrate that proper plasma membrane architecture mediated by MCC/eisosomes is needed to coordinate diverse range of processes in the plasma membrane that promote virulence.

S9:3

A TRANSCRIPTIONAL CIRCUITRY INVOLVING THE MASTER REGULATORS NDT80P, EFG1P AND MEMBERS OF THE HEAT-SHOCK FACTOR-TYPE DNA-BINDING PROTEINS CONTROLS *CANDIDA ALBICANS* MORPHOGENESIS

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Heat shock factor (HSF)-type transcriptional regulators are highly conserved DNA-binding proteins required for adaptation to acute stress. Hsf1p, the prototype HSF-type regulator in yeast controls a variety of important cellular processes reflecting its essential function. The *C. albicans* genome encodes one Hsf1p functional homolog, Cta8p (also named Hsf1p, essential protein), as well as 3 additional members of the HSF-type family displaying high sequence homology with Cta8p: Sfl1p, Sfl2p and Skn7p. Sfl1p and Sfl2p were shown to antagonistically control the *C. albicans* yeast-to-hyphae transition: Sfl1p represses it, whereas Sfl2p activates it in response to temperature. On the other hand, Skn7p imparts *C. albicans* hyphal development in response to oxidative stress. To better understand the function of Sfl1p and Sfl2p, we performed genome-wide location and expression analyses by combining chromatin immunoprecipitation (ChIP) coupled to high-throughput sequencing and microarray transcript profiling. We found that both regulators bind, through divergent motifs, to common targets encoding master regulators of hyphal growth and virulence (e.g. UME6, TEC1, NRG1) or markers of the yeast-form growth (e.g. RME1, RHD1, YWP1) and exert both activating and repressing effects on gene expression, indicating that they carry dual transcriptional regulatory functions. In addition, Sfl2p specifically binds to and turns on the expression of a high proportion of hyphal-specific genes (HSGs; HWP1, HGC1, ECE1, others), revealing a direct link between Sfl2p and hyphal development. Genetic interaction analyses revealed that SFL1 and SFL2 interact with major regulators of hyphal growth and virulence, including EFG1, UME6, TEC1 and BRG1. Strikingly, bioinformatic analyses indicated that the master regulators Efg1p and Ndt180p co-bind with Sfl1p and Sfl2p to the promoter of their targets. We show indeed that Efg1p binds in vivo to the promoter of many Sfl1p and Sfl2p targets and immunoprecipitates with Sfl1p and Sfl2p. Genetic interaction analyses also indicate that SFL1 and SFL2 functionally interact with NDT80. Interestingly, recent ChIP-on-chip and transcript profiling analyses suggested that

the mechanism of Skn7p-mediated transcriptional control is reminiscent of Sfl1p and Sfl2p. Skn7p binds to the promoter of many regulators of hyphal growth and virulence and exerts both activating and repressing effects on gene expression. Bioinformatic analyses similarly suggested that at least Efg1p and Ndt80p co-bind with Skn7p to the promoter of its targets. We propose that the regulatory function of *C. albicans* HSF-type regulators that convert temperature- (Sfl1p and/or Sfl2) or oxidative stress- (Skn7p) sensing inputs into a morphogenesis programming output is part of a transcriptional circuitry implicating intimate functional interactions with the master regulators Efg1p and Ndt80p.

S9:4

A NEW ROLE FOR THE CLASS I CHITIN SYNTHASE ENZYMES DURING POLARISED GROWTH IN *CANDIDA ALBICANS*

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As fungal cells proceed through the cell cycle, they deposit new cell wall material at sites of polarised growth. These sites include the tips of growing buds and hyphae, and sites of septation. Chitin is a structural component of almost all fungal cell walls and septa, and in *Candida albicans*, is synthesised by a family of four chitin synthase enzymes, Chs1 (class II), Chs3 (class IV) and Chs2 and Chs8 (class I). Analysis of mutant phenotypes has given us clues about the roles of these enzymes during growth and cell division. For example, Chs1 is essential and is responsible for the synthesis of the primary septum, and Chs3 synthesises the majority of chitin found in the cell wall as well as the chitin ring at division sites. The localisation of Chs1-YFP and Chs3-YFP in live cells has provided further evidence to support these roles for Chs1 and Chs3. The role of the two class I enzymes (Chs2 and Chs8) is less well understood. Deletion of *CHS2* and *CHS8* results in a reduction of up to 95% of the chitin synthase activity that we can measure *in vitro*, and both enzymes are present at sites of septation before cytokinesis. But what does this mean in terms of the biological function of the class I chitin synthases in *C. albicans*? In *Saccharomyces cerevisiae,* the class I enzyme (*Sc*Chs1) has an important role in sealing the bud before cell separation. Deletion of *ScCHS1* results in a bud lysis phenotype. To assess whether hyphae of a *C. albicans* class I mutant exhibited a cell lysis phenotype, we grew a *chs2*Δ*chs8*Δ strain in medium containing propidium iodide (PI) as a cell viability indicator. We observed intact but dead hyphae which failed to elongate 4 h after induction of hyphal growth, and hyphal tip lysis after 24 h. Dead hyphae were also observed in cells of a *chs3*Δ mutant, but in this case, the mother cells were dead and lysis occurred along the lateral cell wall and not at the hyphal tip. A combination of these two phenotypes was observed in a *chs2*Δ*chs3*Δ mutant. No cell lysis was observed in the corresponding wild type strain. We also investigated the precise localisation of Chs2-YFP and Chs8-YFP in live hyphal cells by fluorescence microscopy. We confirmed that Chs2-YFP was localised to hyphal tips throughout growth. We also describe a unique pattern of localisation for the class I enzymes at septation sites which first appear as a ring, then contract to a spot in the middle of the septum, and later split into two spots, one on either side of the septum. Both enzymes remain in this configuration throughout subsequent cell cycles. Despite the persistent localisation of Chs2-YFP and Chs8-YFP at septa in hyphae, we did not observe any phenotypes that were indicative of a septal defect in *chs2*Δ*chs8*Δ mutant cells. Taken together, these data suggest that Chs2 and Chs8 play an important role in hyphal growth and in maintaining hyphal tip integrity that is distinct from that of Chs3.

S9:5

RSR1-GTPASE CYCLING ACTS TO MAINTAIN POLARIZED GROWTH BY PROMOTING THE EFFICIENT CLUSTERING OF CDC42 DURING *CANDIDA ALBICANS* HYPHAL DEVELOPMENT

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During *Candida albicans* hyphal development, an active cluster of Cdc42 persists at hyphal tips throughout multiple cell cycles. In addition to its impact on hyphal cell morphology, initiation and maintenance of Cdc42 activity at hyphal tips is thought to impact the expression of the hyphal transcriptional program. Our previously published results support the idea that the Ras-like GTPase Rsr1 has a role in focusing Cdc42 activity, which contributes to the establishment and maintenance of hyphal growth. The localization of the Rsr1 GEF (Bud5) at the very apex of hyphal tips, along with the subapical distribution of the Rsr1 GAP (Bud2), sets up potential zones of Rsr1 activity within the hypha. Deletions of Bud2 and Bud5 were used to investigate the roles of Rsr1-GDP and Rsr1-GTP, respectively, during hyphal development, and how Rsr1 cycling contributes to focusing Cdc42 activity at hyphal tips. Deletion of either Bud2 or Bud5 results in wider hyphae with decreased expression of hyphal-specific genes, consistent with the idea that both Rsr1 regulators are important for hyphal morphogenesis. During germ tube induction, loss of Bud5, but not Bud2, was associated with the formation of multiple clusters of Bem1-YFP, a proxy of Cdc42 activity. In addition, cells lacking Bud5 exhibited abortive attempts at daughter cell emergence on the mother cell surface. Loss of Bud2, but not Bud5, caused a broader Bem1-YFP distribution at germ tube tips. These results suggest that Rsr1-GTP (Bud5) acts to reduce the rate of Cdc42 activation during hyphal induction whereas Rsr1-GDP (Bud2) helps to focus Cdc42 activity. Further, in mature hyphae, loss of Bud2, but not Bud5, causes an increase in the frequency of hyphal branching, suggesting that Bud2 is important for limiting Cdc42 activity to a primary focus during hyphal growth maintenance. In addition, loss of either Bud2 or Bud5 results in an inability to maintain localization of Bem1-YFP at the tips of mature hyphae. Together, these data support a model where Rsr1 regulators set up zones of Cdc42 activity during hyphal development. During hyphal induction, Bud2 delimits the region to which Cdc42 activity is localized while Bud5 promotes singularity of Cdc42 clustering by slowing its activation; this in effect creates a sink-like draw for polarity components that conserves Cdc42 and allows continued polarized growth. In addition, in mature hyphae, the subapical localization of Bud2 correlates with a role for Rsr1-GDP in inhibiting spontaneous activation of Cdc42 as cell lengths increase. This inhibitory function of Rsr1-GDP may further help to conserve Cdc42 and maintain constitutive polarized growth of the hypha.

S10A:1

DIRECTIONAL POLARISATION AND SURFACE EXPLORATION BY *C. ALBICANS* HYPHAE

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The hyphae of *Candida albicans* penetrate superficial epithelial cell layers, solid organ tissue and even medical silicones. Proteins that provide directional guidance during morphogenesis and subsequent hyphal tip growth are essential for the effective invasion of underlying substrates. We used a variety of *in vitro* approaches, including electric fields and nanofabricated mazes, to investigate how the cell biology of polarized growth in *C. albicans* translates into specific exploratory behaviour. We have previously shown that calcium influx is important for tropic growth in *C. albicans,* which involves re-orientation of the polarized growth axis. During cell polarization,

GDI activity and formin-mediated actin-cable assembly mediate two mechanisms that focus the active form of the polarity organiser, Cdc42-GTP, at the nascent growth site. Loss of CaRdi1 (the GDP dissociation inhibitor which removes de-activated Cdc42-GDP from the plasma-membrane) or Bnr1, one of the 2 formins involved in actin-cable nucleation, attenuated the cathodal polarization of *C. albicans* cells in an electric field. However, normal responses were completely restored by the addition of exogenous calcium. Calcium influx may therefore act as a directional cue by promoting localization of Cdc42-GTP at the plasma-membrane, compensating for defects in either of its two trafficking pathways. After morphogenesis, the Spitzenkörper, polarisome and exocyst in the tips of adhered hyphae were asymmetrically biased towards the substrate. The Spk responded dynamically to contact with obstacles by re-orienting towards them, a positioning which promoted contour-following and hyphal growth into gaps. Septum-formation re-centralized the Spk within the apex and restored a linear growth trajectory. Thus, the directional growth responses of *C. albicans* hyphae are determined by the interplay between internal cellular growth processes and inputs from the physical environment.

S10A:2

THE ROLE OF KINASES IN HYPHAL DEVELOPMENT

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Much progress has been made in elucidating the signal transduction pathways that mediate hyphal specific patterns of transcription in response to diverse hyphal inducing cues. Moreover, there is a growing understanding of the molecular processes at the tip the underlie that promote the extreme polarised growth of hyphae. However, an important gap in our understanding is how the hyphal specific transcription program leads the establishment these processes. Kinases are key part of the hyphal development program forming a link between gene transcription and polarised growth.
Two novel examples of kinase action will be presented. The first concerns the process of polarised growth at the hyphal tip. Before fusion with the plasma membrane, secretory vesicles are tethered at the hyphal surface by a multiprotein complex called the exocyst. The spatial distribution of the exocyst can explain the pattern of polarised growth of the tip. A key hyphal-specific gene is *HGC*1 which encodes a cyclin partner of the cyclin-dependent kinase, Cdk1. In the absence of Hgc1, germ tube start to form, but are unable to maintain true hyphal growth. A number of targets of Cdk1-Hgc1 have already been identified. Here will show here that Cdk1-Hgc1 targets three residues on the exocyst component Exo84, and that this is essential for efficient hyphal growth. Exo84 phosphorylation results in a reduction in the affinity of Exo84 for phosphatidylserine in the plasma membrane allowing Exo84 to recycle. Interestingly, Exo84 is also phosphorylated by Cdk1 in *S. cerevisiae*, but in this case it results in a cessation of bud growth at mitosis. However, we show that in *C. albicans* growth does not cease at mitosis. The different outcomes of Cdk1 phosphorylation of Exo84 is explained by the different distribution of the phosphorylation sites, presenting an interesting example of how phosphorylation sites have been adapted during evolution to suit the different growth habits of the two species.
The second example concerns the maintenance of hyphal-specific gene expression. In a yeast cell cycle, Fkh2 is responsible for the G2 program of transcription that enables the onset of mitosis. Surprisingly, we have found that the Fkh2 is phosphorylated early after hyphal induction before the cell cycle is initiated. Microarray analysis shows that *fkh2*Δ mutant shows widespread loss of gene expression affecting a diverse array of cell functions. However, when the phospho-acceptor sites in Fkh2 are substituted with non-phosphorylatable alanine residues, the reduction of gene ex-

pression is restricted to a subset of specifically required for virulence such as tissue damage, adherence, biofilm formation and induction of host immune response. Biological assays confirm impairment of these attributes. Thus cell-cycle independent Fkh2 phosphorylation is a hyphal specific event required for the expression of virulence-associated functions.

S10A:3

OPAQUE TO WHITE SWITCHING OCCURS SHARPLY AND BIMODALLY

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Candida albicans can switch between two heritable cell types, termed 'white' and 'opaque.' Each cell type is stable for many generations as switching between them is rare. When switching does occur, it does so without any known changes in the primary sequence of the genome; hence, it is epigenetic. Switching between two cell types induces changes in the expression of hundreds of genes, enabling differing metabolic preferences, cellular morphologies and mating behaviors. While many studies have characterized the regulation of each individual cell type as well as the conditions that induce switching between them, there is limited dynamic information regarding the transition between cell types. Coupling a flow cytometer to a robotic arm, we tracked a cell population with single cell and high time resolution during this transition. We show that the transition from opaque to white cells is indeed switch-like, as a large fraction of the population switches synchronously. Many aspects of this 'dynamic signature' remain constant across a wide range of conditions. However, while many opaque cells switch to the white cell type, the response is bimodal; a fraction of cells remains opaque. The proportion of cells that remains opaque varies with condition; similar to proportional control systems examined in linear dynamics. Tracking the dynamics of individual cells over time has revealed features of opaque to white switching not detectable by bulk population measurements.

S10A:4

USE OF STRUCTURALLY INFORMED MUTANT PROTEINS TO ASSESS THE CONTRIBUTION OF THE PEPTIDE-BINDING CAVITY AND AMYLOID-FORMING REGION TO *CANDIDA ALBICANS* ALS3 ADHESIVE FUNCTION

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The adhesive phenotype of *Candida albicans* contributes to its ability to colonize the host and cause disease. Als proteins are one of the most widely studied *C. albicans* virulence attributes; deletion of *ALS3* produces the greatest reduction in adhesive function. Although adhesive activity is believed to reside within the N-terminal domain of Als proteins (NT-Als), the molecular mechanism of adhesion remains unclear. Previously, we solved the structure of NT-Als and identified its peptide-binding cavity (PBC) as an adhesive mechanism that recognizes free C termini of protein ligands. We used this structure to design mutations in NT-Als3 that test the contribution of the PBC to *C. albicans* adhesion, and assess the adhesive properties of other NT-Als3 regions in the absence of a functional PBC. Structural analysis of purified loss-of-PBC-function mutant proteins showed that the mutations did not alter the general structure or surface properties of NT-Als3. The mutations were incorporated into full-length *ALS3* and integrated into the *ALS3* locus of an *als3/als3* null strain, under control of the native *ALS3* promoter. Immunolabeling with an Als3-specific monoclonal

antibody verified that cell-surface abundance of variant Als3 proteins was comparable to a haploid *ALS3/als3* control strain. Adhesion phenotypes of the PBC-mutant strains were evaluated in assays using freshly collected human buccal epithelial cells, monolayers of pharyngeal epithelial (FaDu) and umbilical vein endothelial (HUVEC) cells, and saliva-coated glass coverslips. Loss of PBC function resulted in an adhesion phenotype that was indistinguishable from the *als3/als3* null strain. The adhesive contribution of the Als3 amyloid-forming-region (AFR) was also tested using these methods. *C. albicans* strains producing cell-surface Als3 in which the amyloidogenic potential was destroyed, showed little contribution of the AFR to adhesion but indicated a potential role for the AFR in aggregation between fungal cells. Als3 protein, in which both the PBC and AFR were mutated, showed an adhesion phenotype indistinguishable from the PBC-mutant strains and null mutant control. Collectively, these results demonstrate the essential and principal role of the PBC in Als3 adhesive function.

S10A:5

MUTATIONAL ANALYSIS OF ESSENTIAL SEPTINS REVEALS LINK BETWEEN SEPTIN-MEDIATED SIGNALING AND THE CAMP PATHWAY

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Septins are highly conserved structural components of animal and fungal cells that have roles in the polarization of cells and cell division. These GTP-binding proteins are stable proteins whose localization and activity is regulated by post-translational modifications. Their essential role in the cell cycle is best understood in the model yeast *Saccharomyces cerevisiae*. In this fungus, septins localize to the presumptive bud site and remain in the bud neck between mother and daughter cells as the cell cycle progresses, serving as a scaffold to direct protein localization to sites of cell separation. Following the conclusion of the cell cycle, these essential proteins rapidly disperse from the bud neck and are recycled to the next presumptive bud. Septins appear to behave similarly in the yeast-like cells of the pathogenic fungus *Candida albicans* and are also essential for cell survival. Septins, however, behave differently in the hyphal cells of *C. albicans*. In this growth form, septin rings remain stable for several cell generations and the behavior of individual septins is altered. While these observations strongly support a role for septins during hyphal growth, we know very little about why their continued presence is important in hyphal cells of *C. albicans*. Five septins ,Cdc3,Cdc10, Cdc11, Cdc12, and Sep7 constitute the mitotic septin ring in the human pathogenic fungus *Candida albicans*. Although the septin ring itself is essential for cell survival, several of the septins are dispensable for growth. Studies of these non-essential septins have been vital to identifying a role for septins in wall integrity, hyphal formation, and pathogenesis in *C. albicans*. While the mutations in non-essential septins have been useful in establishing these phenotypes, the septin defect is so slight that identifying causative associations between septins and downstream effectors has been difficult. We created Decreased Abundance by mRNA Perturbation (DAmP) alleles of essential septins, which display a more severe septin defect than the defect observed in deletions of non-essential septins. The septin-DAmP alleles have allowed us to genetically separate the role of septins in hyphal growth and cell wall integrity and identified the cyclic AMP pathway as a pathway that likely acts in a parallel manner with septins in hyphal morphogenesis.

S10A:6

GENOTYPIC AND PHENOTYPIC VARIABILITY IN A COLLECTION OF CLINICAL *CANDIDA GLABRATA* STRAINS

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Candida glabrata is an important opportunistic human pathogen causing superficial and deep tissue infection. For the pathogenicity of this fungus, a highly dynamic cell wall, adherence to different host tissues and abiotic surfaces and an extensive genomic flexibility are necessary. Also, adherence capacity is mediated through a large number of differentially regulated adhesion-like proteins in the cell wall, and the *C. glabrata* genome harbours a multitude of those potential adhesion genes. We previously reported about a significantly higher colonization rate of the human oral cavity with *Candida glabrata* in the elderly population. Based on the results of questionnaires, a high correlation between *C. glabrata* colonization rates with dentures was found. Based on these results, we here characterized the ability of more than 300 fresh clinical *C. glabrata* strains to adhere to various surfaces and analysed a collection of selected clinical *C. glabrata* strains for genotypic and phenotypic plasticity. The collection strains were subjected to Multi Locus Sequence typing (MLST) and their karyotype was determined by CHEF gel-electrophoresis. A comprehensive phenotypic analyses of all strains was performed, including numerous cell wall related phenotypes, adhesion to abiotic surfaces as well as human cell lines. In addition, reverse transcriptase RT-PCR to some identified adhesins; Epa1, Epa3, Epa6, Epa7, Epa22 and Awp1-7 of selected hyper-adherent strains was conducted. Our results illustrate the multilayered plasticity and heterogeneity of cell wall and adhesion phenotypes that enables *C. glabrata* to adapt to various environmental and host environments.

S10B:1

DRUG RESISTANCE IN *CANDIDA* CLINICAL ISOLATES: A RESOURCE FOR UNEXPECTED PATTERNS

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S10B:2

THE RELATIVE IMPACT ON AZOLE ANTIFUNGAL SUSCEPTIBILITY OF SPECIFIC MUTATIONS IN *ERG11* AMONG CLINICAL ISOLATES OF *CANDIDA ALBICANS*

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In *Candida albicans*, *ERG11*, a key gene of the ergosterol biosynthesis pathway encoding the target of azole antifungals, contributes to azole resistance in a number of ways. Overexpression of *ERG11*, either through an activating mutation in the transcription factor gene *UPC2*, or through increased copy number of chromosome 5, on which *ERG11* resides, results in reduced azole susceptibility. In addition, mutations in *ERG11* that result in an amino acid (aa) substitution have been shown to alter the ability of the azole antifungals to bind to and inhibit Erg11, resulting in resistance to this class of antifungal. Although *ERG11* mutations are frequently observed in clinical isolates, the contribution of specific *ERG11* mutations to azole resistance in *C. albicans* has not been widely explored. We sequenced both alleles of *ERG11* in 63 fluconazole

(FCZ)-resistant clinical isolates [minimum inhibitory concentration (MIC) ≥ 8 µg/mL]. Our analysis revealed that 55 isolates carried at least one mutation in *ERG11*, and we identified 26 distinct positions in which aa substitutions occurred. The number of substitutions in single *ERG11* alleles ranged between 1 (n=28) and 4 (n=3). In total, 31 distinct *ERG11* alleles were recovered with 10 distinct *ERG11* alleles containing only a single aa substitution. Notably, 21 isolates carrying *ERG11* mutations also contained activating mutations in *UPC2*. We selected 19 distinct *ERG11* mutant alleles to characterize by introduction into the wild-type azole-susceptible strain SC5314. Strains were tested for susceptibility to FCZ, itraconazole (ICZ) and voriconazole (VCZ) by standard CLSI methods. Strains homozygous for the aa substitutions Y132F, K143R, F145L, S405F, D446E, G450E and G464S had at least a 4-fold increase in FCZ MIC. Additionally, we characterized several *ERG11* alleles containing multiple mutations. Strains homozygous for the Y132F+K143R combination exhibited a 32-fold increase in FCZ MIC, which decreased susceptibilities beyond either substitution alone. Likewise, the D278N+G464S combination resulted in a 16-fold increase in FCZ MIC. The Y132F+F145L combination increased FCZ, ICZ, and VCZ MICs 16-32-fold, 4-fold, and 16-fold respectively in a strain homozygous for this mutant allele. These findings indicate that mutations in *ERG11* are quite prevalent among azole resistant clinical isolates and that most mutations result in an appreciable change in FCZ and VCZ susceptibilities. Specific combinations of *ERG11* mutations result in decreased azole susceptibility beyond a single mutation alone. Combinations of *ERG11* mutations with activating mutations in *UPC2*, as was observed in many of these isolates, likely synergize in their influence on azole resistance. These findings shed light on the effects of specific *ERG11* mutations on azole resistance and may facilitate better predictions of treatment outcomes in response to azole therapy in the future.

S10B:3

CHROMOSOME 5 MONOSOMY OF *CANDIDA ALBICANS* CONTROLS SUSCEPTIBILITY TO VARIOUS TOXIC AGENTS INCLUDING MAJOR ANTIFUNGALS

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Candida albicans is a prevailing fungal pathogen with a diploid genome that can adapt to environmental stresses by losing or gaining an entire chromosome or a large portion of a chromosome. We have previously found that the loss of one chromosome 5 (Ch5) allows for adaptation to the toxic sugar L-sorbose. The latter is similar to caspofungin and other antifungals from the echinocandins class in that it represses synthesis of the cell wall glucan in fungi. Here, we extended the study of the phenotypes controlled by Ch5 copy number. We examined 57 strains, either disomic or monosomic for Ch5 representing five different genetic backgrounds, and found that the monosomy of Ch5 causes elevated levels of chitin and repressed levels of 1,3-β-glucan components of the cell wall, as well as diminished cellular ergosterol. Increased deposition of chitin in the cell wall could be explained, at least partially, by a twofold down regulation of *CHT2* on the monosomic Ch5 that encodes chitinase and a 1.5-fold up regulation of *CHS7* on Ch1 that encodes chitin synthase. Other important outcomes of Ch5 monosomy consist of susceptibility changes to agents representing four major classes of antifungals. Susceptibility to caspofungin increased or decreased, susceptibility to 5-fluorocytosine decreased, whereas susceptibility to fluconazole and amphotericin B increased. Our results suggest that Ch5 monosomy represents an unrecognized *C. albicans* regulatory strategy that impinges on multiple stress response pathways.

S10B:4

GENETIC INTERACTIONS BETWEEN THE BIOGENESIS OF IRON-SULFUR CLUSTERS AND FLUCONAZOLE TOLERANCE IN SACCHAROMYCES CEREVISIAE AND *CANDIDA ALBICANS*

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The survival and proliferation of fungal cells in the presence of sub-optimal concentrations of azole drugs, known as tolerance, constitutes a risk factor for the development of drug resistance. In fact, tolerance imposes a strong evolutionary pressure toward the selection and fixation of mutations allowing fungal cells to thrive despite the presence of azoles. Screening yeast libraries of dosage and mutation suppressors, we identified the biosynthesis of iron-sulfur clusters, a process occurring in mitochondria, as a major regulator of fluconazole (FLC) tolerance in *S. cerevisiae*. Increased dosage of Mge1, a nucleotide exchange factor for the mitochondrial Ssq1 chaperone, enhances the FLC tolerance of yeast while having no effect on the general susceptibility to the antifungal (no change in MIC). Overproduction of Mge1 results in stabilization of Erg11, the protein target of FLC, and to higher accumulation of ergosterol (Fiori and Van Dijck, submitted). *C. albicans* orf19.2524/orf19.10059 encodes the functional ortholog of yeast Mge1. Contrary to what we observed in baker's yeast, however, overexpression of *CaMGE1* in the reference strain SC5314 decreased FLC tolerance. A FLC-susceptible clinical strain isolated from a patient with oropharyngeal candidiasis (*1*), and characterized by a remarkable tendency to develop resistance to FLC, was found to carry a hypermorphic *CaMGE1* allele (*CaMGE1*^{G619A}) in heterozygosity. In one specific experiment, 13 stable mutants with FLC MIC ≥ 32 µg/ml were isolated upon plating 10^5 cells of this strain on drug-containing plates. In all FLC^R isolates, the *CaMGE1*^{G619A} allele was lost in favor of the wild type one by way of two independent events of mitotic recombination or gene conversion. These genetic rearrangements occurred during cells preculture in the absence of FLC and were transmitted to a small progeny of 7 and 6 daughter cells, respectively. Strikingly, loss of heterozygosity at *CaMGE1* eliminating the hypermorphic allele also occurred in two sequential drug-resistant derivatives of the same FLC-susceptible clinical strain, isolated from the same patient upon FLC treatment. Drug resistance has been attributed to mutations in *ERG11* and *TAC1*, and to the formation of isochromosome 5 in the two *in vivo* isolated mutants (*1*). We found mutations in *ERG11* known to be associated with drug resistance also in our *in vitro*-isolated mutants. However, what appears remarkable in both classes of mutants is the inverse correlation with the heterozygous *CaMGE1*/*CaMGE1*^{G619A} state. Our results suggest the existence of a complex link between *CaMGE1* and resistance to FLC in *C. albicans*. The *CaMGE1*/*CaMGE1*^{G619A} state may provide a balanced polymorphism in which the *CaMGE1*^{G619A} allele could facilitate the onset of resistance, while resulting detrimental to cell fitness once resistance is established. This model is currently under investigation.

S10B:5

AN INHIBITOR OF RIBOSOMAL BIOGENESIS HAS BROAD ANTI-FUNGAL ACTIVITY

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The lack of new antifungal compounds with unique mechanisms of action is a concern for therapeutic management of patients with fungal infections. Current antifungals are few in number and each has its inherent problems. The triazoles are fungistatic, often leading to the selection of drug-resistant isolates and the echinocandins are not of broad-specificity, with resistance also appearing in patient isolates. The apparent reasons for the lack of new development include establishing a

market sufficient in size to cover the costs of development. We have recently utilized a compound library (NIH-NCI Developmental Therapeutics) of more than 2600 small molecule compounds to identify inhibitors. An initial screen with a panel of pathogenic fungi including *Candida* species and *Cryptococcus neoformans* was performed. From this screen, a thiosemicarbazone (RAC726) demonstrated potent (0.1-0.4µg/ml) and broad antifungal activity among isolates that included fluconazole resistant *C. albicans* strains that overexpress either *CDR1* (encoding an ATP-binding cassette drug transporter) or *MDR1* (encoding a major facilitator superfamily transporter). Interestingly, synergy was demonstrated with RAC726 and fluconazole, voriconazole, or itraconazole, and with caspofungin at higher concentrations of RAC726. Furthermore, RAC726 was non-toxic to mouse and human fibroblasts. To understand the mechanism of action, we then performed transcriptome analysis of untreated and treated *C. albicans*. A Gene Set Enrichment Analysis (GESA) was applied to compare the ranked gene lists from the transcript profiles according to the change in their expression to a predefined database of 8123 gene sets. We observed significantly enriched gene sets of ribosome biogenesis functions that were down regulated due to RAC726 treatment. Accordingly, leucine incorporation was reduced in treated cells. A second large cluster of upregulated oxidative stress response genes was noted that probably represents a secondary effect of the compound on cells. In summary, we propose that RAC726 targets the fungal translational machinery and induces ROS stress. The minimum toxicity of RAC726 along with its reasonable inhibitory activity suggests further study is needed including lead optimization.

S10B:6

IN VIVO DEVELOPMENT OF FLUCONAZOLE RESISTANCE IN *CANDIDA PARAPSILOSIS* ISOLATES FROM A PATIENT WITH PERSISTENT BLOODSTREAM INFECTION AND THE DISCOVERY OF A NEW *MRR1* GENE MUTATION

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We report the first case of *in vivo* development of azole resistance in *candida parapsilosis* isolates from a patient with persistent bloodstream infection during long-term antifungal treatment (fluconazole, 200mg qd). The *Candida parapsilosis* isolates were persistently recovered from blood cultures from April 28, 2010 to July 4, 2011. The susceptibility results showed that the last isolated strain became resistant to fluconazole and voriconazole. In the present study, the isolates characterization and the previously reported resistance mechanism was studied. A total of five strains were isolated from the blood cultures of patient A (A1-A5). While two isolates from patient B (B1, B2) in the same ward and one isolate from patient C (C1) in surgical ward during the same hospitalization period were also studied for control study. The activity of fluconazole and voriconazole were confirmed by the broth microdilution (BMD) method following the CLSIM27-A3 guidelines. The microsatellite typing were performed using B5d, CP1, CP4, CP64 as the microsatellite marker to genotype these isolates. To further study the possible resistance mechanism, specific primers were used for PCR amplification of transcription factor *MRR1* which controls the *MDR1* expression. According to the susceptibility results, B1, B2, B3 and C1 strains were susceptible to fluconazole (MICs, 0.5µg/ml) and voriconazole (MICs≤0.008µg/ml). The A1-A4 isolates showed higher MICs but still susceptible to fluconazole (MICs, 2µg/ml) and voriconazole (MICs, 0.03µg/ml). However, A5 isolate was

resistant to fluconazole (MICs, 16µg/ml) and intermediated to voriconazole (MICs, 0.5µg/ml). The microsatellite typing results demonstrated that the five strains from patient A belonged to the same clone. After aligning the eight isolates *MRR1* sequences, a single nucleotide mutation was detected in A1-A4 compared with the *MRR1* sequence of the susceptible strain,T2957C/WT, which results in the replacement of a leucine by a proline (L986P). In A5 isolate, mutations were found in both alleles, T2957C/T2957C. It is likely that the detected mutation may play a key role in *MDR1* overexpression in *C. parapsilosis*. The isolates with mutation in one allele showed higher MICs than wide type isolates, and the isolates with mutations in both alleles exhibited much higher MICs than isolates with mutation in one allele. In conclusion, it is the first case report of *in vivo* induced azole resistant *Candida parapsilosis* after long-term antifungal treatment and the mutations of *MRR1* were detected. The present *in vivo* case and resistance mechanism study supported the previous Portugal's study in which the resistance of *C. parapsilosis* to different azoles could be experimentally induced *in vitro* and also the mutations of *MRR1* were detected.

S10C:1

G1/S TRANSCRIPTION FACTOR COMPLEX COMPOSITION AND FUNCTION IN *CANDIDA ALBICANS*

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The G1/S transition is a critical control point for cell proliferation, and involves essential transcription complexes termed MBF in Schizosaccharomyces pombe, or SBF and MBF in Saccharomyces cerevisiae. Swi4p and Mbp1p comprise the DNA binding elements for SBF and MBF, respectively, in S. cerevisiae, while Swi6p is a common activating component. In the fungal pathogen *Candida albicans*, regulation of the G1/S transition and cell proliferation are not well understood. We previously characterized the *C. albicans* orthologues of Swi6p, Swi4p and Mbp1p, and found that absence of Swi4p and Swi6p strongly and similarly influenced cell proliferation, expression of some G1/S-associated genes, and resulted in filamentous growth. However, absence of Mbp1p had little effect on phenotype. Others have shown that promoters of several G1/S-modulated genes in *C. albicans* contain enriched MBF-binding elements but lack the canonical SBF binding motif. Together this suggests that Swi4p and Swi6p may be components of a single MBF-like complex in *C. albicans*. However, cells lacking Swi6p and Swi4p or Swi4p and Mbp1p were viable, in contrast to the situation in S. cerevisiae, suggesting that additional factors may contribute to G1/S regulation. In order to determine the composition of the putative G1/S transcription complex in *C. albicans*, and identify additional regulators, we tandem-affinity purified Swi4p, Swi6p and Mbp1p, and identified interacting factors using Orbitrap LC/MS. Swi6p-TAP affinity-purified samples from growing yeast cells showed abundant peptides corresponding to Swi6p, Swi4p and Mbp1p. In Swi4p and Mbp1p-affinity-purified complexes, Swi6p or Swi6p and Nrm1p, respectively, were the most enriched proteins. Additional peptides were detected, but at lower levels, suggesting that Swi6p, Swi4p and Mbp1p may be core G1/S complex factors. Reciprocal co-immunoprecipitations confirmed that Swi6p interacts with Swi4p and Mbp1p, but Swi4p and Mbp1p do not physically bind. The results further question the role of Mbp1p and raise the potential for two separate G1/S complexes in *C. albicans*. We next investigated putative Swi4p targets under yeast growth conditions using genome-wide location analysis. Based

on three independent tiling arrays, Swi4p was significantly enriched at promoters of genes associated with several functions, including cell wall growth and cell division. Strikingly, the most abundant group of targets was associated with filamentous growth, and included EFG1. Northern blotting showed that EFG1 expression was moderately reduced in the absence of Swi4p. Furthermore, absence of EFG1 reduced the extent to which swi4Δ/swi4Δ cells formed long filaments. Thus, Swi4p may contribute to the regulation of EFG1 and filamentous morphogenesis, as well as the G1/S transition, suggesting that it may lie at the interface between cell cycle regulation and development in *C. albicans*.

S10C:2

A SINGLE AMINO ACID MUTATION IN IPI1 CONFERS MULTIANTIFUNGAL RESISTANCE WITHOUT AFFECTING VIRULENCE IN *CANDIDA GLABRATA*

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Recent surveillance data have revealed an increased number of *Candida glabrata* clinical isolates that display resistance to not only azoles but also echinocandins. The key mechanisms of antifungal resistance are mutations in the drug targets (e.g., *FKS* genes for echinocandins and *ERG11* for azoles) and the activation of azole efflux pumps; however, these mechanisms do not account for all of the resistant phenotypes of clinical isolates. To further understand the molecular basis of antifungal resistance, *C. glabrata* wild-type cells were continuously exposed to low concentrations of micafungin, and cells that developed an increased micafungin tolerance were selected. Surprisingly, these strains also acquired high-level resistance to azoles including fluconazole, itraconazole, and voriconazole. These phenotypes were irreversible, but no mutations were present in the *FKS* genes, *ERG11*, or the transcriptional regulator of multidrug transporter genes *PDR1*. A complementation assay using a *C. glabrata* cDNA library revealed that a single amino acid substitution in the *IPI1* gene was responsible for the acquired multiantifungal resistance, and the introduction of this mutation into wild-type *C. glabrata* reproduced all of the phenotypes. In *Saccharomyces cerevisiae*, Ipi1 is known to be a component of the Rix1 complex and functions in rRNA processing; however, the *IPI1* gene product has not yet been characterized in pathogenic fungi. Previous work in the Edlind laboratory showed that membrane sphingolipids modulate the echinocandin-Fks interaction. In particular, the accumulation of dihydrosphingosine and phytosphingosine, which are intermediates of the sphingolipid biosynthesis pathway, conferred increased micafungin susceptibility in *C. glabrata*. Conversely, markedly decreased levels of dihydrosphingosine and phytosphingosine were detected in an *ipi1* mutant, possibly accounting at least in part for the decreased micafungin susceptibility of the mutant. On the other hand, the mutant exhibited a 12-fold upregulation of *CDR1*, which encodes a multidrug efflux pump. Moreover, flow cytometric analysis using rhodamine 6G, a known substrate of Cdr1, demonstrated an increased drug efflux in the mutant. Genome-wide expression profiles determined by DNA microarray analysis suggested that Pdr1 signaling was activated in the mutant. In agreement with these findings, the deletion of *CDR1* or *PDR1* abolished azole resistance in the *ipi1* mutant. Importantly, the virulence of the mutant was comparable to that of the wild-type strain in a mouse model of disseminated candidiasis. Our results indicate that decreased susceptibility to different classes of antifungals can be induced by only a single amino acid mutation without affecting virulence, suggesting the possibility that such a mutant may emerge and cause serious diseases in clinical settings.

S10C:3

INTRACELLULAR SURVIVAL OF *CANDIDA GLABRATA* IN MACROPHAGES

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Although the pathogenicity mechanisms of *Candida glabrata* are largely unknown, immune evasion strategies likely play a key role during infection. We and others have shown that *C. glabrata* not only survives, but also replicates within macrophage phagosomes (Seider *et al.*, 2011, J Immunol 187:3072-86). By analyzing phagosome maturation markers and lysosomal tracers, we observed that fungal-containing phagosomes remain in a late endosomal stage and do not proceed to a phagolysosome. Although V-ATPase is abundant in these compartments, they remain negative for staining with the acidotropic dye LysoTracker, and phagosomal hydrolase activity is low. Neither fungal survival, nor inhibition of acidification is influenced by opsonization of *C. glabrata* cells, macrophage stimulation with IFN-γ or vitamin D3, or macrophage M1- versus M2-type differentiation. In contrast, heat killed yeasts are delivered to an acidic phagolysosome. Rising phagosomal pH with chloroquine significantly reduced fungal survival in macrophages, indicating the importance of a balanced phagosomal pH for the intra-phagosomal fate of *C. glabrata*. Western Blot analyses revealed no striking differences in activation of signaling cascades downstream of pattern recognition receptors when comparing viable and heat killed cells. This suggests that initial recognition events of fungal surface components are not responsible for triggering the delivery of viable yeast cells to non-matured phagosomes. We discovered that, similar to *C. albicans*, *C. glabrata* is able to alkalinize its extracellular environment, when growing on amino acids as sole carbon sources *in vitro*. This metabolic process may allow viable fungal cells to actively raise intra-phagosomal pH and consequently block phagosome maturation. To test this hypothesis, we are currently screening for *C. glabrata* mutants with defects in extracellular alkalinization *in vitro*. These mutants will then be analyzed for survival within macrophages and for phagosome acidification. In conclusion, our results support the concept that *C. glabrata* is well-adapted to persistence within macrophages. Active fungal processes which modulate the phagosomal pH may interfere with phagosome maturation, thereby creating a niche for *C. glabrata* replication.

S10C:4

SUPER-RESOLUTION IMAGING OF C-TYPE LECTIN SPATIAL REARRANGEMENT DURING FUNGAL PARTICLE RECOGNITION

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Dendritic cells express DC-SIGN and CD206, C-type lectins (CTLs) that bind a variety of pathogens and facilitate their uptake for subsequent antigen presentation. Both proteins form domains on the tens of nanometer to micrometer scale in the plasma membrane of naïve human immature dendritic cells (DC). We quantitatively analyzed the spatiotemporal kinetics of CTL recruitment to host-fungal particle (zymosan) contact sites using confo-

cal microscopy and 3D reconstruction of the contact area. Dramatic DC-SIGN and CD206 recruitment and co-localization occurs in the first hour of exposure to fungal particles followed by sustained concentration of receptors in the contact sites out to four hours. Receptor recruitment amplitude and kinetics are sensitive to the nature of the fungal particle being recognized, with the *Candida* species pathogens rapidly recruiting and co-localizing significantly more receptor to the contact than *S. cerevisiae* or zymosan particles (*S. cerevisiae*-derived). Quantitative analysis of super-resolution imaging of both DC-SIGN and CD206 demonstrates a shift to clustering over length scales longer than resting CTL nanodomains within contact regions relative to membranes on the same cell but outside of the fungal contact. We conclude that if ordering on the scale of naïve DC nanodomains exists, domains must be packed closely enough to achieve inter-domain edge separations of < 30 nm. Furthermore, the Getis G statistic, a measurement of local clustering, determines that contact sites possess more nanostructure on the ~250-350 nm scale than non-contact regions, while nanostructure below ~250 nm predominates in non-contact regions. This study provides evidence of local receptor spatial rearrangement on the nanoscale that occurs in the plasma membrane upon pathogen binding. Such receptor rearrangement in the plane of the plasma membrane may be significant for achieving receptor densities and cross talk between receptors to drive productive fungal immunity. The authors acknowledge the following funding sources as instrumental to the conduct of this research: NIH 5P50GM085273 (A.N., M.I), GM-041402 (K.J., N.T.), GM-064346 (K.J.), NSF MCB-0641087 (N.T), and a UNM Medical Trust Fund and Tobacco Settlement RAC grant (A.N).

S10C:5

PROBING THE EVOLUTIONARY POTENTIAL OF *CANDIDA ALBICANS* WITH ARTIFICIALLY ACTIVATED TRANSCRIPTION FACTORS

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During colonization and infection of its human host, *Candida albicans* adapts to changes in the environment by reversibly altering its morphology and adjusting its gene expression pattern. In addition, *C. albicans* can generate genetic variants that are better adapted to new conditions. A prominent example is the evolution of drug-resistant strains during antimycotic therapy. Resistance against the widely used antifungal drug fluconazole, which inhibits ergosterol biosynthesis, is frequently caused by gain-of-function mutations in the zinc cluster transcription factors Mrr1, Tac1, and Upc2. These mutations result in constitutive activity of the transcription factors and overexpression of their target genes, including multidrug efflux pumps (MDR1 and CDR1/CDR2) and ergosterol biosynthesis genes. We found that Mrr1, Tac1, and Upc2 can also be rendered constitutively active by C-terminal fusion with the heterologous Gal4 activation domain, suggesting that this may represent a general strategy for the artificial activation of zinc cluster transcription factors. We therefore created a complete library of all 82 *C. albicans* zinc cluster transcription factors in a potentially hyperactive form, which were expressed in the wild-type reference strain SC5314. Screening of this library resulted in the discovery of novel regulators of morphogenesis and resistance to drugs and oxidative stress. Among these were Mrr2, which controls the expression of the major multidrug efflux pump CDR1, as well as several transcription factors that induced the expression of FLU1, an efflux pump that is required for tolerance of the antifungal peptide histatin 5. In many cases, overexpression or deletion of the wild-type gene, two commonly used methods to analyze gene function, did not cause a corresponding

phenotypic alteration. Artificial activation is therefore a highly useful and complementary method to elucidate the role of zinc cluster transcription factors of unknown function. As *C. albicans* frequently acquires natural gain-of-function mutations in these transcriptional regulators under selective pressure, the comprehensive library of strains containing hyperactive forms of all its zinc cluster transcription factors is a valuable resource to probe the evolutionary potential of this fungus when it faces novel environmental challenges.

S10C:6

IRON BINDING NEGATIVELY IMPACTS THE FUNGICIDAL ACTIVITY OF SALIVARY PROTEIN HISTATIN 5 AGAINST *CANDIDA ALBICANS*

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The salivary protein Histatin 5 (Hst 5) is fungicidal towards *Candida albicans*, the causative agent of oropharyngeal candidiasis (OPC) in immunocompromised individuals. Binding of Hst 5 to *C. albicans* cell wall and its subsequent uptake is an important step in this killing since Hst 5 targets have been known to be intracellular. However, its activity in saliva is compromised by salivary protease-mediated degradation and interaction with salivary salts. Hst 5 has also been shown to bind various metals in saliva, namely Cu, Zn, and Ni. Iron is one of the most abundant metals present in saliva and total Fe binding capacity of whole saliva ranges from 0.03-15mg Fe/ml. Surprisingly, interaction of Hst 5 with Fe has not been studied previously. **Objective:** To investigate potential iron-Hst 5 interaction and its role in Hst 5 function. **Methods:** Iron-binding ability of Hst 5 was analyzed by Circular Dichroism (CD) in Tetrafluoroethylene (TFE) after titration with increasing amounts of metal. Candidacidal assay was performed using a micro dilution plate technique to examine *C. albicans* sensitivity to Hst 5 after incubating Hst 5 with different concentrations of iron (Fe-Hst 5) for 1 h. FITC labeled Hst 5 was also incubated with various iron concentrations and the effects on peptide binding and uptake by *C. albicans* were quantified using FACScan. Protease stability of Hst 5 in the presence and absence of iron was measured by mass spectrometry following trypsin digestion. RNA-sequencing was used to compare gene expression between Hst 5 treated and untreated *C. albicans* cells. **Results:** CD results showed that Hst 5 can bind up to 10 equivalents of iron. This binding resulted in loss of alpha-helical secondary structure that is normally observed for Hst 5 in TFE. A significant decrease in the candidacidal ability of Fe-Hst 5 was observed, with increasing iron concentrations being inversely proportional to Hst 5 killing activity. Binding and uptake assays showed that the decrease in killing was likely a result of reduced binding and uptake (10 fold reduction) of Fe-Hst 5 by *C. albicans* cells. Protease stability analysis showed that binding iron protects Hst 5 from proteolytic digestion, offering protection against trypsin-like salivary protease activity. Furthermore, RNA-sequencing results identified changes in iron uptake genes in Hst 5 treated *C. albicans* cells. **Conclusion:** We show for the first time that Hst 5 can bind iron. Furthermore, our findings suggest that consequences of this binding not only affect candidacidal ability and proteolyic stability of Hst 5, but also contribute to a novel killing mechanism involving interference with cellular iron metabolism. Thus salivary iron levels may play an important role in the antifungal activity of innate and exogenously applied antimicrobial peptides during oral fungal infections.

S11:1

CHARACTERIZATION OF EXTRACELLULAR MATRIX MANNAN PRODUCTION, SECRETION, AND ROLE IN *CANDIDA ALBICANS* BIOFILM ANTIFUNGAL RESISTANCE

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Background: *Candida albicans* biofilms are highly drug resistant, due largely to the extracellular matrix surrounding the biofilm cells. The matrix components involved in providing protection from antifungals are unknown, as the specific biochemical composition of the matrix has not been defined until recently. Our group identified the polysaccharide α-mannan as the most abundant carbohydrate component of the *C. albicans* extracellular matrix, and found it exists predominantly as an α-1,6 linked mannose backbone with α-1,2 linked mannose branches. **Methods and Results:** We sought to characterize the production and delivery of matrix mannan, and hypothesized that, as it is present in such high quantities, it may impact the biofilm drug resistance phenotype. To address this hypothesis, we constructed a library of 43 homozygous deletion mutants for genes with proven or putative mannan modification function. Mutant biofilms were screened for matrix mannan content using a combination of gas chromatography and ELISA approaches. 16 of the 43 mutants tested had reduced levels of matrix mannan, ranging from 20-60% reduction compared to wildtype. Most of these were mutants lacking α-1,6 or α-1,2 mannosyltransferases. To investigate the mechanism of mannan delivery to the matrix, we treated biofilms with the secretory pathway inhibitors brefeldin A and monensin. Interestingly, matrix mannan levels were reduced up to 60%, but not eliminated, possibly indicating a compensatory mechanism for matrix mannan secretion. We next examined if matrix mannan plays a role in biofilm drug resistance. When biofilms were treated with α-mannosidases or tunicamycin, an inhibitor of N-glycosylation, levels f susceptibility to the antifungal fluconazole increased. Additionally, 7 of the mutant strains with reduced matrix mannan also had increased susceptibility to fluconazole relative to WT. These same mutant biofilms, when treated with ^{3}H-fluconazole in a sequestration assay, retained lower levels of drug in the matrix. The previously demonstrated impact of another matrix component, β-1,3 glucan, led us to hypothesize that mannan contributes to the structural integrity of the matrix in a cooperative manner. hus, we also measured other matrix components in mannan mutants and unicamycin-treated biofilms. Remarkably, the majority of mutants with educed levels of mannan also had reduction of matrix β-1,3 and β-1,6 lucan, suggesting a functional interaction between matrix polysaccharides. **Conclusions:** We have shown that mannan, an abundant matrix component, is critical for antifungal resistance and overall matrix function. Collectively, these findings provide insight into the newly characterized *C. lbicans* biofilm extracellular matrix, an important factor in biofilm pathogenicity.

S11:2

PATHOGEN RESPONSE TO THE ENVIRONMENT OF INVASIVE INFECTION

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Background. Gene expression dynamics have provided foundational insight into almost all biological processes. Here we apply both knowledge-based and discovery-based approaches to deduce the signals and pathways that drive pathogen gene regulation during invasive *Candida albicans* infection. Methodology/Principal Findings. Our knowledge-based approach employs nanoString probes for numerous environmentally responsive genes; our discovery-based approach employs nanoString probes for all predicted *C. albicans* transcription factor genes. Infection is associated with rapid induction of zinc

and iron limitation response genes, as well as genes characteristic of invasive hyphal cells. There is a more gradual induction of oxidative stress response genes, which coincides with expression of host inflammatory cell reporters. We identify new virulence regulators among highly expressed transcription factor genes, and profiling of attenuated mutants during infection reveals a novel zinc acquisition pathway. Finally, we find that genes induced by the antifungal drug caspofungin during infection correspond to genes that are repressed at the onset of infection.
Conclusions/Significance. The pathogen response circuitry is tailored uniquely during infection, with many relevant regulatory relationships that are not evident during growth in vitro. Notably, repression of a key drug response pathway during infection may reveal the pathogen's Achilles' heel. Our findings support the principle that virulence is an emergent property at the level of gene expression, a property manifested only in the context of host-pathogen interaction.

S11:3

APPEARANCE OF A NOVEL CTG-CLADE SPECIFIC HISTONE H3 VARIANT REGULATES A RECENTLY EVOLVED TRANSCRIPTION CIRCUIT FOR BIOFILM DEVELOPMENT IN *CANDIDA ALBICANS*

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Apart from the core histones - H2A, H2B, H3 and H4 - non-allelic variants of histones play important roles in controlling global gene expression. Among them histone H3 variants, in particular, play a significant role in genome indexing by determining the status of transcription of a specific gene under a particular growth condition. Though *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* possess a single gene coding for only one form of core histone H3, species belongs to the CTG clade, carry three genes expressing two types of core histone H3 variants. In *Candida albicans,* two genes encode an identical protein (H3.major), whereas the other one codes for a variant (H3.minor) with three changes in the amino acid sequence. Based on RT-PCR, subcellular localization and western blot analysis, we show that both histone H3 variants are transcribed and translated in *C. albicans*. A homozygous null mutant of *H3.minor* is viable suggesting its non-essentiality for survival. Global transcriptome analysis of this null mutant suggests an alteration of gene expression associated with several pathways. Intriguingly, several genes involved in filamentation and biofilm formation are up-regulated in the null mutant as compared to the wild type. In depth analysis by qPCR confirms that adhesion genes (such as *ALS3* and *HWP1*) as well as several biofilm-induced genes are over-expressed in the mutant as compared to the wild-type. Quantitative analysis of growth at various stages of biofilm development by confocal microscopy confirms that biofilm formation is faster in the null mutant as compared to the wild-type. In addition, based on the coloration of wild type and mutant colonies on Chromagar, we reveal that mutants are more invasive in nature as compared to the wild type. This invasive property of *H3.minor* mutant is found to be conserved across *Candida* species. Taking together all these observations, we propose that this minor histone H3 is probably involved in creating repressive chromatin for biofilm gene expression during planktonic growth in *C. albicans*.

S11:4

SMALL MOLECULE INHIBITORS OF *CANDIDA ALBICANS* FILAMENTATION THAT IMPAIR BIOFILM FORMATION ON MULTIPLE SURFACES

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By high-throughput phenotypic screening of small molecules, we identified compounds that

inhibit adhesion of *C. albicans* to polystyrene. Our lead candidate compound also inhibits binding of *C. albicans* to cultured human epithelial cells, the yeast-to-hyphal morphological transition, induction of the hyphal-specific HWP1 promoter, biofilm formation on silicone mesh, pathogenesis in a nematode infection model, and alters fungal morphology in a mouse mucosal infection assay. We termed this compound filastatin, based on its strong inhibition of filamentation, and use chemical genetic experiments to show that it acts downstream of multiple signaling pathways (Fazly et al., PNAS 2013). We will present new data on the ability of filistatin and related compounds to affect Candida biofilm formation on a variety of surfaces.

S11:5

MODULATION OF *CANDIDA ALBICANS* VIRULENCE BY ORAL COMMENSAL BACTERIA

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Candida albicans is a commensal colonizer of the alimentary tract mucosa, where it coexists with diverse bacterial communities. In the human oral cavity Mitis group streptococci are among the most ubiquitous and abundant colonizers. Being notoriously resistant to broad spectrum prophylactic antibiotics, these organisms are frequently co-isolated with *C. albicans* as suspected pathogens from the blood of immunosuppressed patients, or the sputum of cystic fibrosis patients. However it is not known whether their co-existence leads to pathogenic synergy. Pathogenic interactions of *C. albicans* with members of this bacterial group (*Streptococcus oralis*, *Streptococcus sanguinis*, *Streptococcus gordonii)* were investigated using two novel mucosal disease models. 1) A flow cell model that allows mucosal biofilm formation on oral and esophageal organotypic cultures, under salivary flow. 2) An oral co-infection model in immunosuppressed mice. When inoculated alone, streptococci did not form mucosal biofilms in either model and were cleared efficiently by all mice. When co-inoculated, *C. albicans* promoted the ability of streptococci to form mucosal biofilms and to colonize the mouse oral and gastrointestinal tract, while it did not affect their growth planktonically. Consistent with this, *S. oralis* and *C. albicans* co-infection in mice significantly augmented the frequency and size of oral thrush tongue lesions. Although streptococci did not affect growth of *C. albicans*, they increased its ability to invade organotypic models of the oral and esophageal mucosae under salivary flow, and promoted deep organ dissemination in mice. *C. albicans* genes associated with mucosal adhesion and invasion (hwp1, als3, rim101, ssa1) were strongly upregulated by the presence of streptococci both in vitro and in vivo. During co-infection *Candida* invaded the oral mucosa mostly paracellularly and reduced E-cadherin protein levels in mucosal epithelial junctions significantly more, compared to *C. albicans* alone. Co-infection with *S. oralis* and a *rim101-/- C. albicans* mutant showed that mucosal invasion and E-cadherin degradation is dependent on this transcriptional pathway. Whole mouse genome tongue microarray analysis revealed that when compared to animals infected with one organism, the doubly-infected animals had genes in the major categories of neutrophilic response/chemotaxis/inflammation significantly upregulated, indicative of an exaggerated inflammatory response. This response was partly dependent on TLR2 signaling since oral lesions, transcription of proinflammatory genes and neutrophil infiltration, were attenuated in $TLR2^{-/-}$ animals. In summary, our work identified a previously unrecognized, multifactorial pathogenic synergy between oral commensal bacteria and *C. albicans* with implications for the pathogenic potential of *C. albicans* in the upper gastrointestinal tract.

S12:1

C-TYPE LECTINS AND ANTI-CANDIDA IMMUNITY

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The last few decades has seen a tremendous increase in our understanding of the mechanisms underlying the development of protective anti-fungal immunity. Key among these discoveries is the identification of pattern recognition receptors (or PRRs) expressed by immune cells which recognise conserved fungal components, such as beta-glucans and mannans. Recognition of these structures by PRRs, particularly by members of the C-type lectin receptor (CLR) family, triggers intracellular signalling cascades that initiate a variety of cellular and inflammatory responses, and induce the development of pathogen specific adaptive immunity. We now understand that innate recognition by CLRs is essential for the development of protective immunity to these pathogens. In this presentation, I will cover the key developments in our understanding of the function and roles of these receptors in the context of anti-Candida immunity, highlighting recent achievements.

S12:2

IS MAPK SIGNALLING IN CANDIDA ALBICANS INVOLVED IN THE COLONIZATION OF MOUSE GUT?

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S12:3

C. ALBICANS SOD5 REPRESENTS A NEW FAMILY OF EXTRACELLULAR COPPER ONLY SUPEROXIDE DISMUTASE ENZYMES REQUIRED FOR PROTECTION FROM HOST DERIVED OXIDATIVE STRESS

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Copper/zinc superoxide dismutase (Cu/Zn SOD) enzymes are key intracellular antioxidants that catalyze the disproportionation of superoxide radicals to hydrogen peroxide and water. Cu/Zn SODs function as homodimers with each monomer containing a catalytic copper ion, a zinc ion, an intramolecular disulfide, an invariant arginine, and an electrostatic loop. In addition to intracellular SOD1, *C. albicans* genome encodes three genes that are predicted to be extracellular GPI-anchored Cu/Zn SODs. These SODs have been shown to be important virulence factors (Fradin et al, 2005, Frohner et al, 2009). Our studies focus on SOD5, since it has been shown to be the most important of the extracellular SODs for protection from oxidative stress during infection (Fradin et al, 2005, Frohner et al, 2009). In collaboration with the Hart lab, we have determined the crystal structure of SOD5. We show that SOD5 retains the conserved catalytic copper ion, the intramolecular disulfide, and invariant arginine found in Cu/Zn SODs; however, it functions as a monomer and lacks residues that coordinate the zinc ion and an electrostatic loop. This raised the question as to the true function of SOD5. Through pulse radiolysis measurements, we found SOD5 is indeed a SOD that reacts with superoxide at rates that approach diffusion limits comparable to conventional

Cu/Zn SODs. We show that SOD5 expressed in *C. albicans* accumulates outside the cell in the apo form with an oxidized disulfide and can be rapidly activated with the addition of extracellular copper. Such activation by extracellular copper may be important in the "copper burst" of host immunity. We have found that extracellular copper only SODs similar to SOD5 are found in many pathogenic fungi. Thus, SOD5 is the representative member of a new family of eukaryotic extracellular copper only SODs evolved for pathogen defense.

S12:4

C. ALBICANS ECE1 IS A NOVEL PORE-FORMING TOXIN CRITICAL FOR ACTIVATING THE EPITHELIAL CELL 'DANGER' RESPONSE

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A host's ability to discriminate between commensal and pathogenic microbes is key to homeostasis and immunity. Previously, we demonstrated a MAPK-specific mechanism enabling epithelial cells (ECs) to discriminate between invasive and colonising C.albicans by responding specifically to hyphae. Here, we show this EC 'danger' response mechanism is targeted against the hyphal protein, Ece1p. Unlike wild-type C.albicans, infection of ECs with an ece1Δ/Δ null mutant does not activate the 'danger' response (p38/c-Fos, ERK1/2/pMKP1, cytokines) or cause damage, despite growing as hyphae and invading ECs. In contrast, overexpression of ece1 in an avirulent non-filamentous mutant restores activation of the 'danger' response. Further, stimulation of ECs with recombinant Ece1p induced damage and the 'danger' response. Analysis of EC responses to fragments of Ece1p identified the region responsible for Ece1p activity. Stimulation of ECs with this peptide (Ece1p3) resulted in phosphorylation of MKP1 as well as activation of all three MAPK pathways, NF-kB and Akt signaling, and cytokine release. Further, stimulation of ECs and other cells with Ece1p3 induced release of the damage marker, LDH. Confirmation of the damage-inducing effects of Ece1p3 was provided when treatment of artificial phospholipid bilayers with this peptide resulted in the formation of pores, whilst scanning electron microscopy revealed the presence of ring-like structures (reminiscent of pores) in Ece1p3-treated ECs, suggesting that Ece1p3 may function as a pore-forming toxin. To identify the clinical relevance of these data we investigated the role of Ece1p in mucosal infections. Utilizing a zebra fish swim bladder model of invasion and infection, we demonstrated that the ece1Δ/Δ null mutant shows reduced ability to induce cell damage in the swim bladder or to recruit neutrophils to the infected organ. Likewise, in a murine model of oropharyngeal candidiasis, ece1Δ/Δ was unable to successfully infect and appeared avirulent. Finally, intravenous inoculation of zebra fish with ece1α/α demonstrating increased survival compared with wild-type infected fish. These data provide evidence that Ece1p is a virulence factor for mucosal candidiasis and could be targeted by ECs to enable discrimination between yeast and hyphal forms of *C. albicans* via activation of the MAPK 'danger' response. The data also suggest that Ece1p can function as a pore forming toxin (the first to be identified in a human pathogenic fungi), acting to damage cells. Further the activity of this protein can be localized to a 32 amino acid region. Ece1p and its active region, Ece1p3, therefore represent a novel target for future therapies to treat chronic candidiasis, as well as a target for the development of an effective mucosal vaccine.

S12:5

BLOCKING TWO-COMPONENT SIGNALLING ENHANCES *CANDIDA ALBICANS* VIRULENCE AND STIMULATES A NOVEL MECHANISM TO SURVIVE SAPK HYPER-ACTIVATION

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The Ypd1 phosphorelay protein is a vital constituent of fungal two-component related signal transduction pathways. Inactivation of Ypd1 in *Saccharomyces cerevisiae* results in programmed cell death due to the sustained activation of the 'p38-related' Hog1 stress-activated protein kinase (SAPK). Furthermore, two-component signalling proteins are not found in animals and thus Ypd1 is considered to be a prime antifungal target. However, here we challenge this conclusion and report that the major fungal pathogen of humans, *Candida albicans*, can survive sustained activation of the Hog1 SAPK triggered by loss of Ypd1. Moreover, drug-induced down-regulation of Ypd1 during a mouse model of systemic infection enhances the virulence of *C. albicans*. We find that *C. albicans* cells lacking Ypd1 adapt to long-term SAPK activation by significantly lowering *HOG1* mRNA levels. Strikingly, this Hog1-deficient state is rapidly reversed following stress exposure as Hog1 levels and stress resistance are restored. Thus *C. albicans* has developed unprecedented mechanisms to actively reduce cellular Hog1 levels to allow survival of long-term Hog1 activation, while retaining Hog1-mediated adaptive responses. Collectively, our findings negate the generality of targeting Ypd1 proteins in antifungal strategies and reveal a novel cellular adaptation mechanism to sustained SAPK activation.

Poster Abstracts

1A

ROLES OF THE WOR2 TRANSCRIPTION FACTOR IN N-ACETYLGLUCOSAMINE-INDUCED WHITE-OPAQUE SWITCHING AND FILAMENTATION IN *CANDIDA ALBICANS*

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Candida albicans, a major opportunistic fungal pathogen of humans, can spontaneously undergo white-to-opaque switching, a prerequisite of mating. The phenotypes of white and opaque cells are heritable and bistable. The zinc-finger transcription factor Wor2 (**W**hite **O**paque **R**egulator **2**) has previously been identified as an essential regulator of white-to-opaque switching under standard laboratory culture conditions. Deletion of *WOR2* locks cells in the white phase. In this study, we report that N-acetylglucosamine (GlcNAc) can induce white-to-opaque switching in the *wor2/wor2* null mutant and stabilizes the opaque phenotype of *C. albicans*. Moreover, overexpression of *RAS1V13* (the activating form of *RAS1*) hypersensitizes white cells of the *wor2/wor2* mutant to GlcNAc. These results suggest that Wor2 is not required for opaque cell formation at least under some culture conditions. We propose that GlcNAc rewires the transcriptional regulating circuitry of white-opaque switching in *C. albicans*. We have further found that the *wor2/wor2* null mutant undergoes more robust filamentation in response to a numble of inducers. The differential abilities of the wild type and *wor2/wor2* mutant in filamentation may be related to their white-to-opaque switching ability.

2B

COMPARATIVE EVOLUTION OF MORPHOLOGICAL REGULATORY MECHANISMS IN *CANDIDA* SPECIES

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Morphological transitions play an important role in virulence and virulence-related processes of a wide variety of pathogenic fungi, including *Candida albicans*, the most frequently isolated human fungal pathogen. While environmental signals, transcriptional regulators and target genes associated with *C. albicans* morphogenesis are well-characterized, considerably little is known about morphological regulatory mechanisms, and the extent to which they are evolutionarily conserved, in the less pathogenic and less filamentous non-*albicans Candida* species (NACS). We have identified specific optimal filament-inducing conditions for three NACS (*C. tropicalis*, *C. parapsilosis* and *C. guilliermondii*), which are very limited, suggesting that these species may be adapted for niche-specific filamentation in the host. While many *C. albicans* filament-specific genes are evolutionarily conserved in NACS, we have found that only a subset of selected genes was induced upon filamentation in *C. tropicalis*, *C. parapsilosis* and *C. guilliermondii*. One of the genes showing conserved expression was *UME6*, which encodes a key filament-specific transcriptional regulator of *C. albicans* hyphal development. Interestingly, constitutive high-level expression of *UME6* was sufficient to drive increased filamentation and biofilm formation, as well as partly restore conserved filament-specific gene expression in both *C. tropicalis* and *C. parapsilosis*. This finding suggests that evolutionary differences in filamentation ability among pathogenic *Candida* species may be partially attributed to alterations in the expression level of a

conserved filamentous growth machinery. In contrast to *UME6*, *NRG1*, encoding an important repressor of *C. albicans* filamentation, was down-regulated in filament-inducing vs. non-inducing conditions only in *C. albicans*, but not in the three NACS. In addition, deletion of *NRG1* resulted in enhanced filamentous growth of *C. tropicalis* and *C. parapsilosis*, but not *C. guilliermondii*, suggesting that this regulator plays only a partly conserved role in controlling NACS filamentation. Overall, our results suggest that *C. albicans* morphological regulatory mechanisms are partially conserved in NACS and have evolved to respond to more specific sets of host environmental cues.

3C

SYSTEMATIC INVESTIGATION OF BIOLOGICAL CIRCUITS THAT COUPLE CELL GROWTH AND DIVISION IN THE OPPORTUNISTIC YEAST CANDIDA ALBICANS

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The basis for commitment to cell division in late G1 phase, called Start in yeast and the Restriction Point in metazoans, is a critical but still poorly understood aspect of eukaryotic cell proliferation. All eukaryotic cells must grow to a critical cell size before commitment to division occurs. This size threshold couples cell growth to division and thereby establishes long-term size homeostasis. So far, mechanisms involved cell size homeostasis in fungal pathogens are not known. A comprehensive collection of Candida albicans mutants (representing more than 60% of non-essential genome) was screened for cell size defect using a Z2-Coulter Counter (Beckman). We determined cell size distributions for the complete set of 3000 gene deletion strains and identified 200 abnormally small or large mutants. Our screen uncovers conserved mechanisms that were previously reported in S. cerevisiae and metazoans, in addition to novel regulatory circuits that govern critical cell size at Start specifically in C. albicans. We also found that genes encoding component of the stress-activated protein kinases Hog1, including HOG1, PBS2, SSK2, YPD1 and SLN1 are required for cell size homeostasis. All aspect of Start, including the activation of G1-cyclins, SBF-dependent transcription, bud emergence and DNA replication initiation were accelerated in hog1 mutant implying that Hog1 pathway is a negative regulator of Start. The transcriptional profile of G1-elutrated cells of hog1 demonstrated that Hog1p is required for proper activation of genes of different functional categories related to both mitochondrial and cytosolic translation (ribosome biogenesis, processing of rRNA and tRNA charging). Other regulators of cell size homeostasis will be discussed and a comprehensive genetic connectivity with cell cycle and growth regulators will be shown. From an evolutionary perspective, our study also provided a framework to compare and understand how regulatory pathways that couple cell growth to cell division evolve especially in a context of a pathogenic fungus compared to saprophytic fungi. We also reported the rewiring of many regulatory circuits that control cell size between C. albicans and S. cerevisiae suggesting that cell size control is plastic and species-specific and could be druggable in pathogenic fungi.

4A

ALTERNATIVE EVOLUTIONARY ENDPOINTS IN HYPOXIC REGULATION OF CANDIDA GLABRATA AND RELATED SPECIES.

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The ability to respond to hypoxia (low oxygen) is important for both pathogenic and non-

pathogenic species. One of the most important responses is the upregulation of ergosterol synthesis genes in hypoxic conditions. Expression of ergosterol genes also determines sensitivity to azole drugs. In filamentous fungi (e.g. *Aspergillus fumigatus*) expression of ergosterol genes is regulated by SREBPs (Sterol Regulatory Element Binding Proteins), whereas in *Candida albicans* and *Saccharomyces cerevisiae* this role is carried out by the Upc2 transcription factor. In the *Saccharomyces* clade (e.g. *S. cerevisiae*, *Candida glabrata*, *Naumovozyma castellii*) there are two paralogs of *UPC2* (*UPC2* and *ECM22*) resulting from the whole genome duplication (WGD) event. We used a combination of gene knockouts and RNA-seq analysis to explore the roles of *UPC2* and *ECM22* in these species. Whereas Upc2 is the main regulator of ergosterol genes in *S. cerevisiae*, we find that this role is taken by Ecm22 in *N. castellii*. Upc2 and Ecm22 most likely sense oxygen levels via lowering sterol levels. However, hypoxic regulation in *S. cerevisiae* also responds to heme levels, via the activity of the Hap1 transcription factor, and the Rox1 repressor. The role of Rox1 in hypoxic regulation has only been explored in *S. cerevisiae*. We found that *ROX1* underwent an ancient gene duplication that pre-dates the WGD in the *Saccharomyces* clade. During subsequent evolutionary events, in some species only *ROX1* was retained (e.g. *S. cerevisiae*), in some only its paralog *ROX2* was retained (e.g. *Candida albicans*), and in others, both *ROX1* and *ROX2* were retained (e.g. *N. castellii*). We find that the *ROX1* paralog retains a role in hypoxic regulation, whereas *ROX2* has other functions. However, in *C. glabrata* an unusual event has occurred, and *ROX2* replaces *ROX1* as the hypoxic regulator. Our approach emphasizes the power of comparative analysis of fungal genomes for exploring the evolution of transcriptional regulation, particularly in relation to the hypoxic response and drug resistance.

5B

THE DISCORDANCE BETWEEN EPIDEMIOLOGICALLY RELATED INVASIVE AND FLUCONAZOLE RESISTANT *CANDIDA TROPICALIS* ISOLATES HIGHLIGHT THE NECESSITY TO FURTHER OPTIMIZE, STANDARDIZE AND VALIDATE *CANDIDA TROPICALIS* MULTILOCUS SEQUENCE TYPING

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The utility of *Candida tropicalis* Multilocus sequence typing (MLST) remains unsettled because of the high degree of sequence type difference demonstrated in strains with close epidemiological links. We obtained 48 *C. tropicalis* isolates from the China Invasive Fungal Surveillance Net (CHIF-NET) 2010 study, including a subset of a rare cluster of six fluconazole resistant isolates from the same intensive care unit of a northeast China hospital. We applied MLST to these epidemiologically linked isolates and made comparison to international and regional *C. tropicalis* strains. Primer pairs were redesigned because of the PCR failure using published primers. MLST discriminated 40 diploid sequence types (DSTs) among 48 isolates, including 36 novel DSTs, while *XYR1* gene showed the highest discriminatory power. Following review of medical records and isolates' phylogenetic analysis results, three of the six geographic clustered fluconazole resistant isolates were interpreted as in-hospital transmission, whilst the other three were not. When the study isolates' DSTs were compared to previously reported *C. tropicalis* isolates, there was poor type alignment with regional strains. We recommend that if at least four of six homologous MLST target genes, with the remaining variant alleles demonstrating limited heterozygous/homozygous difference, then clinical and epidemiological data should be incorporated for a comprehensive interpretation

of these results. The failure of the *C. tropicalis* MLST method to relate highly epidemiologically linked isolates within the study subset, as well as the absence of meaningful links to the regional strains, suggests the current MLST method should be critically reassessed.

6C

SPECIES DISTRIBUTION AND ANTIFUNGAL SUSCEPTIBILITY PROFILES AMONG CANDIDA SPP. IN RUSSIA

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Objectives: To study pathogens of invasive candidiasis (IC) distribution and the susceptibility to fluconazole and voriconazole. **Methods:** A total of 134 clinical isolates from patients with IC from different hospitals of Russia during 2011-2013 were studied. *Candida* spp. were identified using MALDI-TOF mass-spectrometry and DNA-sequencing of D1/D2 regions of DNA. In vitro susceptibilities to fluconazole and voriconazole were studied by CLSI M27-A3 method (microdilution) with interpretation criteria CLSI M27-S4 (December, 2012). **Results:** Etiologic agents of IC included 8 species: *C.albicans* (52,3%), *C.parapsilosis* (16,4%), *C. glabrata* (13,5%), *C.tropicalis* (10,4%), *C.krusei* (3,7%), *C.guilliermondii* (1,5%), *C.pararugosa* (1,5%), and *C.dubliniensis* (0,7%). In vitro to fluconazole were susceptible 102 (77,9 %) strains, susceptible dose dependent (SDD) - 19 (14.5%), and resistance (R) - 10 (7.6%), MICs for one strain of *C.dubliniensis* and two strains of *C.pararugosa* were respectively 0.125 µg/ml and 1 µg/ml. In *Candida albicans* were susceptible 68/70 (97.2%) and R - 2 (2.8%). In *Candida* non-*albicans* were susceptible 34 (55.7%) strains; SDD were 19 (31.2%) and R - 8 (13,1%). All 18 *C.glabrata* isolates were SDD to fluconazole. Eighteen *C.parapsilosis* strains were identified as susceptible, SDD - 1 strain, R - 3. All 14 *C.tropicalis* and 2 *C.guilliermondii* strains were susceptible to fluconazole. We found that 133 (99.3 %) strains were susceptible and 1 (0,7%) strain was SDD (*C.tropicalis*) to voriconazole. **Conclusion:** The most common species among etiologic agents of invasive candidiasis in Russia was *C. albicans* (52,3%) followed by *C.parapsilosis* (16,4%) and *C. glabrata* (13,5%). Using new interpretative criteria of CLSI M27-A3 we found that 77,9 % *Candida* spp. strains from patients with invasive candidiasis in Russia were susceptible to fluconazole and 99.3% - to voriconazole.

7A

PHENOTYPIC ALTERATIONS DERIVED FROM LOSS OF HETEROZYGOSITY IN GENETICALLY MANIPULATED AND LABORATORY MAINTAINED CANDIDA ALBICANS STRAINS

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During our characterization of the KU70 ORF from C. albicans we found that both heterozygous (KU70/ku70-α) and homozygous (ku70-α/ku70-α) disruptants generated from strain CAI4 using the URA blaster exhibited MMS- and thermo-sensitivity when compared to strain CAF2-1, regardless they were Uri+ or Uri-. We found that strain CAI4 (Uri-) is MMS- and temperature-sensitive compared to its parental strain CAF2 (Uri+) and therefore disruptants in any gene in CAI4 or its derivatives display both phenotypes. In addition, MMS-induced filaments from strain CAF2-1 were shorter than from strain CAI4. CAI4 strain did not regain the MMS- or the thermoresistance of CAF2-1 following reintroduction of URA3 in its own locus. From the three Uri- strains generated by Fonzi and Irwin (1993), only CAI4 was MMS sensitive, whereas the other two, CAF4-2 and CAF3-1 were MMS

resistant. However, CAF3-1 displayed severe thermosensitivity, higher than CAF4-2 and CAI4, suggesting that both phenotypes are unlinked. Strain CAI-4, but not CAF3-1 or CAF4-2, is homozygous for a short region of Chr3R (Abbey et al., 2011) suggesting that this homozygosity is important for the MMS sensitivity of strain CAI4. Accordingly, induction of LOH at Chr3R of CAF4-2 yield in derivatives with the MMS sensitivity of CAI4. In CAI4, the Chr3R homozygous region includes ORFs SNF5 and POL1, whose deletants in S. cerevisiae are MMS sensitive. However, strains hemizygous for each of these genes did not show MMS sensitivity regardless the allele disrupted, indicating that MMS sensitivity is either unrelated to SNF5 and POL1 or it needs the simultaneous presence of two weak alleles of one of them. Spontaneous or induced homozygosis of a large fragment of Chr2L increased the MMS sensitivity of CAI4 and CAF4-2 derivatives with segmental homozygosis in Chr3R whereas the same LOH event did not alter MMS sensitivity of strain CAF4-2 (Chr3R heterozygous), suggesting the existence of "sign epitasis". Our results also call for caution during the phenotypic characterization of the Uri+ version of C. albicans mutants using the CAF2-1 strain as a wild type control. ACKNOWLEDGEMENTS: This research was supported by grants SAF2010-19848 from Ministerio de Ciencia e Innovación (Spanish Government) and Ayuda a grupos CCV014 from Junta de Extremadura. REFERENCES: Fonzi W.A. and Irwin M.Y. (1993) Genetics, 134, 717-728. Abbey D., Hickman M., Gresham D. and Berman J. (2011) G3, 1, 523-530

8B

RAPID AND CONTINUED EVOLUTION OF THE TELOMERE-ASSOCIATED GENE (TLO) FAMILY IN CANDIDA ALBICANS.

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The telomere-associated gene (*TLO*) family underwent a rapid expansion from one or two members in closely related *Candida* species to fourteen members in *C. albicans* strain SC5314. The fourteen *TLO* gene family members have diverged significantly within the SC5314 genome to produce three distinct clades (α, β, and γ), each with unique functional characteristics. Tloα and Tloβ clade members localize primarily to the nucleus where Tloα proteins are incorporated into the Mediator transcriptional complex. In contrast, Tloγ proteins localize to both the mitochondria and the nucleus although their molecular function has not been explored. To determine if *TLO* genes from these three clades have further diversified in *C. albicans*, we analyzed the subtelomeres of 21 clinical isolates to identify the *TLO* genes present, as well as their relative positions on each chromosome. The number of *TLO*s in these strains varied from 9 to 15 family members. The relative frequency of the two most common clades, *TLOα* and *TLOγ*, varied significantly between strains, while all strains harbored a single *TLOβ* member. Importantly, sequencing revealed entirely novel *TLO* gene architectures and, thus, the existence of novel *TLO* clades. A greater number of unique *TLOα* clade sequences were identified compared to *TLOγ* clade, supporting it as the most diverse clade. The 3' end of the *TLO* genes contained the vast majority of sequence polymorphisms, whereas the 5' Med2 domain was highly conserved among all family members. The genome positions of *TLOβ* and *TLOγ* family members were generally conserved, but *TLOα* members were highly mobile, often being found on different chromosome arms in different strains. Therefore, the *TLO* gene family has continued to undergo significant evolution since *C. albicans* speciation, including frequent movement between chromosome arms. Together, this expansion and evolution has produced a large heterogeneous family of proteins that are predicted to impact gene expression dependent on the particular Tlo protein that is incorporated into the Mediator transcriptional complex.

9C

PHENOTYPIC ANALYSIS OF CUG CLADE SPECIES

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Non-*albicans* species are responsible for about half of all cases of disseminated candidiasis, a proportion that has been rising, yet little effort has been expended to understand the biology of these other species. Half of the non-albicans infections are caused by species in the CUG clade (which excludes the more distantly related *Candida* glabrata); together with *C. albicans*, 75-80% of cases of disseminated candidiasis are caused by the CUG clade. Within this clade, however, there is a wide spectrum of clinical significance amongst the species from commonly isolated species, such as *C. albicans* and *C. tropicalis*, to less common ones, such as *C. guillermondii* and *C. lusitaniae*, to rare pathogens such as *C. famata* (*Debaromyces hansenii*). This range of pathogenic potential offers a tool to understand virulence adaptations that underlie the success of *C. albicans* and *C. tropicalis* relative to the other species. We have begun phenotypic characterization, testing the eight CUG species with complete genome sequence in host-relevant stresses including oxidative, osmotic, nitrosative, pH, and salt. In general, stress resistance correlated with pathogenesis, though there were exceptions to this pattern; *C. lusitaniae* grew nearly as well as *C. albicans* in most stress conditions. Further, we have assessed cellular morphology in numerous conditions that induce hyphal development in *C. albicans*. Some form of filamentous growth was observed in all species except *C. guillermondii* and *D. hansenii*, most commonly under nitrogen starvation or when embedded in the agar and was most robust in the most pathogenic species. Virulence potential was not well correlated with adhesion using a polystyrene-crystal violet biofilm model. In contrast, the phenotypic differences were most strongly correlated with virulence during interactions with murine macrophages, in which the most pathogenic species, particularly *C. albicans*, were more likely to form hyphae, survive phagocytosis and damage the macrophages. Interaction with host phagocytes appears to be predictive of virulence and offers a facile model for understanding the pathogenicity adaptations within this species clade.

10A

FUNCTIONAL ANALYSIS OF THE *EFG1* PROMOTER IN THE WHITE-OPAQUE TRANSITION OF *CANDIDA ALBICANS* BY GENERATING A SERIES OF DELETION DERIVATIVES CONTROLLING THE LUCIFERASE GENE

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The present-day method of choice to explore transcriptional circuitries is the use of genome-wide ChIP-chip analyzes in combination with genome-wide transcriptional profiling comparing wild-type and mutant strains. This powerful method has been extremely valuable in generating a general genome-wide picture of transcriptional circuitries, but may not be optimum for defining specific interactions that actually regulate differential gene expression in the white-opaque transition. It is usually taken for granted that finding a binding pick for a transcription factor in the promoter region of a target gene, in association with an expression defect in a strain with a deletion for this transcription factor, is proof that the transcription factor regulates gene expression by binding to the identified promoter region. This assumption is rarely tested. We used the data recently published by Hernday et al. (2013, Molecular Microbiology, vol. 90) on the role of *EFG1* in the transcriptional network controlling white-opaque switching in *C. albicans* and compared them with those

of Lachke et al. (2003, Molecular Microbiology, vol. 48), who performed an analysis of deletion derivatives of the 2,320 bp region upstream of *EFG1* in white-opaque switching. Two regions in the promoter analyzed by Lachke et al. were shown to bind to 5 of the 6 transcription factors analyzed by Hernday et al., in the white or opaque phenotypes. Region number 1, where Efg1 and Czf1 were shown to bind by Hernday et al. in the white phase, was found to correspond to a precipitous decrease in expression in the white phase, by Lachke et al., when removed. In contrast, the same region, where Wor1, Wor2, Efg1 and Czf1 where shown to bind in the opaque phase, and region number 2 where Wor1, Wor2 and Wor3 where shown to bind in the opaque phase, did not affect expression when removed in the opaque phase. This indicates that ChIP-chip binding does not necessarily lead to altered expression. However, the length of the intergenic region between *EFG1* and its closest upstream neighboring gene is about 10,000 bp. We therefore have expanded the functional analysis of the *EFG1* promoter to its full-length (10 kb) by systematically deleting the 10 regions identified from the data of Hernday et al., and tested their role in the regulation of *EFG1*.

11B

DIVERGENT REGULATION: HIGHLIGHTING DIFFERENCES IN WHITE-OPAQUE SWITCHING BETWEEN CANDIDA TROPICALIS AND CANDIDA ALBICANS

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Phenotypic switching allows for rapid transitions between alternative cell states and is important in pathogenic fungi for colonization and infection of different host niches. In *Candida albicans*, the white-opaque switch plays a central role in regulating the program of sexual mating and interactions with the mammalian host. White-opaque switching is controlled by the a1/alpha2 complex encoded at the *MTL* (mating-type-like) locus, which enables **a** and alpha cells to easily switch from the white state to the mating-competent opaque state. We recently demonstrated that the related pathogen *C. tropicalis* also undergoes a white-opaque phenotypic switch. Interestingly, unlike *C. albicans*, *C. tropicalis* cells undergo similar white-to-opaque switching frequencies in all three cell types (**a**, alpha, and **a**/alpha), and thus switching appears independent of *MTL* control. Transcriptional profiling of *C. tropicalis* white and opaque cells reveals significant overlap between switch-regulated genes in *MTL* homozygous and *MTL* heterozygous cells, although twice as many genes are white-opaque regulated in **a**/alpha cells as in **a** cells. Our work establishes Wor1 as the master regulator of switching in *C. tropicalis*; deletion of *WOR1* locks cells in the white state, while *WOR1* overexpression induces opaque formation. *WOR1* overexpression also promotes both filamentous growth and biofilm formation in *C. tropicalis,* independent of the white-opaque switch. These results demonstrate an expansive role for *C. tropicalis* Wor1, including the regulation of processes necessary for infection of the mammalian host. Interestingly, despite the conservation of *WOR1* as the master regulator of the white-opaque switch in *C. albicans* and *C. tropicalis*, preliminary results indicate that other genes regulating the *C. albicans* switch do not play equivalent roles in *C. tropicalis*. We propose that alteration of the white-opaque regulatory circuit might account for notable phenotypic differences in *C. tropicalis* compared to *C. albicans*, including the surprising existence of a stable "intermediate" phenotype that is neither white nor opaque.

12C

GENOTYPIC VARIATION IN THE ALS3 ADHESIN ACQUIRED DURING HOST-PATHOGEN INTERACTIONS

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Among the cell wall proteins, the ALS (agglutinin-like sequence) gene family, and in particular ALS3 is of special interest because it not only contributes to adhesion and fungal antigenic variation but is also a hypha-specific gene and therefore crucial for the formation of mature biofilms. In addition, Als3 interacts with host cadherins thereby inducing endocytosis by host cells. All Als proteins contain immunoglobulin (Ig)-like and threonine-rich domains at the N-terminus, and a serine and threonine rich region at the C-terminus allowing glycosylation of the proteins. The central domain consists of multiple tandem copies of a 108 bp repeat and can vary in length between and within the ALS genes. Importantly, higher numbers of repeats have been associated with increased adherence. To study the role of ALS3 during host-pathogen interactions, we first looked for variation in the length of tandem repeats in 229 and 311 isolates recovered from an oropharyngeal (OPC) and a systemic infection (BSI) model, respectively. The progenitor strains possess ALS3 alleles readily separated by gel electrophoresis due to differences in tandem repeat number. The survey of size variation of the tandem repeat sequence within the ALS3 gene revealed LOH (retaining only one of the alleles) in 13% of OPC and 14% of BSI isolates analyzed. No bands of novel size were detected. Interestingly, while the ALS3 allele with more tandem repeats was preferentially retained in the OPC isolates, BSI isolates more often retained the shorter allele, suggesting host-specific retention of ALS3 alleles after LOH. Isolates that lost either allele, presumable due to loss of heterozygosity (LOH), were further analyzed by SNP-RFLP to determine the extent of LOH along the Chr (ChrR). SNP-RFLP analysis for 4 ChrR markers revealed that most OPC isolates (93%) exhibited whole Chr LOH consistent with the idea that growth in the oral environment promotes chromosome loss and reduplication events. In contrast, only 32% of BSI isolates exhibited whole Chr LOH, consistent with the idea that growth in the blood stream promotes chromosome loss and reduplication to a lesser degree and recombination events to a greater degree than the OPC model. Sequence analysis of the 5' and 3' region of ALS3 for all LOH isolates revealed point mutations in the majority of isolates, albeit all of which were synonymous. In addition, sequence analysis of ALS3 for isolates previously shown to exhibit altered biofilm formation compared to the progenitor strain identified one isolate with multiple non-synonymous amino acid changes near the GPI-signal sequence. Quantitative RT-PCR is underway to assess whether sequence changes in ALS3 resulted in a change in Als3 expression, which could potentially have a direct impact on antigenic variation and host-pathogen interactions.

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IDENTIFICATION AND SUSCEPTIBILITY PATTERN OF CANDIDA SPP. ISOLATES FROM PATIENTS ADMITTED AT THE CENTRAL HOSPITAL OF MAPUTO, MOZAMBIQUE.

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Introduction: The Candida species have been implicated in superficial and systemic mycoses, mostly in immunocompromised patients or on prolonged treatment with antibiotics. Data on fungal infections at the Maputo Central Hospital in Mozambique and in general are scarce, which limits define appropriate strategies for control and prevention. This study was conducted to give a snapshot view on candida spp. in urban hospital of Mozambique. **Objective:** Identify isolates of Candida spp. from samples of patients admitted at the Central Hospital of Maputo to species level and determine the susceptibility to antifungal agents available at the Hospital. **Method:** 35 isolates of Candida spp. previously isolated from various clinical specimens in the Laboratory of Microbiology of the Maputo Central Hospital, and stored were recovered on Sabouraud dextrose agar (Accumix, Tulip Diagnostic, India).

Candida species were identified by conventional techniques (filamentation, and microcultivation VITEK2 ® system (BioMérieux, France)). The susceptibility to antifungal agents was determined using the VITEK2 ® system. **Results:** A total of 35 isolates of Candida spp. analyzed, and the predominant specie was Candida albicans isolates 26 (74%), followed by Candida lusitaniae and Candida glabrata isolates each with 2 (6%),Candida parapsilosis, Candida tropicalis, Candida magnoliae, Candida norvegenesis and Candida famata with 1 isolated each (3%). All C.albicans isolates (n = 24), C. parapsilosis (n = 1) and C. norvegenesis (n = 1) were susceptible to fluconazole and amphotericin B. The two C.lusitaniae strains, one was susceptible to fluconazole and amphotericin B and another was moderately resistant to fluconazole and amphotericin B. One C. glabrata was susceptible to fluconazole and amphotericin B, another intermediate to fluconazole and susceptible to amphotericin B. C.tropicalis, and C.magnoliae C.famata were not tested to antifungal agents. **Conclusions:** This is a pioneering study, being the first time that identifies Candida spp. to the species level in the country and determines their susceptibility to antifungals. C. albicans was the most frequent and more sensitive to antifungal agents available in Mozambique. There was a great diversity of non-albicans Candida and variety of patterns of susceptibility to antifungal agents. There is a need to implement a routine basis to identify to species level and susceptibility testing for yeast in clinical laboratories of the National Health System in Mozambique, to enable the administration of appropriate therapy, monitoring and prevention of the emergence of resistant strains. The study has as its limitation the small sample size and the lack of patient's clinical information

14B

HIGH LEVELS OF GENOTYPIC VARIATION AND AMPLIFICATION OF A SPECIFIC CHROMOSOME 6 HOMOLOG IN PASSAGED ISOLATES OF C. ALBICANS

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During the course of infection, C. albicans encounters many different host environments to which it must adapt rapidly to ensure growth and survival. Furthermore, it must be able to cope with alterations in established niches during long-term persistence in the host. We are just beginning to understand the mechanisms by which C. albicans adapts to different host environments in vivo, whether these changes are triggered by exposure to the mammalian host, or whether the fungus itself modulates antigenic properties through variations of surface proteins. To understand what genotypic and phenotypic changes C. albicans undergoes during host-pathogen interactions, we passaged the fungus using an oropharyngeal (OPC) mouse model of infection. We identified many isolates with significant alterations in virulence-associated phenotypes, such as growth at host temperature and biofilm formation. The types of alterations in these virulence-associated phenotypes were suggestive of a decrease in virulence potential. To identify the genotypic changes that underlie the observed changes in virulence-associated phenotypes DNA content (ploidy) was determined by flow cytometry. Notably, 10% of all isolates with a virulence-associated phenotype exhibited altered ploidy compared to the diploid progenitor strain. Karyotype analysis by CHEF identified gross chromosomal rearrangements (GCRs) in 50.8% of surveyed isolates. Of these, 36% had at least one shift in Chr size, consistent with loss of heterozygosity and/or major repeat sequence contractions/expansions, and 35% of isolates had at least one amplification event. Comparative genome hybridization analysis of

a few OPC isolates confirmed that an increase in band intensity was indeed consistent with an increase in ploidy. Interestingly, only 8% of isolates had both types of changes. Surprisingly, ~30% of all isolates showed amplification of Chr6, and in particular the longer homolog of Chr6. Because Chr6 is particularly enriched for multiple virulence gene families, including agglutinin-like sequences, secreted proteinases and phospholipases, the amplification of several virulence genes via a single Chr non-disjunction event likely represents a rapid mechanism of C. albicans adaptation to fluctuating host environments.

15C

EXPLORING FUNGAL DIVERSITY IN CYSTIC FIBROSIS PATIENT LUNGS

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Fungal pathogens cause life threatening infectious diseases, affecting billions and killing at least 1.5 million people per year. Many patient populations are vulnerable to these eukaryotic pathogens, as is the case with cystic fibrosis (CF) patients. CF is a genetic disorder characterized by abnormal ion transport across epithelial cell membranes, causing thick mucus accumulation in the lungs, providing a suitable environment for commensal microorganisms such as Pseudomonas aeruginosa and Burkholderia species to flourish. Chronic infections lead to respiratory tract complications, often eventually causing patient death. Fungi are often recovered together with bacteria from CF patient sputum samples, but there has been limited analysis of fungal diversity and impact in the context of CF patients compared to their bacterial counterparts. Our research focuses on Candida isolates from CF patient sputum samples by characterizing their diversity and the evolution over the course of clinical exacerbations. To date, we have characterized 1208 Candida isolates from 25 CF patients based on species identity, growth kinetics and morphology, and susceptibility to antifungal drugs. Of the 1208 Candida isolates, the majority were C. albicans, while other species such as C. glabrata, C. tropicalis, and C. parapsilosis were identified as well. We discovered multiple C. albicans isolates from different CF patients that showed filamentous growth under standard conditions that normally promote yeast form growth. Using whole genome sequencing of two yeast isolates and two filamentous isolates recovered from the same patient, we identified mutations in two transcription factors that were specific to the filamentous isolates. One filamentous isolate had a non-synonymous mutation in the DNA binding domain of NRG1, and the other had a nonsense mutation in WOR2, leading to a truncated protein product. Functional analyses to determine if these mutations are responsible for the filamentation phenotype are currently underway. Our ultimate goal is to more broadly assess fungal diversity within CF patient lungs. Additional phenotypic analyses will focus on Aspergillus species, which are known to cause complications in CF patients. This will be complemented by fungal microbiome analysis based on sequencing of the internal transcribed spacer 1 (ITS1) region amplified from sputum samples. Taken together, this work will illuminate the diversity of fungal species in CF patient lungs, and how the fungal community changes over the course of clinical exacerbation.

16A

TRANSCRIPTIONAL REGULATION OF MITOCHONDRIAL FUNCTIONS IN CANDIDA ALBICANS

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We have previously demonstrated that reduction in the activity of the electron transport system Complex I in the Candida albicans goa1Δ results in dysfunctional mitochondria. Compared to parental and gene-reconstituted strains, ATP synthesis is reduced and cells undergo a loss in chronological aging that is associated with high levels of reactive oxidant species (ROS). Greater killing of the mutant by human neutrophils and avirulence follow loss of these functions. By microarray, we demonstrate a significant reduction in peroxisomal activities including non-glucose carbon metabolism, lipid oxidation and in MSF transporters. To identify regulatory proteins of GOA1, a transcription factor knockout library (TRKO) was screened for phenotypes that indicate dysfunctional mitochondria. GOA1 transcription was then assessed in each of these TRKO mutants with similar phenotypes as the goa1Δ. We found that TRKO mutants of RBF1, HFL1 and DPB4 were unable to grow on glycerol and had reduced oxygen consumption, and mitochondrial ETC Complex I activity, similar to the goa1Δ mutant. GOA1 transcription in each of these TRKO mutants was significantly reduced. The array profiles of rbf1Δ and hfl1Δ TRKOs were more similar to goa1Δ mutant and to each other. The dpb4Δ displayed down regulation in mitochondrial DNA synthesis genes. Our studies demonstrate common and gene-specific regulatory functions (direct or indirect) for each TRKO, including carbon and phospholipid metabolism, stress adaptation, cell wall synthesis, transporter efflux, rRNA processing and nuclear/mtDNA replication. We conclude that these TRs regulate a number of gene groups that apparently integrate energy formation with other metabolic events and cell processes such as cell wall construction. Of the interest, the TRs are fungal-specific, warranting their consideration as antifungal drug targets.

17B

NOVEL FUNCTIONS IN PATHOGENESIS OF EFFLUX GENES, *CDR1* AND *CDR2*, IN AN AZOLE-RESISTANT CLINICAL *CANDIDA ALBICANS* ISOLATE

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Efflux pumps *CDR1* and *CDR2* are overexpressed in an azole-resistant clinical isolate, Gu5. To investigate the direct contribution of *CDR1* and *CDR2* in Gu5, we have constructed mutant cells containing *CDR1* or/and *CDR2* null mutations. First of all, deletions on *CDR1* but not *CDR2* alone increased the sensitivity to antifungal drugs. Furthermore, inactivation of *CDR2* in *cdr1/cdr1* mutant cells significantly increased sensitivity to drugs. The expression profile comparison showed that the expression of 116 genes, involved in adhesion, biofilm formation, drug resistance, stress response, virulence, and others, were reduced when both *CDR1* and *CDR2* were deleted, suggesting that Cdr1p and Cdr2p have multiple functions in pathogenesis. We found that in serum-containing media, the *cdr1/cdr1 cdr2/cdr2* double mutant cells formed germ tube similar to parental Gu5. However, they were defective in hyphal extension and adhesion. Further assessment in a zebrafish bath infection model revealed the novel functions of Cdr1p and Cdr2p in adhesion, hyphal extension, and virulence in the azole-resistant clinical *C. albicans* isolate.

18C

ROLE OF THE CGHAA1-DEPENDENT REGULATORY SYSTEM IN C. GLABRATA RESPONSE AND TOLERANCE TO ACETIC AND LACTIC ACIDS

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To successfully colonize the acidic vaginal tract C. glabrata has to cope with various stresses including the presence of acetic and lactic acids, produced by the bacteria that co-colonize this niche and whose role in restraining the progress of Candida infections is recognized. Little is known about the molecular mechanisms governing the adaptive responses of C. glabrata to acetic and lactic acids, although the identification of these players is expected to provide a highly interesting set of novel biological targets to control the ability of this yeast to colonize the human host, either as a commensal or a pathogen. In Saccharomyces cerevisae, the response and tolerance to acetic acid is largely controlled by the Haa1 transcription factor [1,2,3]. In this work it is demonstrated, for the first time, the crucial role exerted by Haa1-signalling pathway in the response of C. glabrata to stress induced by acetic or lactic acids. The elimination of CgHAA1 dramatically increases C. glabrata susceptibility to these acids, but has no effect on growth when a strong acid was used to acidify the growth medium. Approximately 140 C. glabrata up-regulated genes in response to acetic acid stress were found to be regulated, directly or indirectly, by CgHaa1, based on results of a transcriptomic analysis. Functional clustering of the CgHaa1-target genes indicates an enrichment of those involved in carbohydrate metabolism, transport, cell wall maintenance, regulation of internal pH and nucleic acid processing. Several of the CgHaa1-regulated genes were found to contribute to maximal C. glabrata tolerance to acetic acid including CgGAD1, encoding a glutamate decarboxylase; CgTPO2/3, encoding a drug efflux pump of the Major Facilitator Superfamily; and CgYPS1, encoding a cell wall aspartyl protease. Our results show that the CgHaa1-signalling pathway increases C. glabrata tolerance to acetic acid by reducing the internal accumulation of the acid and by up-regulating the activity of the plasma membrane proton pumping-ATPase, two essential features for a robust weak acid response. The relevance of the CgHaa1 signalling pathway in the context of the extreme tolerance to acetic acid exhibited by vaginal clinical C. glabrata isolates is also discussed.
[1] Mira NP, Becker J and Sá-Correia I, OMICS:14, 587-601, (2010) [2] Mira NP, Teixeira MC and Sá-Correia I, OMICS:14, 525-40, (2010) [3] Mira NP et al., Nucleic Acids Res., 16, 6896-907, (2011)

19A

C. ALBICANS VMA2 IS NECESSARY FOR V-ATPASE ACTIVITY AND FUNCTION AND CONTRIBUTES TO FILAMENTATION, SECRETION, AND AUTOPHAGY

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Candida albicans is a major cause of hospital-acquired infections, yet current methods for treating *C. albicans* infection remain limited. The *C. albicans* vacuole is central to several critical biological processes and has been suggested as a drug target for the development of new antifungal drugs. An essential component of vacuolar biogenesis and function is

the V-ATPase proton pump, a multi-subunit complex responsible for the acidification of internal organelles. V-ATPase is composed of two subunits, V_o and V_1: V_o is embedded in the organellar membrane and is the site of proton transport, and V_1 is the catalytic head of the complex, responsible for ATP hydrolysis. V-ATPase V_1 is composed of the V_1A and V_1B subunits. We therefore constructed a tetracycline-regulatable *VMA2* mutant, strain tetR-*VMA2*, in order to study the contribution of the V-ATPase V_1B subunit to *C. albicans* pathobiology. Inhibition of *VMA2* expression with doxycycline in the tetR-*VMA2* strain led to the inability to grow at alkaline pH characteristic of the *vma* phenotype. *VMA2* inhibition decreased resistance to a variety of stress conditions, including calcium, cold temperature, caspofungin, and growth on non-fermentable carbon sources. Interestingly, we found that *VMA2* inhibition increased resistance to fluconazole. Of note, inhibition of *VMA2* increased susceptibility to oxidative stress; oxidative stress response is of particular interest as it has been shown to be important for host colonization in *C. albicans*. Following repression of *VMA2*, V-ATPase did not fully assemble at the vacuolar membrane and was impaired in both proton transport and ATPase-specific activities. Moreover, V-ATPase inactivation by *VMA2* repression led to vacuolar alkalinization. Repression of *VMA2* interfered with vacuolar biogenesis and resulted in abnormal vacuolar morphology, including marked accumulation of membranous structures within the vacuole. *VMA2* inhibition also inhibited key virulence-related traits in *C. albicans*, including filamentation and the secretion of both aspartyl proteases and lipases. Further, we studied the role of *VMA2* in autophagy, the breakdown and recycling of cellular material, and found that repression of *VMA2* resulted in delayed autophagy. These studies demonstrate the diverse role of V-ATPase in *C. albicans* cell biology and support the potential of V-ATPase as an antifungal drug target.

20B

GLOBAL ANALYSIS OF HSP90 CO-CHAPERONES AND CLIENT PROTEINS IN CANDIDA ALBICANS DRUG RESISTANCE, THERMOTOLERANCE, AND MORPHOGENESIS.

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Hsp90 is an ATP-dependent molecular chaperone that acts to stabilize components of signal transduction cascades, especially those involved in adaptation to stress. Recently, we pioneered a chemical genomic approach to map the first Hsp90 chaperone network in a pathogen. Prior to our work, a global analysis of Hsp90 interactors had only been achieved in the model yeast Saccharomyces cerevisiae, where Hsp90 interacts with ~10% of the proteome. Our study revealed that the Candida albicans Hsp90 interaction network demonstrates remarkable environmental plasticity, and identified novel effectors upstream and downstream of Hsp90, as well as network rewiring over evolutionary time. It also revealed novel fungal-specific targets that regulate drug resistance and morphogenesis, a key virulence trait, with broad therapeutic potential for treating life-threatening fungal infections. Our pilot screen covered ~10% of the genome. We have recently screened an additional ~10% of the genome, and identified 153 Hsp90 genetic interactors. Many of these interactors play a role in drug sensitivity and morphogenesis, including Stt4, Pep7, and Erg5, all of which are Hsp90 interactors in multiple environmental conditions. To complement the genetic analysis, we performed proteomic analysis to determine the set of proteins that interact with Hsp90 and those that interact with specific co-chaperones, which are thought to mediate recognition of client proteins and modulate function of the Hsp90 chaperone machine. We

have expanded our analysis to assess the role of several conserved Hsp90 co-chaperones in drug resistance, thermotolerance, and morphogenesis. This work illuminates the genetic and physical interactions that underpin regulatory circuitry that is of central importance for fungal development, drug resistance, and disease.

21C

THE ROLE OF PHOSPHATIDYLSERINE IN CANDIDA ALBICANS VIRULENCE

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In hospitalized patients with neutropenia, C. albicans is the fourth leading cause of systemic bloodstream infections, which have a mortality rate of approximately 30%. Currently, the drugs available to treat fungal infections are limited, with some that have serious side effects and others that are less effective due to drug resistance. Therefore, it is crucial to discover new antifungal targets that are conserved in fungi, and characterize how inhibition or deletion of these targets affects the virulence of this pathogen. The phosphatidylserine synthase of C. albicans, Cho1p, appears to be a good drug target as a mutant lacking this enzyme (cho1Δ/Δ) is avirulent in animal models of Candida infections and the protein is not conserved in humans. We discovered that phosphatidylserine affects C. albicans' virulence trait expression, cell wall integrity, immune evasion, and the ability to grow in certain nutrient limiting conditions. These phenotypes may affect virulence and are being explored to determine the role of phosphatidylserine in fungal virulence. Agglutinin-like sequence (ALS) proteins are cell wall adhesins that are vital for C. albicans attachment to host cells. These adhesins allow C. albicans to bind to host ligands expressed on epithelial cells, aiding in tissue invasion and damage. Several of these adhesins are expressed only during filamentous growth and biofilm formation, both of which are factors in the progression of candidiasis. We discovered that the cho1Δ/Δ mutation results in a reduction in Als3p protein expression on hyphae, correlating with reduced adhesion of C. albicans to mammalian epithelial cells. The outer layer of C. albicans' cell wall contains mannosylated proteins (mannoproteins) that mask the fungus from innate immunity recognition. An important pathogen associated molecular pattern that is recognized by phagocytic cells is beta-1,3-glucan, a polysaccharide located beneath this mannoprotein layer. The Dectin-1 receptor that is expressed by neutrophils and macrophages recognizes beta-1,3 glucan, which mediates phagocytosis and destruction of C. albicans. We discovered the cho1Δ/Δ mutant has increased exposure of beta-1,3 glucan, and an increased binding of Dectin-1, suggesting that the cho1Δ/Δ mutant is more susceptible to innate immune system recognition. Phosphatidylserine is a precursor for the de novo pathway that synthesizes the essential phospholipid phosphatidylethanolamine. We are exploring the role of phosphatidylethanolamine acting downstream of phosphatidylserine versus the affects of phosphatidylserine itself on these phenotypes. These phenotypes combined may be responsible for the mutants' avirulence.

22A

CELL SURFACE REGULATION IN CANDIDA ALBICANS BY THE ACE2 SIGNALING PATHWAY

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The transcription factor Ace2 is found in many fungal genomes and positively regulates expression of genes involved in cell separation and cell wall biogenesis. Recently Ace2 was shown to regulate transcription of *PMT* genes encoding protein-*O*-mannosyltransferases in *Candida albicans* [1]. *C. albicans* mutants lacking Ace2 are unable to overcome defects in cell wall structures. To investigate the function

of Ace2 for regulation of *PMT* genes, genome-wide chromatin immunoprecipitation (ChIP) on chip analyses were used to explore binding of Ace2 to target sequences. The results indicate that Ace2 mainly localizes to promoter regions of genes under normal growth conditions, while Ace2 binds to open reading frames in the presence of the *N*-glycosylation inhibitor tunicamycin. Promoter regions of identified genes were enriched with the nucleotide sequence GCTGG representing a possible Ace2 binding motif; in contrast, binding to open reading frames of Ace2 under stress conditions did not reveal a consensus binding motif. Direct binding to *PMT*-promoter regions could not be detected in any condition, suggesting that Ace2 regulates *PMT* transcription indirectly. Focusing on Ace2 target genes encoding putative transcription factors, transcript Levels of *ZCF21* [2] were found to be upregulated under glycostructure-damaging conditions. Analysis of the *zcf21* mutant revealed that Zcf21 is involved in transcriptional Repression of *PMT1* encoding the Pmt1 isoform. Consistant with this observation, the *zcf21* mutant is highly resistant to the cell wall perturbating agents tunicamycin and caspofungin. [1]Cantero PD *et al.*, (2011) Damage to the glycoshield activates *PMT*-directed O-mannosylation via the Msb2-Cek1 pathway in *Candida albicans.* Mol Microbiol 80: 715-725.[2]Perez JC *et al.*, (2013). *Candida albicans* commensalism and pathogenicity are intertwined traits directed by a tightly knit transcriptional regulatory circuit. PLoS Biol 11:e1001510.

23B

ROLE OF THE LATE SECRETORY PROTEINS SEC15P AND SNC2P IN *CANDIDA ALBICANS* BIOLOGY AND VIRULENCE

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Candida albicans is an opportunistic fungal pathogen that causes invasive infections at a high mortality rate. *Candida albicans* virulence is associated with multiple phenotypes, including (i) secretion of aspartyl proteases (Saps), which are degradative enzymes that assist in adherence and tissue invasion and ii) biofilm formation, which promotes intravascular infection and dissemination. In prior studies, we have shown that an intact pre-vacuolar secretory pathway is required for secretion of Saps, biofilm formation, and virulence. In these experiments, we studied two of the components of the late secretory pathway: a) *SEC15*, which is one of the first elements of the exocyst complex responsible for mediating polarized targeting of vesicles to active sites of exocytosis, and b) *SNC2*, a v-SNARE protein involved in the fusion between Golgi-derived secretory vesicles with the plasma membrane. Therefore, we hypothesized that *SEC15* and *SNC2* may play essential key role in *C. albicans* virulence-related processes. We generated conditional mutant strains in which each gene (*SEC15* and *SNC2*) was placed under the control of a tetracycline-regulated promoter. In the presence of doxycycline (repressed state), the tetR-SNC2 mutant strain was fully viable at 24 hours, whereas cell death of the tetR-SEC15 mutant strain occurred after 6 hours. Prior to loss of cell viability in the repressed state, the tetR-SEC15 strain was markedly defective in virulence-related phenotypes such as secretion of Saps and lipases, adherence, biofilm formation, and filamentation. In contrast, the tetR-SNC2 mutant strain in the repressed state was not defective in these virulence-associated phenotypes, except for a minor defect in filamentation and delayed secretion of Saps, thus suggesting functional compensation by an unknown trafficking component.. Taken together, these studies indicate distinct roles for the late secretory proteins Sec15p and Snc2p in *C. albicans* biology and virulence.

24C

AGGREGATION OF CANDIDA ALBICANS IN PLASMA LEADS TO DRUG RESISTANCE.

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Candida albicans, an opportunistic fungal pathogen of humans, undergoes hyphal morphogenesis in response to the effects of the physical environment. Previous research shows that contact with a surface, such as during growth within an agar matrix, triggers contact-dependent filamentation in a Dfi1p-dependent manner. This research identified additional conditions for Dfi1p-dependent filamentation and found that these conditions gave rise to the formation of aggregates. Results showed that similar aggregation occurred in blood products, that these aggregates were drug resistant, and determined the physical forces that cause aggregation and Dfi1p-dependent filamentation. Dfi1p-dependent filamentation occurred during growth in liquid medium containing polyethylene glycol. Under these conditions rapid and reversible aggregation of C. albicans cells occurred. Aggregates formed within seconds of the cells being placed in the polymer solution. Alternative polymers, including dextran and polyvinylpyrrolidone, caused neither aggregation nor filamentation. The concentrations and molecular weights of polyethylene glycol that led to aggregation and filamentation were those that are to be optimal for the physical process of depletion attraction. In the human body, high molecular weight blood plasma proteins also cause depletion-mediated aggregation of red blood cells. Therefore blood and blood products, such as plasma and serum, were tested and found to cause aggregation of C. albicans cells in a similar manner to the aggregation seen in the presence of polyethylene glycol. This aggregation occurred both at the physiological temperature of 37°C and at 25°C. The absence of filamentation at the lower temperature allowed the nature of these aggregates to be probed without the complicating effects of hyphae. Aggregates formed in either polyethylene glycol or blood products at either physiological or lower temperature were highly resistant to the antifungal drug fluconazole. Therefore, C. albicans aggregates that formed in blood products had some of the features of biofilm cells. The fluconazole resistance of C. albicans aggregates may be a cause of treatment failure.

25A

PHOSPHO-REGULATION OF THE CANDIDA ALBICANS HYPHAL REPRESSOR NRG1 BY THE CELL WALL BIOSYNTHESIS KINASE CBK1

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The ability of *C. albicans* to reversibly switch between different morphological forms contributes to its virulence as a human fungal pathogen. In *C. albicans*, hyphal development is subject to both positive and negative regulation. The negative regulation of hyphal development is mediated by a repression pathway that involves the recruitment of the co-repressor Tup1 to the DNA by the DNA-binding protein Nrg1, the negative regulator of filamentous growth. In *C. albicans*, cells that lack Nrg1 are characterised by a constitutive filamentous phenotype. The aim of this study was to investigate the molecular mechanisms by which Nrg1 repression is relieved, with a particular focus on phosphorylation. This study assessed the possible phosphorylation-dependent regulation of Nrg1 by the cell wall biosynthesis kinase (Cbk1), which is a conserved kinase absolutely required for polarised growth. Three Cbk1 consensus target sites in Nrg1, S200, T251 and T281 were identified. Two of these Cbk1 phosphorylation sites, T251 and T281, are located in the DNA-binding

domain of Nrg1, specifically at the end of each zinc finger motif of this protein. We proposed a model in which Cbk1 phosphorylates Nrg1 at these three sites in order to modulate its DNA-binding ability, relieving the repression of hyphal specific genes and allowing the yeast to hyphae morphological switch. We tested our model by targeting the three Cbk1 consensus sites in Nrg1 to generate non-phosphorylatable and phospho-mimetic versions of them. The phospho-mimetic mutations released Nrg1 repression and also showed an unexpected nuclear localisation, suggesting that even when the protein was unable to leave the nucleus, it was not functional. We also found that the mutation of the Cbk1 consensus sites in Nrg1 abolished binding to the promoter of hyphal specific genes, leading to de-repression of these genes. We demonstrated that the phosphorylation of Nrg1 at these sites is important to regulate its function.

26B

BLEOMYCIN DETOXIFICATION IN *CANDIDA ALBICANS* IS NOT SOLELY ATTRIBUTED TO LAP3P FUNCTION

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Candida albicans typically resides as a normal commensal organism, but can be induced to become a severe opportunistic pathogen upon induction of hyphal stuctures. Although there is a sizable collection of data regarding the transition from budding yeast to invasive hyphal forms, the complete mechanism is not completely understood. Proteolysis is known to play a vital role in cellular metabolic processes. Upon induction of germ tubes, *C. albicans* concomitantly up-regulates the production of secreted aspartyl proteases (Saps). Even though there appears to be a strong correlation between proteolysis and virulence of *Candida* species, the vast majority of the experimental findings to date have focused on secreted proteases. Roles for *intracellular* proteases in the biology and virulence of *Candida* species remain largely undiscovered. The *Saccharomyces cerevisiae LAP3* gene is a protease that was originally isolated based on a genetic approach in which mutant cells possessing a decreased ability to hydrolyze an aminopeptidase substrate were screened. Subsequent work has provided evidence that Lap3p is widely distributed throughout nature and plays a role in numerous cellular processes, including detoxification of bleomycin. *C. albicans* demonstrates an increased tolerance to the anticancer glycopeptide bleomycin when compared to other eukaryotes. To date, the *C. albicans* Lap3p remains uncharacterized, and its function is largely inferred from sequence homology to Lap3p found in other organisms. Since *Candida* Lap3p has been hypothesized to play a role bleomycin detoxification, we utilized a series of experimental approaches to address this hypothesis. *C. albicans LAP3* was cloned from genomic DNA into a *S. cerevisiae* protein expression vector and transformed into a *S. cerevisiae* strain deleted for the *LAP3* gene. Using spots of serial-diluted transformants, along with a Lap3p specific enzyme assay, we discovered that incorporation of the *Candida LAP3* was functionally capable of replacing the *Saccharomyces* protein, and caused no detectable negative effects on growth of baker's yeast. Through the use of enzyme inhibitor studies we found that the *Candida* Lap3p functions as a cysteine protease, similar to its orthologous proteins from various eukaryotes. Sensitivity of the varying *S. cerevisiae* strains to increasing concentrations of bleomycin was addressed both on solid and liquid media. Introduction of the *C. albicans* Lap3p, even when overexpressed, did not significantly increase the resistance of *S. cerevisiae* to bleomycin. The results described in this work not only represent the first evidence of cloning of the *C. albicans LAP3* gene, but also that this gene can serve as a functional equivalent of the *S. cerevisiae LAP3*. Additionally, we provide the first set of data to demonstrate that the *LAP3* gene is not the sole *C. albicans* factor involved in bleomycin detoxification.

27C

ROLE OF ZCF2 IN THE TRANSCRIPTIONAL RESPONSE OF CANDIDA ALBICANS TO SULFITE

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Candida albicans is an opportunistic fungal pathogen that is highly resistant to different oxidative stresses. Among these oxidants is sulfite, which is produced by C. albicans, sulfate-reducing commensal microbes, and neutrophils. To find candidate genes required for sulfite resistance, a collection of transcription factor deletion mutants from Homann et al. was screened for increased sensitivity to sulfite. From this collection, only the mutant lacking the zinc cluster transcription factor gene ZCF2 was specifically sensitive to sulfite. Hennicke et al showed that ZCF2 is required for cysteine induction of expression of SSU1, a putative sulfite exporter. To test for an adaptive response to sulfite, we exposed C. albicans cultures to subtoxic levels of sulfite and subsequently challenged cells with higher, toxic levels of sulfite. Sulfite-pretreated wild type cultures showed a greater resistance to sulfite than control cultures. In contrast, ΔΔzcf2 cultures showed little or no adaptive response to sulfite stress. To analyze the transcriptional response of C. albicans to sulfite, cultures in rich media (YEPD) of wild type or ΔΔzcf2 strains were treated for 15 min with a subtoxic level of sulfite and compared to control cultures for differential mRNA expression by ratios of cDNA hybridization to C. albicans genomic DNA microarrays. 32 genes showed 8-fold or higher expression in wild type cultures treated with sulfite versus control conditions. Based on CGD-based annotation of the C. albicans genes or SGD-based annotation of their S. cerevisiae orthologs, most of the genes strongly induced by in C. albicans could be grouped into four functional sets: alternative nitrogen catabolism, sulfite removal or detoxification, oligopeptide transport, and alternative oxidase. In addition, 26 of the 30 genes showing reduction in expression of 8-fold or more with sulfite treatment are predicted to have a role in ribosome biogenesis. Using the same protocol, we tested for differential gene expression between sulfite and control cultures of the ΔΔzcf2 strain. Here, the alternative nitrogen catabolism and sulfite removal or detoxification gene sets showed little or no induction by sulfite treatment, yet the oligopeptide transport, alternative oxidase, and ribosome biogenesis gene sets showed similar levels of differential expression as observed for wild type cultures. These data suggest a specific role for the putative transcription factor Zcf2 in protecting C. albicans against toxic effects of sulfite on nitrogen catabolism.

28A

CREATION OF CONSTRUCTS TO ANALYZE STRUCTURE-FUNCTION IN THE ESSENTIAL SEPTIN CDC3 IN CANDIDA ALBICANS

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Septins are a family of GTP-binding proteins conserved in fungi and animals that are often found at sites of cell separation. Best studied in the budding yeast *Saccharomyces cerevisiae*, the five mitotic septins of this organism form a ring at the interface between the mother and daughter cell. This ring establishes mother-daughter polarity by binding proteins preferentially at one side of the ring or the other; it serves as a barrier to diffusion across the plasma membrane between mother and daughter cell and within the bud neck itself; and it serves as a scaffold for proteins required for cytokinesis. In the human pathogenic fungus *Candida albicans*, septins have vital, but poorly understood roles in filamentation, pathogenesis and cell wall integrity. Septins are composed of variable N- and C-terminal domains, a polybasic region in the N-terminus that binds to phosphatidylinositol-(4,5)-bisphosphate (PIP2), a GTP-binding domain, and in

most cases, a septin-unique coiled-coil domain. Structure-function studies in *S. cerevisiae* have demonstrated that septins can be regulated by nucleotide binding, sumoylation, acetylation, phosphorylation, and dephosphorylation at sites that are frequently not conserved in *C. albicans* septins. Thus, we know very little about how *C. albicans* septins are regulated or what residues or domains contribute to their function in cell wall integrity and filamentation. We have created constructs to analyze structure-function of the essential septin *CDC3* in *C. albicans* as well as tagging construct for *CDC3* that may be useful in other applications as well.

29B

DEVELOPING HIGH-THROUGHPUT ASSAYS TO IDENTIFY AND CHARACTERIZE SMALL MOLECULES THAT TARGET THE CANDIDA ALBICANS VACUOLE.

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The fungal vacuole supports a variety of cellular functions that are critical for *Candida albicans* survival within, as well as invasion of the mammalian host. This includes central roles in resisting stresses exerted by host defense mechanisms, as well as hyphal growth that is associated with tissue invasion and injury. As such, we hypothesize that small molecules that disrupt vacuolar function can provide novel and potentially efficacious antifungal therapies. Using a previously developed high-throughput screen we have identified 82 potential Vacuole Disrupting chemical Agents (VDAs) to date. To facilitate the classification of these VDAs and uncover their mechanism of action, we have developed a number of secondary assays that examine distinct aspects of vacuolar function. Herein we describe an assay to detect defects in Golgi-to-vacuole trafficking in *C. albicans*, using a versatile and sensitive GFP reporter based upon the vacuolar hydrolase Carboxypeptidase Y (CPY). In *Saccharomyces*, CPY is synthesized as an inactive precursor with a pre-pro-peptide containing a vacuolar sorting signal that is removed upon delivery to the vacuole. We have identified the *C. albicans* CPY pre-pro-peptide (CPP), and produced a CPP-GFP-fusion construct. Fluorescence microscopy revealed that CPP efficiently targets GFP to the *C. albicans* vacuole, where the fusion protein is cleaved to release GFP, as evidenced by molecular mass shifts on western blots. However, CPP-GFP is mislocalized and remains uncleaved in two *C. albicans* vacuolar trafficking mutants, *vps21Δ/Δ* and *ypt72Δ/Δ*. Furthermore, CPP-GFP can be detected in the culture supernatant of the two vacuolar trafficking mutants but not the isogenic control strain, presumably due to missorting of CPP-GFP into the secretory pathway. These results validate the use of our CPP-GFP reporter to detect Golgi-to-vacuolar trafficking defects in *C. albicans*. Initial studies have demonstrated that CPP-GFP distribution is affected by several of the VDAs that were identified using our primary screening assay. At the time of submission, we are further refining the system to produce a CPP-Luciferase fusion that will enable us to directly detect and quantify CPP missorting into the culture supernatant, greatly enhancing the efficiency and sensitivity of the assay.

30C

THE PHOSPHORYLATED SR DOMAIN OF *C. ALBICANS* MRNA-BINDING PROTEIN SLR1 INFLUENCES ITS NUCLEAR AND HYPHAL TIP LOCALIZATION

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Post-transcriptional regulation of gene expression by RNA-binding proteins has been implicated in polar growth of many eukaryotes. We recently found that absence of *C. albicans* SR-like RNA-binding protein Slr1 slows yeast and hyphal growth, decreases the ability of

C. albicans to damage host cells *in vitro* and lowers virulence in a model of disseminated candidiasis. SR (serine-arginine-rich) proteins regulate gene expression by influencing processes from mRNA splicing to RNA transport and their function and localization are frequently controlled by phosphorylation. We therefore tested whether Slr1 is phosphorylated in the C-terminal SR domain and whether this domain is important for Slr1 localization and function. Immunoblot analysis of phosphatase-treated Slr1-GFP revealed two bands that migrate faster than untreated Slr1-GFP, indicating its phosphorylation *in vivo*. Mutant slr1-6SA-GFP, in which the 6 serines within the SR domain are replaced with alanine, migrates similarly to dephosphorylated Slr1-GFP. Thus the C-terminal SR domain likely encompasses all Slr1 phosphorylation sites. Whereas wild-type Slr1-GFP co-localizes with DAPI staining in nuclei of yeast-form and hyphal cells, slr1-6SA-GFP is primarily found in cytoplasmic foci, appearing particularly concentrated at the tip of hyphal cells. The hyphal tip focus partially overlaps with an FM4-64-stained region at the tip, indicating the proximity of slr1-6SA-GFP to the Spitzenkörper, a vesicle-rich structure implicated in hyphal tip growth. This localization suggests that the mutant slr1 protein may travel with mRNA-protein complexes targeted to the hyphal tip. To test this hypothesis, slr1-6SA-GFP was expressed in cells with and without the She3 mRNA transport protein. The percentage of hyphal tips with slr1-6SA-GFP foci is over three-fold lower in cells lacking She3. The copurification of She3 with the type V myosin Myo2 and the ability of actin disruptor cytochalasin A to partially disperse the slr1-6SA-GFP hyphal tip foci further support the possibility that the mutant slr1 protein and She3 are found in complexes transported to the hyphal tip. Neither hyphal extension nor generation time in YPD broth cultures is decreased by the presence of slr1-6SA-GFP as the sole copy of Slr1. Therefore, our results indicate that the C-terminal SR domain of Slr1 is phosphorylated and affects intracellular localization, but that its phosphorylation is not required for the role of Slr1 in *C. albicans* growth. Studies to identify specific phosphorylation sites and to explore the impact of SA mutations on more specific Slr1 functions and molecular interactions are ongoing.

31A

PROTEOMIC ANALYSIS OF SECRETED PROTEINS AND EXTRACELLULAR VESICLES FROM *CANDIDA ALBICANS* SC5314 AND *ECM33*Δ STRAINS

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Candida albicans secretes a considerable number of proteins involved in different processes such as virulence, cell wall organization, interaction with host and biofilm formation. In recent years extracellular vesicles have also been described as a general mechanism in the molecular traffic across the cell wall to the extracellular space in fungi, including *C. albicans*, being important contributors in the pathogenic process. In order to analyze the extracellular proteome of *C. albicans* SC5314, cell-free culture supernatants were separated into vesicles (visualized by TEM) and vesicle-free fractions. The samples were analyzed by LC-MS/MS (Orbitarp), obtaining 74 and 64 proteins with two or more peptides in vesicle and vesicle-free preparations, respectively. Interestingly, differential proteins identified only in vesicle-free samples were enriched in secreted proteins whereas cytoplasm proteins were more abundant in the samples from vesicles. Furthermore, previous surfome analysis showed that *ecm33*Δ mutant displayed a larger number of proteins that the wild type strain at the surface; but proteins involved in cell wall organization were less abundant in the mutant surfome. In order to investigate the relevance of Ecm33p function in the anchoring of proteins to the cell wall, we also carried out a comparative analysis of *C. albicans* SC5314

and *ecm33*Δ secretomes. The results of this analysis showed that *ecm33*Δ secreted more proteins than the wild type. Moreover, in the sample from SC5314 vesicles 74 proteins were detected (with two or more peptides and in at least two replicas) against 158 proteins identified in the mutant strain. In the vesicle-free preparation the numbers of proteins detected were 64 versus 109 in wt and mutant, respectively. The functional classification and subcellular localization of these proteins showed interesting results. Some of these proteins are being analyzed using deleted mutant strains.

32B

FUNCTIONAL AMYLOIDS IN FUNGAL DISEASES

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Previously we have shown that Als5p, a *Candida albicans* cell wall adhesins, contains an amyloid forming sequence which is conserved in other Als family members. These sequences are critical for soluble peptide and protein amyloid fibril formation. The Als5p amyloid sequence is critical for cell adhesion and biofilm formation (Garcia *et al*, PLoS ONE e17632). In a *C. elegans* infection model Als5p amyloid interactions function in colonization to induce a commensal-like state (Bois *et al* Euk. Cell 12:703). However assays thus far have not addressed the existence of fungal amyloids in the human host. We therefore, hypothesized that these amyloid interactions are present during colonization and are necessary for host-fungal interactions. Here we used autopsy sections from patients with invasive candidiasis, aspergillosis and coccidiomycosis to determine the presence of fungal amyloid interactions in the host. We show that fungal surface amyloids are present in autopsy tissue sections from patients inflicted with candidiasis as well as the other fungi. The fungi present in these candidiasis patients are also positive for amyloid binding dyes, such as thioflavin T, thioflavin S, Congo red and a sequence-specific fluorescent amyloid peptide probe. In the infected tissue and *in vitro*, amyloid-expressing fungi were coated with human serum amyloid P component (SAP), a pattern recognition receptor with anti-inflammatory function. Thus in infected human tissue, fungal amyloids contribute to anti-inflammatory responses. These data collectively may contribute in identifying novel treatments, diagnostics and prophylactics needed to combat fungal diseases.
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33C

DISSOCIATION OF *CANDIDA ALBICANS* BIOFILMS BY ANTI-AMYLOID COMPOUNDS

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Fungal biofilms represent an increasing strain on resources in the clinic, where fungal contamination of indwelling catheters has become pervasive. Along with this increase, an alarming amount of fungi are becoming drug resistant. This situation highlights the need for investment in the development of new antifungal chemotherapy regimens to help combat invasive fungal disease caused by biofilms. Since most current anti-fungal drugs target some aspect of the cells' genetic machinery, we propose that other compartments in addition to genetics be exploited in the development of new biofilm eradication strategies. Here we present preliminary evidence in support of the hypothesis that protein-protein interactions of amyloid forming adhesins in the extracellular compartment of *C. albicans* biofilms provide

a promising target for intervention. Biofilms were grown for 24 hours in RPMI-MOPS (pH 7.4) prior to treatment. To ascertain the presence of amyloids in mature *C. albicans* biofilms, we stained the biofilms with thioflavin-T, (300 nM) followed by epi-fluorescence microscopy. For dissociation experiments, thioflavin-T was used at concentrations ranging from 10 to 100 uM, for 24 hours, after which the metabolic activity of the biofilm was assayed by XTT. We further screened several plant based polyphenols for anti-amyloid activity against *Candida albicans* biofilms. We used a combination of metabolic assays and confocal microscopy in our analysis of biofilm response to anti-amyloid compounds. Treatment of mature *C. albicans* biofilms with thioflavin-T and xanthochymol resulted in significant decrease in biofilm viability as measured by XTT assay. Confocal microscopy analysis revealed a dramatic reduction in hypha in the presence of anti-amyloid compounds used in these experiments. Further, there are synergistic interactions between the anti-amyloid xanthochymol and fluconazole against fungal biofilms. Based on these experiments we propose that the extracellular compartment of fungal biofilms containing amyloid forming adhesins represents a credible target for intervention against established fungal biofilms.

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34A

CANDIDA ISOLATES AND THEIR SENSITIVITY PATTERN IN JAIPUR

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THE RETROSPECTIVE STUDY WAS CONDUCTED IN A TERTIARY DIAGNOSTIC CENTRE[PIRAMAL DIAGNOSTICS] FROM 2006-2010. SAMPLES SUSPECTED OF BEING CANDIDA SAMPLES WERE CULTURED ON CHOCOLATE AGAR, BLOOD AGAR, MAC CONKEY AGAR, SABOURAUDS AGAR WITH AND WITHOUT ANTIBIOTICS. WHITE SMOOTH COLONIES WERE OBTAINED ON SABOURAUDS AGAR ,BLOOD AND CHOCOLATE AGAR. SMEARS WERE MADE AND GRAM STAINING WAS DONE. IF BUDDING YEAST CELLS WERE SEEN , SUBCULTURED ON HICHROM MEDIA .ALSO GERM TUBE TEST DONE. ABOUT 1000 CANDIDA STRAINS WERE ISOLATED OUT OF THESE 250 BUDDING YEAST CELLS WERE FINALLY IDENTIFIED TO SPECIES LEVEL AND SENSITIVITY DONE ON API 32 C [BIOMERIUX] AND API ATB FUNGUS 3[BIOMERIUX] USING MINI API. MIC OF <1 mg/L WAS TAKEN SENSITIVE FOR AMPHOTERICIN B ,< 8 mg/L FOR FLUCANAZOLE,< 1 mg/L FOR VORICANAZOLE ,<0.12 mg/L FOR ITRACANAZOLE AND < 4 mg /L FOR FLUCYTOSINE. MANUAL SENSITIVITY WAS ALSO PERFORMED BY DISC DIFFUSION TESTING AS DESCRIBED IN CLSI DOCUMENT M44-A (14). AGAR PLATES CONTAINING MULLER HINTON AGAR SUPPLEMENTED WITH 2% GLUCOSE AND 0.5 µg OF METHYLENE BLUE PER ml . FLUCONAZOLE (25 µg) AND VORICONAZOLE (1 µg) DISCS WERE PLACED ON THE SURFACE OF INOCULATED PLATES AND READ AT 18 to 24 hr .TO FIND OUT THE TREND OF CANDIDA IN INDIAN SCENERIO MAXIMUM CASES WERE NONALBICANS OF WHICH CANDIDA TROPICALIS WAS MOST COMMONLY ISOLATED. NEXT COMMON WAS C. ALBICANS.MOST COMMON ISOLATED STRAINS WERE SENSITIVE TO ANTIFUNGALS SHOWING RESISTANT PATTERN HAS NOT EMERGED IN CANDIDA AND NO CHANGE OF TRENDS IN 4 YEARS.

35B

ISOLATION AND EVALUATION OF CANDIDA SPECIES AMONG PREGNANT WOMEN IN OBAFEMI AWOLOWO UNIVERSITY TEACHING HOSPITAL, ILE-IFE.NIGERIA

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Background: Pregnant women are more susceptible to both vaginal colonization and infection by yeast. Untreated Candida infection can lead to very serious health and medical problems and is worsened while pregnant. **Objective:** This study aimed at isolating and evaluating the carriage rates of Candida species among pregnant women in Ile-Ife southwestern Nigeria. **Methods:** The High vaginal swabs collected were inoculated onto Sabroud dextrose agar (SDA) and incubated aerobically at 370c for 24hrs. Candida species were checked for on the SDA medium. Gram staining and Germ-tube test was performed on the yeast isolates to confirm Candida albican, the morphologic and biochemical characteristics of other Candida species were also carried. **Results:** Ninety-nine antenatal clinic attendees at Obafemi Awolowo University Teaching Hospital were recruited for this study. Out of the 99 with mean age of 30.17 women recruited, 49.5% with mean age of 30.59 were positive for Candida albican infection. Other species implicated are C. pseudotropicalis 9(9.1%), C. krusei 15(15.2%), C.parapsolosis 1(1.0%), C. stellatoides 4(4.0%), C. tropicalis 8(8.1%), and unknown 13(13.1%). The age range 30-39years had the highest prevalence of C.albican 30(30.3%) and C.krusei 10(10.1%) but C.tropicalis 5(5, 1%), C.stellatoides 3(3.0%) C. pseudotropicalis 5(5.1%) were prevalent in age range 20-29years. Multigravid had the highest the highest rate of C.albican of 14(50.0%) whereas, parity range 1-2 within multigravid had highest occurrence of C. albican in age range 30-39years with 24(33.8%). Zero (0) parity within primigravid had C.albican with 23(31.9%). **Conclusion:** The percentage of Candida albican among 99 infected pregnant women was 49.5% emphasizing the importance of routine screening of pregnant women thereby assisting in prevention of invasive neonatal candidal infection. Thorough medical examination and culture of HVS is highly recommended for pregnant women to ensure detection of vulvovaginal infection by candida species among the immunosupressed person. Appropriate health and proper and well coordinated sex education is needed to reduce candidal infection.
Keywords: Isolation and Evaluation, Candida species, pregnant women, Nigeria.

36C

INVASIVE CANDIDIASIS IN INTENSIVE CARE UNITS OF THE TERTIARY HOSPITAL IN ST. PETERSBURG, RUSSIA.

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Invasive candidiasis (IC) is a severe infection with high mortality. **Objectives:** We analyzed the etiology, clinical signs and symptoms, and results of treatment of invasive candidiasis patients in two ICUs of a tertiary hospital in St. Petersburg, Russia. **Methods:** The prospective study was conducted during one year period: from May, 2012 till June, 2013 yrs. We examined the patients for the presence of lesions (CT, MRI, echocardiography, ophthalmoscopy). We used multifactory analysis by Statistic 6.1 for Windows 7. Diagnosis of IC was made according to EORTC/MSG criteria, 2008. **Results:** During the study period 4874 patients were hospitalized in two ICU departments: surgical and medical. We revealed 10 patients with IC (rate - 2.1/1000). The median age of patients was 55 years (range 27-71), male - 60%, female - 40%. Majority of IC

cases were observed in medical ICU (60%). IC developed after median -15 (7-44 days) of stay in the ICU. We isolated the pathogen from the blood - 80% of patients, central venous catheter - 20% and aspirate from the abdominal cavity - 30%. The etiology of IC: C. albicans - 70%, C. glabrata - 30%, C. krusei -10%, C. tropicalis - 10%. We isolated two pathogens in one sample from 20% of patients. The lesions were not found, except one patient who developed a hepatic lesion. All patients received antifungal therapy - fluconazole (50%), voriconazole (50%), amphotericin B deoxycholate (10%). Duration of treatment was median - 21 (9-58 days). Overall survival at 12 week was 50%. Negative prognostic factor of the 12th week survival was: C. glabrata. **Conclusion:** The frequency of invasive candidiasis in ICUs in tertiary hospital St. Petersburg, Russia was 2.1/1000. The main etiological agent was C. albicans (70%). Fluconazole and voriconazole were main antifungal agents. Twelve week overall survival was 50%. Negative prognostic factor of the 12th week survival was: C. glabrata.

37A

COMBINATION OF INVASIVE CANDIDIASIS AND INVASIVE ASPERGILLOSIS IN HEMATOLOGICAL PATIENTS.

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Objective: Invasive candidiasis (IA) and aspergillosis (IA) are leading causes of morbidity and mortality in haematological patients. Publications about combination of IC and IA in haematological patients are limited. **Methods:** The prospective study was conducted during the period 2012-2013 y.y. Diagnosis of IC and IA was made according to EORTC/MSG criteria (2008). **Results:** We observed 3 hematological patients with IC and IA. The mean age of our patients was 42 years (range 36-58), male and female ratio 1:2. Underlying conditions were: acute myeloid leukemia - 2, non-Hodgkin's lymphoma - 1. Diagnosis of IC was confirmed by culture of blood. The agents of IC were C.krusei, C. parapsilosis and unidentified Candida. Test «Platelia Aspergillus EIA» (Bio-Rad) was positive in all of patients in blood and BAL. A. flavus in BAL - 1 patient. The main sites of IA and IC were lungs (100%), sinuses (67%), skin and soft tissues - (33%). More then one organs were affected in all of patients. Antifungal therapy was performed all of patients: voriconazole - 100%, fluconazole - 67%, amphotericin B deoxycholate - 67%%, caspofungin - 67%%, amphotericin B lipid complex - 33%, micafungin - 33%. Combination therapy voriconazole + amphotericin B, voriconazole+ caspofungin, voriconazole+ micafungin was used for 67% patients. Duration of antifungal therapy was 11 - 167 days (median - 120). Necrosectomy of nasal soft tissues was performed in 1 patients. Overall survival at 12 weeks was 2/3 of patients. **Conclusion:** Combination therapy can be successful in the treatment of combination of invasive candidiasis and aspergillosis in haematological patients.

38B

THE FIRST CASE OF SUCCESSFUL TREATMENT OF A WOMAN WITH CANDIDA PERITONITIS AND PNEUMOCYSTIS PNEUMONIA IN THE POSTPARTUM PERIOD

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Objectives: The aim is - to demonstrate the first case mixt-mycotic infections: Pneumocystosis (pneumonia) and candidiasis (peritonitis) in the postpartum women without typical risk

factors. **Methods:** The diagnosis invasive mycosis was made according to EORTC/MSG criteria (2008). **Case report:** Patient B., 33 y., hospitalized to the intensive care unit of the Leningrad Regional Hospital with a diagnosis: birth III, at 34-35 weeks' gestation, severe preeclampsia, chronic placental insufficiency subcompensated syndrome, fetal growth retardation, the state after laparotomy (cesarean section in the lower segment , gisteroektomiya with tubektomiya 24/10/09), the pulmonary edema, anemia, chronic pyelonephritis in the acute phase. Objectively: a severe condition, mechanical ventilation. Body temperature - 39 °C. Blood analysis: leukocytes - 7,7 • 109 / L (neutrophils - 86%, lymphocytes - 12%), hemoglobin -98 g / l, platelets -147 • 109 / L, ESR -35 mm / h., fibrinogen - 1.9 g / l. Urine analysis: proteinuria, cylindruria. Test of procalcitonin - 1.33 ng / ml (normal 0-0.5). In samples urine and bronchoalveolar lavage was obtained by growing C. albicans. Empirical antimycotic therapy was started - fluconazole 600 mg / day. Central venous catheter was replaced. After 2 days we had a culture C. albicans of the abdomen drainage and dose of fluconazole was increased to 800 mg / day. On the sixth day hospitalization the patient developed recurrent pulmonary edema. CT - interstitial-alveolar infiltrations of both lungs. On the bronchoscopy - catarrhal endobronhitis. Excluded virus and bacterial aetiology of pneumonia. The acid-base status - arterial hypoxemia (43 mm. Hg). Arterial oxygen saturation decreased (77%). Completed upper tracheotomy and drainage of both lungs. Analysis of risk factors and clinical data (severe respiratory distress, decrease in peripheral blood of oxygen, diffuse widespread changes in the lungs and prolonged lymphocytopenia (0,9 • 109/l), no effect on a wide range of antibiotic) the preliminary diagnosis was - pneumonia caused by Pneumocystis jiroveci. Started therapy of co-trimoxazole (20 mg/kg/day). After 2 days - normalized blood gas and temperature. In follow-up studies of the different samples (blood, urine, BAL, discharge from the drainage) fungi were found. The total duration of treatment of peritonitis (C. albicans) - 33 days, pneumonia (Pneumocystis jiroveci) - 28 days. **Conclusions:** in complicated pregnancies, multiple organ failure, operative delivery with increasing the volume of surgery, presence of immune deficiency in the postpartum period, to consider the possibility of developing invasive fungal infections.

39C

CANDIDA ALBICANS ENDARTERITIS AND KIDNEY ALLOGRAFT INFECTION AFTER RENAL TRANSPLANTATION

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A 53 year old lady underwent deceased donor renal transplant on Dec 22. She suffered delayed graft function requiring intermittent hemodialysis. Her renal function recovered, and she was discharged 11 days later. Her estimated renal function was at 82%. In early Feb, she had a soft tissue infection that was treated with IV antibiotic administered via a venous catheter (PICC line). In March, she was admitted for acute renal failure. She was afebrile, but looked anxious and unwell. Her physical exam was benign, and the allograft kidney was non-tender. Renal biopsy showed no rejection. An ultrasound of the allograft showed tight stenosis of the renal artery anastomosis. A CT scan of the abdomen showed evidence of allograft pyelonephritis. Three sets of blood cultures grew *C. albicans*. She underwent exploratory laparotomy that showed dense adhesions and inflammation around the allograft kidney, and a near-ruptured mycotic aneurysm at the renal artery anastomosis. Infected aneurysm and vessel were removed and bypass was performed using a cadaveric iliac artery. Histopathology of the resected artery showed arteritis with focal necrotizing inflammation and overlying microthombus formation with invasive yeast and pseudohyphae. She was treated with fluconazole for 8 wks, but never recovered renal function. She subsequently underwent allograft removal. This case

of *C. albicans* endarteritis (inner artery lining infection) at the donor-recipient anastomosis illustrates an uncommon but fatal complication after renal transplant. Most cases occurred within 3 mos of transplant, and resulted in aneurysm rupture and death; allograft failure was the norm among survivors. The source of infection in our case is unclear, but may have originated from PICC-associated bloodstream infection (BSI). Graft contamination during harvest is another possibility. The kidney is the last organ to be harvested during multi-organ recruitment; prolonged harvesting time leading to gut ischemia can pre-dispose to *Candida* transmigration and kidney infection. Regardless of the source, once *C. albicans* accesses the bloodstream it can adhere to and invade the endothelial cell lining of blood vessels to infect deeper tissues and cause organ dissemination. Several mechanisms that enhance *C. albicans* crossing through the endothelial cell lining have been postulated: leukocyte phagocytosis and transport, passage between endothelial cells, and endocytosis by endothelial cells. The latter mechanism has been a major focus of investigation. For example, *C. albicans* hypha-specific surface protein Als3 is an adhesin and mediates attachment to endothelial cells. Als3 also serves as an invasin that binds to host cell receptors such as E-cadherin and N-cadherin and induces endocytosis. Since *Candida* is normally cleared rapidly from the blood, strategies to prevent blood vessel invasion may prevent organ complications of BSI.

40A

ASH1 CONTROLS THE PENETRABILITY OF PATHOGENIC BIOFILMS TO IMMUNE CELLS AND THE DISSEMINATION OF DAUGHTER CELLS FROM CANDIDA ALBICANS BIOFILMS IN VITRO

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Biofilms are surface-associated microbial communities embedded within a matrix of extracellular polymers. Biofilms are refractory to antimicrobial drugs and penetration by polymorphonuclear leukocytes (PMNs). This strategy for colonization is thought to enable microbes to maintain a protected anti-microbial, drug resistant environment for growth. Ash1 is a daughter- and hyphal tip-cell localized transcription fctor that promotes filamentous growth and virulence in C. albicans. Transcript profiling (Harcus et al., 2004) of Ras/cAMP/PKA pathway mutants (ras1, cyr1 and efg1) suggest that Ash1 functions downstream of adenylyl cyclase. ASH1 expression is upregulated in ras1 mutants grown under hypha-inducing conditions and in cyr1 mutants grown as yeast or hyphae, indicating that ASH1 expression is repressed in response to Ras/cAMP signaling, an important pathway for virulence and biofilm formation in Candida. Using an in vitro model of biofilm formation, we have developed an assay for determining the number of daughter cells released from biofilms into the surrounding medium. We found that the biofilms formed by ash1 mutants fail to release daughter cells into the culture medium. Instead, ash1 mutant biofilms retained yeast-form daughter cells on the surface of the biofilms. We examined these biofilms for the ability to resist immune cell penetration and found that human PMNs readily penetrate ash1 mutant biofilms whereas control strains resist penetration by PMNs. Our current goal is to identify the target genes regulated by Ash1 during biofilm formation. This work was supported by the Developmental Studies Hybridoma Bank at the University of Iowa, Iowa City, USA.

41B

CANDIDA AND YEAST-YEAST INTERACTIONS, IN VITRO AND IN VIVO

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At a given time, the healthy human gut microbiome contains from zero to twelve or more detectable fungal species. Candida yeasts

dominate and may be considered endemic to the human microbiome, but they rarely exist in isolation. Multiple Candida species, or multiple strains of a single species, may coexist; while other autochthonous and allochthonous yeasts may influence community composition and host well-being. Amplicon pyrosequencing of fungi from the feces of healthy humans suggests that competitive exclusion between ecologically similar species and strains affects species abundance; additionally, killer toxins from food-processing yeasts inhibit the growth of C. albicans, C. tropicalis and other opportunistic pathogens in vitro and may play a role in vivo. The possibility of using natural interactions between yeasts to shift the gut microbiota in a desired direction is discussed.

42C

CANDIDA-BACTERIA INTERACTION IN THE ORAL CAVITY; PARTNERSHIP IN HEALTH AND DISEASE!

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The fungus Candida albicans is a common colonizer of the oral cavity, not only in disease but also in health. Within the healthy oral cavity many hundreds of bacterial species are present in the microbiome. Candida thus meets many different species of bacteria in the oral cavity and they influence each other directly through physical and chemical interactions. The response of Candida to bacteria is very diverse and this "interactome" could play important roles in maintaining health or developing disease. We hypothesize that Candida physically interacts with bacteria through different molecular mechanisms. For instance, we previously showed that Staphylococcus aureus interacts with Als3p of C. albicans, while Pseudomonas aeruginosa binds to chitin in the hyphal cell wall. In the present study we determined the physical interactions between Candida albicans and a selection of oral bacteria using microfluidics combined with time-lapse microscopy. Hyphae of C. albicans where grown in the microfluidics channels and adhesion of green fluorescent bacteria, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans and Streptococcus mutans was analyzed in real time. All bacteria adhered to hyphae and/or to yeast cells be it to different extends. In contrast to S. aureus and other Streptococcus spp, S. mutans did not interact with C. albicans through Als3p. In conclusion, different bacteria interact with C. albicans through distinct molecular mechanisms and might influence the composition of the oral microbiome maintaining health or leading to disease.

43A

INTERSPECIES INTERACTIONS BETWEEN *CANDIDA GLABRATA* AND *CANDIDA ALBICANS* IN OROPHARYNGEAL CANDIDIASIS

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Oropharyngeal candidiasis (OPC) is predominantly associated with *Candida albicans*; although *Candida glabrata* is the most frequently co-isolated Candida species in OPC. Single species OPC infection by *C. glabrata* is rare *in vivo* suggesting that interactions between these two species promote co-infection. **Objective:** Determine whether the presence of both *C. albicans* and *C. glabrata* species promotes adhesion or biofilm formation *in vitro*, and whether co-infection of both Candida species in a murine OPC model alters infection levels of the partner organism. **Methods:** Co-adhesion of *C. albicans* (yeast and hyphae) with *C. glabrata* was observed using fluorescent microscopy and with sedimentation assays. Saliva coated plastic plates were used to measure biofilm mass of single and dual species biofilms. For *in vivo* studies, a murine model of OPC was used. Mice (C57BL/6 female, 4-6 weeks old) were immunosuppressed with cortisone and infected sublingually with

C. albicans or *C. glabrata* alone, or with both species concurrently. *C. glabrata* was also infected sublingually in mice that had one day or two day pre-established *C. albicans* infections. Colony Forming Units (CFUs) were determined by plating homogenized tongue tissues 5 days post infection on Chromagar to differentiate each species. **Results:** No co-adhesion between planktonic yeast *C. albicans* and *C. glabrata* was observed; however *C. glabrata* adhered selectively to *C. albicans* hyphae (9.1±0.2 *C. glabrata* cells/10 µm hyphae). *C. glabrata* sedimentation increased by 1.8 fold in the presence of *C. albicans* hyphae. Dual species biofilm mass exhibited an increase in dry weight (2.7 ± 0.2 mg $p<0.001$) compared to single species biofilms of *C. albicans* (2.1±0.1 mg) or *C. glabrata* (2.1 ± 0.2 mg). *C. glabrata* infection alone had negligible colonization of tongue tissues ($7.0X10^2$ CFU/g); however colonization increased by one log-fold ($3X10^3$ CFU/g, $P\leq 0.02$) upon co-infection with *C. albicans.* Pre-established *C. albicans* infection further increased subsequent *C. glabrata* colonization ($3.5X10^4$ CFU/g, $P\leq 0.0001$) showing the importance of *C. albicans* infection for *C. glabrata* colonization *in vivo*. **Conclusions:** *C. glabrata* selectively adheres to *C. albicans* hyphae and enhances dual species biofilm formation *in vitro*. *In vivo* OPC murine infection by *C. albicans* is needed for *C. glabrata* colonization, suggesting that *C. glabrata* is a secondary colonizer in human OPC. Defining binding partner adhesins in both species will help to identify novel drugs to inhibit or disrupt adhesion. This work was supported by NIH DE10641 and DE022720.

44B

INVESTIGATION ON THE ROLES OF CELL WALL PROTEINS, BGL2 AND ECM33, IN THE BIOFILM FORMATION OF *CANDIDA ALBICANS*

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The biofilm formation of *Candida albicans* is a trigger to induce the pathogenicity and the adhesion of this yeast to the hosts is thought to be necessary to form the biofilm on the surface. Then, understanding of the biofilm formation mechanism of *C. albicans* is one of the important things for medical application. Previously, our group showed that Bgl2 (1, 3 -beta-glucanosyltransferase) and Ecm33 (GPI-anchored protein) of *C. albicans* related to the adhesion to the surface of teeth via saliva in oral cavity. On the other hand, it was previously demonstrated by other researchers that disruption of *ECM33* gene reduced *C. albicans* biofilm formation on engineered human oral mucosa tissue. In this work, to investigate the roles of Bgl2 and Ecm33 in the biofilm formation of *C. albicans* on the surface of artificial devices, we tried to analyze the biofilm formation of *bgl2*, *ecm33* and *bgl2ecm33* disruptants of *C. albicans* in the polystyrene plates. XTT(2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl) -5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) reduction assay was used for measurement of the metabolic activity of biofilm cells *in vitro*. Moreover, we also analyzed transcriptional expression of ALS family genes known as adhesion-related genes by using RT-PCR since these mutants might show different expression levels of *ALS* family genes from the wild type strain. The *bgl2Δ* formed almost the same amount of biofilms as the wild type strain, while, by using SEM, it showed a delay of yeast-to-hypha transition during the biofilm formation. The biofilm amount of the *bgl2Δ/ ecm33Δ* decreased compared with that of the *ecm33Δ* as well as the wild type. Disruption of both *ECM33* and *BGL2* led to reduction of the biofilm formation of *C. albicans* remarkably. On the other hand, *ALS1* and *ALS5* were overexpressed drastically in the *bgl2Δ/ ecm33Δ*, so that the difference of *ALS* gene expression did not influence the biofilm formation of these disruptants. From our results, Ecm33 played a vital role in the biofilm formation of *C. albicans* while Bgl2 contributed to the yeast-to-hypha transition during the biofilm formation.

45C

CANDIDA ALBICANS HSP90 REGULATES BIOFILM DEVELOPMENT IN CONCERT WITH TUP1

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The molecular chaperone Hsp90 plays a central role in the regulation of *Candida albicans* virulence traits, such as morphogenesis and drug resistance in planktonic and biofilm cellular states. To date, only two physical interactors have been described in *C. albicans,* but given Hsp90's critical role as a protein hub that modulates the genotype to phenotype translation it is imperative to understand how it regulates gene function on a global level. Thus, we mapped the first *C. albicans* Hsp90 genetic interaction network by screening a mutant library for hypersensitivity to Hsp90 inhibition in six environmental conditions. This screen revealed 226 interactors, most of which are highly specific and only required for growth in one or two environmental conditions. This led us to hypothesize that they function downstream of Hsp90 and are important for growth in only a subset of those conditions influenced by Hsp90. In this case, the interactors would depend upon the chaperone for stability and activation, which we demonstrated to be the case for Hog1 and others. Screening our network yielded five transcription factors (Bcr1, Mig1, Tec1, Tup1, Upc2) that had previously been implicated in biofilm development. We asked, how do Hsp90 genetic interactions affect *C. albicans* biofilm development? While Hsp90 depletion itself does not affect biofilm metabolic activity or dry weight, Hsp90 depletion in *tup1/tup1* and *tec1/tec1* mutants significantly increased biofilm dry weight, verifying their interactions with Hsp90. We next sought to determine if Hsp90 interacts directly with either transcription factor. Indeed, Hsp90 and Tup1 co-immunoprecipitate with each other, validating Tup1 to be a novel *C. albicans* Hsp90 physical interactor. This interaction remained stable at different temperatures and developmental stages. The functional consequences of the Hsp90-Tup1 interaction are being explored. We previously showed that Hsp90 affects gene expression levels of its genetic interactors. We hypothesized that Hsp90 regulation of gene expression differs between planktonic and biofilm cells. qRT-PCR revealed that Hsp90 modulates transcription factor gene expression in planktonic but not in biofilm cultures. Comparison of Hsp90 levels between cells grown planktonically and as biofilms, revealed that Hsp90 gene expression and protein levels are significantly lower in biofilms. Lastly, we screened transcription factor mutants for filamentous growth in response to Hsp90 depletion. Surprisingly, the *bcr1/bcr1* mutant demonstrated enhanced filamentation in response to reduced Hsp90 levels with filaments resembling those of the *tup1/tup1* mutant, a known repressor of Bcr1 function. Our work illuminates new regulatory connections between Hsp90 and key regulators of biofilm development, with implications for understanding divergent cellular regulation in distinct cellular states.

46A

CHARACTERIZATION OF HUMAN INTESTINAL FUNGAL COMMUNITIES USING A VALIDATED, COMBINED QPCR-TARGETED SEQUENCING STRATEGY

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Fungi, in particular Candida species, are important pathogens in premature infants, causing infections associated with high mortality and significant morbidity in survivors. In neonates, the intestine is a primary site for Candida colonization and invasion. Despite the importance of fungi in neonatal disease, there have been relatively few studies on how fungal

microbiomes are established, are modulated by clinical practices in the NICU, and are related to neonatal disease. To this end, we developed strategies to characterize fungal community structure and quantitatively evaluate Candida species in the intestine. Due to the very low relative amount of intestinal fungi as compared to bacteria, we initially screened specimens for the presence of fungal DNA by qPCR using pan-fungal primers that target the fungal rDNA locus of a broad range of fungi. These primers exhibited low false positive and negative rates and were sensitive in detecting ~ 1 C. albicans rDNA copy/reaction. Eleven (100%) fecal samples were positive by qPCR in at least 2 of 3 triplicate analyses, and subsequently processed for sequencing. Fungal sequences were amplified using barcoded primers developed to target the fungal ITS2 locus and paired-end sequencing was performed using the Illumina MiSeq platform. A custom fungal ITS2 database was created for use with a newly developed fungal-optimized bioinformatics pipeline that uses Mothur software. The workflow was validated using negative (meconium, expected to be sterile) and positive (meconium spiked with 4 known fungi) controls. Samples from infants were sequenced in triplicate (separate DNA isolations) to identify fungal taxa within the sample and the amount of experimental variation present. An average of 27,553 fungal reads (range 43-678,161) was obtained per sample. C. albicans was the most abundant taxon identified (56-99% of reads, present in all replicates of 100% of samples); all samples were also positive by qPCR using C. albicans-specific primers. C. krusei and C. parapsilosis were identified by sequencing in several samples, but only by species-specific qPCR in 1 sample, highlighting a challenge inherent in the study of low-abundance organisms. Fungal diversity (Shannon indices) in the samples ranged from 2.25 (most diverse, asymptomatic older infant) to 0.79 (least diverse, newborn with mucocutaneous candidiasis). In the latter case, 99% of sequence reads matched to C. albicans and analysis by qPCR found ~1x106 C. albicans cells/gm feces. Overall, a modest amount of variation was observed among replicate sequencing results underlining the importance of replication in metagenomic analyses. Combined results from qPCR and targeted sequencing of fungal ITS2 amplicons provide a more informative picture of fungal community structure in the intestine than either strategy alone and indicate that the infant intestine may contain diverse fungal species.

47B

CASPOFUNGIN INDUCES INCREASED HETEROGENEITY OF C. ALBICANS HYPHAL TIP DYNAMICS

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Candida albicans is a fungal commensal and opportunistic pathogen that forms biofilms on mucosal surfaces and medical devices. Biofilm development is characterized by distinct phases of initial adherence onto a surface, the development of a complex three-dimensional structure that is comprised of both cells and secreted extracellular matrix and the eventual maturation of the biofilm over the course of 24-48 hours. These structures contribute to the virulence of fungal pathogens, such as C. albicans, by facilitating drug resistance, colonization of tissues, and invasion. Live cell fluorescent imaging of C. albicans biofilms in a microfluidic culture system allows us to measure the dynamics of hyphal growth over time and biofilm development on a single cell level. This method facilitates accurate measurements of growth of C. albicans hyphal growth in biofilms under different experimental conditions. We have conducted experiments in early-stage spreading biofilms to compare control, untreated populations of C. albicans (SC5314) with populations treated with a sub-MIC concentration of caspofungin (0.015 ug/mL). Our results reveal differences in hyphal morphology and growth rate in the presence

and absence of drug. We hypothesized that partial inhibition of β-glucan synthases by sub-MIC caspofungin would slow the rate of hyphal elongation. After a single hour of sub-MIC caspofungin treatment, we are able to quantify differences in the rate of extension for C. albicans hyphae cultured in caspofungin from our controls. The distribution of hyphal extension rates became more heterogeneous in the presence of sub-MIC caspofungin, exhibiting both a long tail of more rapidly extending hyphae and also a population that grew much more slowly relative to controls. We also quantified differences in cell wall chitin content after switching growing hyphae to medium containing sub-MIC caspofungin or control medium without drug. We observed significantly higher cell wall chitin content at 2 hours post-caspofungin treatment, but chitin levels were equivalent at 3 hours post-treatment. From these results, we conclude that sub-MIC caspofungin treatment leads to increased heterogeneity in hyphal growth behavior and a transient increase in chitin deposition cell wall stress response. These may represent adaptive responses to improve population fitness under stress. Finally, we are building and validating a computational algorithm, implemented in Matlab, to identify and track individual hyphal tips based on the local rate of surface extension in 3 dimensions for a variety of biofilm dynamics measurements. This approach is automated and readily scalable to the large number of growing hyphae present in a biofilm. The authors acknowledge funding through NIH 5P50GM085273 as instrumental to the conduct of this research.

48C

THE ROLE OF ZFU2 IN YEAST CELL ADHERENCE AND BIOFILM FORMATION

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Candida albicans is a commensal fungus that resides on mucosal surfaces of most humans. It is the major fungal pathogen of humans with mortality rates as high as 30% in device-associated infections. One of the predominant causes of *C. albicans* infections is its ability to grow as a biofilm on the surface of medically implanted devices. Our work has focused on the effects of yeast cell adherence in the creation and maintenance of biofilms. Previously, we created a new reproducible adherence assay that led to the identification of thirty *C. albicans* transcription factors that govern adherence to silicone. Expression profiling and gene overexpression allowed for these regulators to be grouped into functional categories. Both approaches indicate that a large number of the adherence regulators converge to control adherence through a limited number of mechanisms. Here we investigate the role of the new transcription factor Zfu2. Unlike many of the other regulators of adherence we identified, Zfu2 has not been extensively studied and appears to regulate adherence and biofilm formation in a mechanism distinct from the other regulators. Our research revealed that *in vitro* a *ZFU2* deletion mutant is unable to form a biofilm and a complement of the deletion restores biofilm formation. Confocal microscopic images showed a rudimentary biofilm composed primarily of yeast cells, and the mutant biofilms had a 3-fold reduction in biomass. To test the significance of our observations to infection, *in vivo* catheter infection model biofilm assays were performed. The *zfu2Δ/Δ* mutant had a severe biofilm defect *in vivo*, and this defect was reversed by complementation with one wild-type copy of *ZFU2*. The study of this unique regulation holds the promise of identifying new mechanisms and mediators of adherence and biofilm formation.

49A

HIGH PREVALENCE OF *CANDIDA DUBLINIENSIS* IN LOWER RESPIRATORY SECRETIONS FROM CYSTIC FIBROSIS PATIENTS MAY BE RELATED TO INCREASED ADHERENCE PROPERTIES

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Cystic fibrosis (CF) is one of the common genetic disorders causing lung diseases in children and adults worldwide. The presence of thick and viscous mucus enables an entrapment of different air-borne microorganisms, such as bacteria and fungi. Recent investigations have shown that *Candida* spp. were the most common yeasts isolated from the lower respiratory tract of CF patients with *C. albicans* being the leading pathogen. Two identification methods, CHROMagar Candida and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOFMS) for differentiation of *Candida* spp. confirmed the different distribution between the tested isolates. A cohort of 20 CF patients was included in this study. From these patients, 25 clinical samples were collected (sputum, pharyngeal swab, BAL) and 26 isolates of *Candida* spp. were identified by MALDI-TOF MS. *C. dubliniensis* was most prevalent species (n=18, 69%) isolated mainly from sputum. This species showed the strongest adherence to a silicone substrate under constant flow when compared to other species of *Candida*. In the majority of cases *C. dubliniensis* was isolated in combination with *Pseudomonas aeruginosa* and *Staphylococcus aureus*.The species appear to be able to survive and coexist with bacteria. The data presented here shows that the presence of *C. dubliniensis* in the lower airways of CF patients may be related to increased adherence properties.

50B

ADHERENCE AND BIOFILM FORMATION OF MEDICALLY RELEVANT ARTHROCONIDIAL YEASTS AND CANDIDA SPECIES

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Candida infections are an important cause of morbidity and mortality in hospitalized patients, especially immunocompromised individuals, and those hospitalized with serious underlying diseases, such as hemato-oncological malignancies. The incidence of candidemia is growing and has dramatically increased within the past two decades. Such infections have been attributed to the common practice of prolonged hospitalization of highly susceptible patients receiving advanced medical treatment. In order to colonize the host and cause disease, arthroconidial yeasts such as (Trichosporon and Magnusiomyces) as well as Candida species must avoid being killed by host defense. Here, we sought to identify one of the main factors participating in disease mechanisms by assaying the adherence properties and biofilm formation ability of a number of different pathogenic fungi. Arthroconidial yeasts and Candida species were isolated from the clinical specimens using standard laboratory procedures and were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), sequence analysis of the D1-D2 domains of the large subunit ribosomal DNA (LSU rDNA) and the ITS1 and ITS2 regions of the rDNA. To investigate how yeast form cell adherence may contribute to the occurrence of different pathogenic fungi isolated in the clinical setting we utilized a previously established adherence assay. The assay examines the ability of yeast form cells to adhere to a silicone substrate under controlled flow, temperature and nutrient conditions. Standard in vitro biofilm formation biomass assays were performed to understand

the possible correlation of a particular fungi's biofilm density and its observed rates of infection in the clinical setting. Interestingly, the data shows that C. dubliniensis has the highest adherence property of the fungi assayed. Arthroconidial Non-Candida yeasts have statistically lower biofilm biomass than C. albicans and C. dubliniensis. These findings suggest that increased biofilm biomass correlate with the frequency of the species being isolated in the clinical setting in Qatar.

51C

INTESTINAL MICROBIOTA SHAPED BY AN AGRARIAN DIET MAY PREVENT FROM CANDIDA ALBICANS COLONIZATION

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In industrialized countries, Candida albicans is the predominant commensal yeast of the human intestine with ~40% prevalence in healthy adults. We recently observed a highly divergent colonization pattern, in a remote homogenous Amerindian (Wayampi) community from French Guiana, where C. albicans prevalence was <7%. A hypothesis to explain this challenging finding is that the intestinal microbiota of this community, shaped by a traditional diet and way of life, presents a specific pattern preventing C. albicans colonization. To study this hypothesis, we analyzed the intestinal microbiota of 23 adults from this community. Three of these individuals carried C. albicans, while the other 20 were C. albicans free. Using 16S rDNA gene pyrosequencing, we recovered 2,500 sequences per sample. Overall the microbiota was rather homogeneous among the subjects studied, with a higher abundance of Bacteroidetes (45% of Operational Taxonomic Units identified/ individual) compared to Firmicutes (40%), as opposed to previous observations made in European subjects using the same techniques. In most samples, Prevotella genus (Bacteroidetes phylum) was the most abundant taxon (>25%), which featured an intestinal microbiota shaped by an agrarian diet. Indeed, Prevotella is known to contain a set of genes allowing for cellulose hydrolysis. Various other genera, Faecalibacterium, Ruminococcacae, Lachnospiraceae, Ruminobacter, Succinivibrio, all with specific abilities to maximize metabolic energy extraction from vegetal polysaccharides, were also highly represented. Finally, the microbiota patterns of the 3 C. albicans carriers did not differ significantly from that of the 20 non-carriers in terms of composition and taxon abundance. Altogether, this study suggests that an intestinal microbiota shaped to produce the highest amount of energy from a vegetarian diet with high fiber intake, as observed in agrarian societies, could correlate with an unexpected under-representation of C. albicans intestinal carriage.

52A

COMPLEX AND COOPERATIVE EXTRACELLULAR MATRIX COMPONENTS OF CANDIDA ALBICANS BIOFILMS IMPAIR ANTIFUNGAL DRUG PENETRATION

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Background: Matrix is a distinct biofilm feature shown to play numerous roles in biofilm survival. Detailed composition remains a knowledge gap. Previous study of the *Candida* matrix has identified β 1,3 glucan, DNA, and protein. The goal of the present studies was to provide a comprehensive investigation of matrix composition. **Methods:** A rollerbottle system was used to produce 60m^2 of *C. albicans* biofilm. Matrix was collected by vortex and waterbath sonication followed by dialysis and lyophilization. Carbohydrate analysis was performed using a combination of GC, GC/

MS, NMR, ELISA, Glucatell, and SAXS. Lipidomics and proteomics were undertaken by GC and LC/MS. Matrix DNA was isolated, cloned and sequenced. Monoclonal antibodies were used to search for polysaccharide components in intact biofilms in vitro and in vivo with microscopy. Fluorescent DNA and lipid stains were imilarly utilized. NMR was used to explore an interaction between antifungal rug and matrix components. **Results: Q**antitative matrix assessment identified proteins (55%) and neutral crbohydrates (~25%), as well as lipids (15%), and DNA (5%). Polysaccharide nalysis revealed 3 molecules: α-1,6 mannose with α-1,2 linked mannose branches 85%), β-1,6 glucan (13%), and β-1,3 glucan (surprisingly <1%). SAXS mdeling suggested an interaction between mannan and β-1,6 glucan. Each mlecule was also identified in the matrix of intact in vitro and clinical bofilms. Glycerolipids and sphingolipids were identified in lipid analysis. Ergosterol was the only sterol identified. The matrix proteome consisted of more than 400 proteins. The major functional was carbohydrate and amino acid metabolism. The putative function of several enzymes would be predicted to modify the matrix polysaccharides. The in vivo proteome found a smaller number of these components. NMR demonstrated an interaction between aggregate matrix and fluconazole. The spectroscopy pattern suggests an interaction with multiple matrix components. Analysis of individual matrix polysaccharide did not demonstrate an interaction, consistent with a cooperative matrix component model. **Conclusions:** Matrix analyses revealed complexity and included all major macromolecular classes. Finding 3 polysaccharides and interaction of two represent an unusual feature compared to most microbial species. Several of these molecules were also identified in clinically relevant biofilms in vivo. Structure-function analysis suggests collaborative function of matrix constituents for the drug resistance phenotype.

53B

β 1,6 GLUCAN IN THE *C. ALBICANS* BIOFILM MATRIX COOPERATES WITH OTHER MATRIX CONSTITUENTS TO PRODUCE A DRUG RESISTANCE PHENOTYPE

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Background: Infections due to *Candida* biofilms are generally recalcitrant to most antifungal therapy. One mechanism that accounts for this phenotype is reduced drug penetration due to the extracellular matrix. Previous studies link matrix β1,3 glucan concentration to this antifungal drug sequestration. Our recent studies identified additional matrix components, including β 1,6 glucan, which represents more than 10% of the matrix polysaccharide. We sought to characterize the production and delivery of matrix β 1,6 glucan and examine its role in biofilm formation and drug resistance. **Methods: and Results:** To address this hypothesis, we constructed a library of 8 homozygous deletion mutants for genes with putative β 1,6 glucan production or modification function. Mutants were screened for adherence, biofilm formation and SEM architecture. Each of the mutants exhibited normal biofilm formation. Mutant biofilms were screened for matrix β 1,6 glucan content using an ELISA. Three of the mutants (KRE5, BIG1, SKN1) tested had reduced levels of matrix β 1,6 glucan, with a 30-60% reduction compared to wildtype. We next determined if matrix β 1,6 glucan contributes to the biofilm drug resistance phenomenon. Each of the mutant strains with reduced matrix β 1,6 glucan also had increased susceptibility to fluconazole and amphotericin. However, planktonic MICs were similar for mutants and the WT strain. In vivo biofilm drug testing corroborated the in vitro results. Two of these same mutant biofilms retained lower levels of drug in the matrix (>50% reduction) in a ^{3}H-fluconazole sequestration assay (KRE5 and

BIG1). The previously demonstrated impact of another matrix component, β-1,3 glucan led us to postulate a physical and structural interaction among matrix constituents. Thus, we also measured matrix β 1,3 glucan and mannan in the β 1,6 glucan mutants. Remarkably, the majority of mutants with reduced levels of β 1,6 glucan also exhibited a reduction of matrix β-1,3 and mannan, consistent with interaction among matrix polysaccharides. **Conclusion:** We have shown that β 1,6 glucan plays a role in biofilm associated antifungal resistance and have identified several genetic components of the matrix β 1,6 glucan pathway. Furthermore, these studies suggest a model of cooperative interaction of matrix components for biofilm function.

54C

MOONLIGHTING TRANSCRIPTION FACTOR: A BIOFILM-SPECIFIC ROLE FOR *C. ALBICANS* UPC2 DURING BIOFILM GROWTH

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Background: UPC2 is a *Candida albicans* transcription factor known for its roles in regulating ergosterol synthesis and associated triazole susceptibility in planktonic cells. We identified UPC2 in a biofilm drug susceptibility screen of Zn finger TF mutants. We theorized that this was related to the changes in membrane sterol content demonstrated in previous planktonic studies. **Methods:** A 232 null mutant zinc finger transcription factor library was screened for fluconazole susceptibility relative to the WT strain during biofilm growth. Strains with increased susceptibility were screened for adherence, mature biofilm formation, and extracellular matrix composition. For UPC2, we also assessed changes in membrane sterols during planktonic and biofilm growth. Quantitative real-time RT-PCR was then used to screen for alterations in expression for genes of interest in the mutant from the parent strain. **Results:** The UPC2 null mutant exhibited marked susceptibility to fluconazole during biofilm growth (70% reduction). In vivo studies in the rat catheter model were congruent with this phenotype. Surprisingly, biochemical analysis demonstrated no change in membrane ergosterol concentration between mutant and parent strains when grown as biofilms. However, matrix β-1,3 glucan, β-1,5 glucan and mannan concentrations in the mutant were markedly reduced to by more than 50% compared WT levels, respectively. Select transcriptional analysis of the mutant and WT biofilms demonstrated a multi-fold reduction in expression for matrix production/modification associated genes. The β-1,3 glucan synthase FKS1 was reduced 1.79 fold. The β-1,6 glucan genes tested (BIG1, KRE1, KRE5, KRE6, and SKN1) were reduced 2.63, 1.54, 2.94, 3.85 and 3.23 fold respectively. Finally, mannan associated genes tested (ALG11, MNN4-4, MNN9, MNN11, and VRG4) also had significant multifold reductions of 2.0, 3.57, 2.33, 2.38 and 3.23 fold respectively. With the exception of VRG4, all gene expression levels tested in planktonic RNA only show a maximum reduction of 1.41. **Conclusion:** These studies suggest that UPC2 has environmentally distinct role in biofilm compared to planktonic infection. Current studies are exploring potential biofilm associated binding partners.

55A

DYNAMICS OF MIXED *CANDIDA* SPECIES BIOFILMS IN RESPONSE TO ANTIFUNGAL TREATMENT

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In many immunocompromised patients, *Candida* spp. biofilms are associated with oral candidiasis, the most common opportunistic fungal infection in humans. These biofilms

are more resistant to antifungal treatment than single, planktonic cells. However, the mechanisms and factors that contribute to this resistance are not well-understood. Although *C. albicans* is the predominant organism found in patients with oral thrush, there is an increasing incidence of oral colonization and infections caused by non-*albicans Candida* spp. (*C. glabrata, C. dubliniensis* and *C. tropicalis*). The emergence of non-*albicans Candida* species that are intrinsically drug resistant or rapidly develop drug resistance is clinically relevant as conventional doses of antifungals are frequently ineffective at treating infections caused by these species. While single-species *Candida* biofilms have been well-studied, considerably less is known about the dynamics of *Candida* spp. in mixed biofilms, especially in response to antifungal treatment. In order to address this question, we have recently developed a highly accurate quantitative PCR-based approach to determine the precise composition of mixed *Candida* spp. biofilms. We generated pre-formed, mixed biofilms using three different sets of *Candida* strains (general laboratory strains as well as susceptible and resistant clinical isolates), and determined the species composition of these biofilms in the presence and absence of a clinically relevant concentration of the widely used antifungal, fluconazole (FLC, 32 μg/mL). In monospecies biofilms generated from general laboratory strains and susceptible clinical isolates, FLC exposure favored the growth of *C. glabrata* and *C. tropicalis*. For these same sets of strains, the quantity of *C. dubliniensis* monospecies biofilm remained consistent while that of *C. albicans* decreased following treatment with FLC. We observed that in a mixed biofilm generated from clinical isolates consisting of three or more species, *C. albicans* lost dominance in the presence of FLC. Surprisingly, there was no significant difference in the total amount of a mixed biofilm consisting of *C. albicans, C. glabrata, C. dubliniensis* and *C. tropicalis* following drug exposure for all sets of strains. Overall, our data suggest that FLC favors growth of certain non-*albicans Candida* species and may not be an effective treatment for pre-formed, mixed biofilms. In addition to providing new information about the dynamics of mixed *Candida* species biofilms, these studies will also pave the way for the development of more effective antifungals.

56B

MATURE CANDIDA GLABRATA BIOFILM DEVELOPMENT IN A SUBCUTANEOUS RAT MODEL.

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So far, biofilm studies have been mostly dedicated to a major human fungal pathogen - C. albicans, but little is known about the virulence factors that allow non-C. albicans species, such as C. glabrata to colonize the human host and to cause infections. Herewith, we translated our C. albicans rat subcutaneous biofilm model to study in vivo mature C. glabrata biofilms. Biofilms were developed inside serum-coated triple-lumen polyurethane catheter pieces. In the subcutaneous model, the implant of multiple Candida-infected devices to the back part of the animal provides a big advantage as it allows us to study several biofilms in one animal. Mature C. glabrata biofilms developed within the first 48 h and the amount of biofilm fungal biomass remained stable up to 6 days. Architecturally, mature C. glabrata biofilms consist of a thick network of yeast cells embedded in extracellualr matrix, as documented by scanning electron microscopy and confocal scanning laser microscopy. Next, we analysed the effect of echinocandins on in vivo mature C. glabrata biofilms by daily intravenous injection of micafungin (10 mg/kg of body weight), caspofungin (5 mg/kg of body weight) and anidulafungin (10 mg/kg of body weight). Fluconazole (125 mg/kg of body weight) was used as a control. In vivo biofilms were equally susceptible to echinocandin drugs, whereas fluconazole remained inneffective. The C. glabrata adhesion process and biofilm

development are regulated by group of genes belonging to the EPA and AWP gene families. Because of this reason, we analyzed the transcriptional profile of EPA1, EPA3, EPA6 and AWP1 - AWP7 in in vivo biofilms formed for 6 days. EPA3, EPA6, AWP2, AWP3 and AWP5 were upregulated in in vivo biofilms compared to in vitro biofilms (6 days biofilm formation on polyurethane catheter pieces placed in 24-well polystyrene plates). To our knowledge, this is the first study, which described in vivo C. glabrata biofilm development in detail and provide an insight into the susceptibility profile, as well as the gene expression machinery of biofilm formation.

57C

IN VITRO ADHESION AND BIOFILM FORMATION IN C. GLABRATA IS AFFECTED DIFFERENTIALLY IN DIFFERENT CULTURE MEDIA AND EXPOSURE TO HYPOXIA (1% O2) AND HYPOXIA MIMETIC COCL2

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C. glabrata, a nondimorphic, haploid yeast accounts for 12 to 15% of all successful Candida infections particularly blood stream infections (Pfaller et al., 2010; Kaur et al., 2005; Jandric & Schuller, 2011;) with the mortality rate of 30% (Klevay et al., 2009). During infection in host, the pathogen faces environmental challenges that are crucial for their survival and virulence. One such challenge is hypoxia, to which both pathogen and host tissue are exposed. Pathogen requires adaptations to hypoxic condition for surviving the hypoxic environment of host i.e. 2.5 to 9.0 % O2 in healthy tissues and ≤ 1% in tumors and wounds (Grahl et al., 2012).). Several mechanisms for hypoxia adaptations and virulence during hypoxia in C. albicans have been investigated (Chung et al., 2012; Grahl et al., 2012; Symnott et al., 2010; Ernst & Tieker, 2009). Hypoxia is found to be associated tightly with the virulence factors and responses to antifungal drugs in C. albicans. The mechanisms of hypoxic adaptations and interrelationship of hypoxia with virulence in the C. glabrata remain unaddressed. CoCl2 in sublethal concentrations mimics hypoxia like state in S. cerevisiae (Vasconcelles et al., 2001; Kumar et al., 2011), Cryptococcus neoformans (Lee et al., 2007) and mammalian systems (Wang & Semenza, 1995). To support our hypothesis, we have investigated the effects of hypoxia (1% O2 in atmospheric chamber) and hypoxia mimetic CoCl2 (0.75mM) on adhesion and biofilm formation in wild type C. glabrata (MTCC3019). RT-PCR data has shown the upregulation of TDH3 a marker of hypoxia (Synnott et al., 2010) after exposure to 1% O2 and 0.75mM CoCl2. Cells were exposed to 1% O2 using hypoxia chamber (Conditions: CO2:5%, O2: 1% and remaining N2 gas). Adhesion (at 370C for 90 minutes) was quantified by XTT- reduction assay on 450nm in 96 well polystyrene plate following Raut et al., 2013. Adhesion ability was found independent of media and different stresses taken here in this study. Biofilm assay was performed following Tsang et al., 2012 in 96 well polystyrene plates in YNB and RPMI at 370C for 60 hours. Metabolic activity of biofilm in 1% O2 was dramatically enhanced (4 times of control) in RPMI. CoCl2 did not affect biofilm formation in RPMI. This study shows that hypoxia (1% O2) has enhanced the biofilm formation by C. glabrata in RPMI medium remarkably without affecting adherence on polystyrene surface. Though hypoxia mimetic in terms of metabolic pathways but CoCl2 did not affect biofilm formation and adherence significantly in either media. Enhanced activity of biofilm might be due to upregulation of EPA6 upon hypoxic exposure (Dormergue & Cormack, 2009). Kucharikova et al., 2011 have shown that EPA6 expression is reduced in the YNB media and adhesion remains unaffected by type of medium in C. glabrata due to inability of pathogen for hyphal transition. This is the first report specifically on effect of hypoxia and CoCl2 on adhesion & biofilm.

58A

CANDIDA ALBICANS AUGMENTS STAPHYLOCOCCAL VIRULENCE BY ACTIVATING THE AGR QUORUM SENSING SYSTEM

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The polymorphic fungus *Candida albicans* and the ubiquitous bacterial pathogen *Staphylococcus aureus* are responsible for a myriad of human diseases, often co-infecting critically ill or immunocompromised patients. Using a murine model of fungal-bacterial intra-abdominal infection (IAI), initial studies have demonstrated that infection with either *C. albicans* or *S. aureus* alone is non-lethal; however, co-infection with both species led to dramatic increases in mortality and microbial burden. The precise mechanism for the observed infectious synergism remains undefined, but previous work has suggested involvement of *S. aureus* toxin production. To that end, we hypothesized that *C. albicans* may augment the expression of a repertoire of *S. aureus* virulence factors, including toxin production. To test this hypothesis, cultures of *S. aureus* (strain JE2), *C. albicans*, or both organisms together were grown under planktonic and biofilm growth conditions. Cell-free supernatants from polymicrobial cultures showed increased hemolysis *in vitro*. Use of an isogenic *S. aureus* Δ*agrA* mutant confirmed that enhanced hemolysin production was *agrA*-dependent. Fractionation of the supernatant demonstrated increased production and lytic activity of two secreted proteins, consistent with hemolytic patterns of alpha and delta(-like) hemolysin. Both of these toxins are partly regulated by the staphylococcal *agr* quorum sensing system. Use of a GFP-reporter plasmid demonstrated enhanced *agr* activity in co-culture as compared to monomicrobial growth, and qPCR and Western blot analysis of *agr*-regulated genes further supported these results. Lastly, Swiss-Webster mice were intraperitoneally inoculated with sub-lethal doses of *C. albicans* + *S. aureus* (JE2), *C. albicans* + *S. aureus* (Δ*agrA*), or *S. aureus* (JE2) alone and monitored for morbidity and mortality. Alarmingly, 80% of the mice co-inoculated with *C. albicans* + JE2 succumbed by d 1 post-inoculation, while only 30% of the mice co-inoculated with *C. albicans* + Δ*agrA* succumbed to the infection but not until significantly later (d 8 post-inoculation). All mice inoculated with JE2 alone survived throughout the infection time course. In summary, *C. albicans* augments *S. aureus* virulence factor production *in vitro* via activation of the *agr* quorum sensing system. Furthermore, rapid mortality observed during *C. albicans*-*S. aureus* IAI occurs via an *agrA*-dependent mechanism. These results further elucidate the complex interaction between *C. albicans* and *S. aureus* during polymicrobial growth and provide further insight into the high mortality rates observed during fungal-bacterial polymicrobial intra-abdominal infections.

59B

CANDIDA ANTIBIOLFIM AND CYTOTOXICITY OF COLLOIDAL SILVER NANOPARTICLES

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The effect of different colloidal silver nanoparticles (SNP) on ATCC Candida albicans and Candida glabrata cells as well as the cytotoxicity of mouse fibroblasts (cell line L929) was evaluated in this study. SNP of average size 5 nm were synthesized by the reduction of silver nitrate through sodium citrate and were stabilized with ammonia (SNP-A) or polyvinylpyrrolidone (SNP-P). Minimal inhibitory

concentration (MIC) tests were performed for C. albicans (n=2) and C. glabrata (n=2) grown in suspension following the CLSI microbroth dilution method. SNP were applied on adhered cells (2 h) or biofilms (48 h) and after 24 h of contact their effect was assessed by colony forming units (CFUs) enumeration. To evaluate the cytotoxicity, L929 cells were exposure to SNP (0.1-100 µg/mL), and after 6, 24 and 48h MTT assay was performed. MIC results showed that SNP were fungicidal against all strains tested at very low concentrations (0.4-3.3 µg/mL). They were highly effective on adhered C. glabrata and respective biofilms. On C. albicans the effect was not so evident but there was also a reduction in the number of biofilm viable cells. Regarding cytotoxicity, silver nanoparticles inhibited the fibroblasts cells viability in a concentration-dependent manner. The type of stabilizing agent interfered on the cytotoxicity of SNP being SNP-A more toxic to L929. In summary, SNP in lower concentrations might have potential to be an effective alternative to conventional antifungal agents for future therapies in Candida-associated denture stomatitis.

60C

EFFECTS OF COMBINATION ANTIMICROBIALS ON *CANDIDA GLABRATA* AND *STAPHYLOCOCCUS AUREUS* BIOFILMS

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Backgroud: Polymicrobial biofilms (PB) are capable of acting as reservoirs for a variety of microorganisms. Previous studies with *C. albicans* (*Ca*) and *S. aureus* (*Sa*) biofilms showed that *Ca* and *Sa* coexist synergistically in mixed microbial biofilm and the antimicrobial drug susceptibility of PB is varied from that of monomicrobial biofilm (MB). Since no such studies have been undertaken with *C. glabrata* (*Cg*)/*Sa* biofilms we investigated the effects of selected antimicrobials on *Cg/Sa* biofilms. **Methods:** MB and PB of *Cg* ATCC 90030 and *Sa* ATCC 43300 were developed in 96-well flat bottom microtiter plates in either BHI (*Sa*) or SD (*Cg*) broth for 24 h at 35 °C. The biofilms were washed and exposed to vancomycin (VAN), ceftaroline (CFT) and daptomycin (DAP) alone and in combination with caspofungin (CFG) for 24 h. The drug-treated biofilms were washed, resuspended and the effect of drug treatment was determined by CFU assay. **Results:** *Sa* MB was more susceptible to VAN (1 log CFU reduction) compared to PM biofilm (0.5 log CFU reduction). When VAN was used in combination with CFG, there was a 2 logs CFU reduction in MB and 1 log CFU reduction in PB. CFT alone produced 0.5 log CFU reductions in both PB and MB of *Sa,* whereas with CFG, there was a 2 log and 0.5 log reduction of CFU in MB and PB, respectively. DAP provided a 0.5 log CFU reduction in MB and a 1 log CFU reduction in PB, but with CFG, a 2 log reduction in MB and a 0.5 log reduction in PB, respectively was obtained. For *Cg,* there was significant CFU reduction in MB (2 log) compared to PB (1 log) when treated with CFG. In combination with VAN similar results were obtained, whereas in combination with CFT and DAP, there were also similar reductions of 1 log CFU each. **Conclusions:** Two-drug combinations with CFG/VAN, CFT or DAP were more effective against MB of *Sa* compared with *Sa/Cg* PB. For *Cg* MB and PB, combination antimicrobials had a minor effect. This is in sharp contrast to previous studies with *Sa/Ca* PB which showed that combination treatment with an antifungal and antibacterial drugs resulted in improved activity against these resistant communities.

61A

SILVER COLLOIDAL NANOPARTICLES: EFFECT ON CANDIDA CELLS AND SUBCUTANEOUS TISSUE OF RATS

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This study investigated the effect of different colloidal silver nanoparticles (SNP) on oral Candida albicans and Candida glabrata cells and on the subcutaneous connective tissue reaction of rats. SNP of average size 5 nm were synthesized by the reduction of silver nitrate through sodium citrate and were stabilized with ammonia (SNP-A) or polyvinylpyrrolidone (SNP-P). Minimal inhibitory concentration (MIC) assays were performed using the microdilution methodology. The antibiofilm activity of SNP was determined by total colony forming units enumeration. The tissue reaction was carried out with polyethylene tubes containing silver nanoparticles (1.0 µg/mL; 540 µg/mL) implanted in the dorsal connective tissue of Wistar rats for 7, 15, 30, 60, and 90 days. The specimens were stained with hematoxylin and eosin and qualitative and quantitative evaluations of the reaction were carried out. MIC results showed that all SNP colloidal suspensions were fungicidal against the tested strains at very low concentrations (0.4-3.3 µg ml-1). In general, all SNP suspensions promoted significant log10 reduction of the mean number of cultivable biofilm cells after exposure to silver concentrations at or higher than 108 µg ml-1. The results showed the particle size and the type of stabilizing agent used did not interfere in the antifungal activity of SN against Candida biofilms. Histological examination showed SNP at 540 µg/mL induced significant tissue reaction on 30 and 60 days after implantation compared to the controls groups (fibrin and saline 0.9%) at the same periods. The inflammatory responses caused by SNP at 1.0 µg/ml, NH3 at 0.13 x 10-3 mol/L and PVP at 0.19 g/L solutions were similar to the controls groups in all experimental periods. Although for both SNP-A and SNP-P at 540 µg/mL have induced significant inflammatory response in rat's subcutaneous tissue, in the future these particles at lower concentrations may contribute to the development of new strategies for the improvement of oral health and quality of life particularly of the complete denture wearers.

62B

REVISITING THE ROLES OF TEC1 AND CPH1 IN THE FORMATION OF COMPLEX MTL-HOMOZYGOUS BIOFILMS IN CANDIDA ALBICANS.

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We previously discovered that MTL-homozygous, mating-incompetent white cells responded to pheromone, but not through the mating response pathway, as is the base of mating-competent opaque cells. Instead, they respond by forming a complex biofilm after 48 hr on elastomer in RPMI 1640 medium at 25°C or 37°C, in air or in 5% to 20% CO2. This response is mediated by the same MAP kinase pathway that mediates the opaque cell mating response, but targets a different major transcription factor, Tec1. Recently, the transcription factor target of the pathway we identified was challenged by Lin et al. (2013; PLoS Pathog 9(4):e100330). However, these researchers used conditions that did not support the formation of a complex biofilm, but rather one architecturally un-complex, consisting primarily of a thick yeast cell polylayer with greatly diminished hyphae and matrix

formation (Daniels et al., 2013; Eukaryot Cell 12(10):1389). Here we further explore the role of Cph1 and Tec1 in MTL-homozygous complex biofilm formation on elastomer, in RPMI 1640 medium, using mutants that have been re-verified by sequencing, and using high resolution two-photon laser scanning confocal microscopy to examine biofilm architecture. We show that the tec1/tec1 mutant forms an architecturally defective, thin biofilm, with a defective basal layer, sparse short randomly oriented hyphae, and dramatically reduced matrix, as we previously published. In contrast, the cph1/cph1 mutant forms an architecturally complex biofilm, with a thick basal layer of cells, and a thick upper layer representing 80% of the biofilm and composed of vertical hyphae encased in dense matrix. Only subtle differences were observed between cph1/cph1 and wild type biofilms, as previously reported (Yi et al, 2008; Mol Biol Cell 19(3):957). We further find that depending on the production lot and vendor of the tissue culture plastic used in the adhesion assay we originally developed, differences between cph1/cph1 and tec1/tec1 cells could be reversed, suggesting this assay, which we abandoned two years ago, is not reliable. These results confirm our former conclusion that Tec1 plays a major role and Cph1 a minor one, in complex biofilm formation.

63C

ROLE OF CANDIDAL ACETALDEHYDE PRODUCTION IN PERSISTENT BACTERIAL INFECTIONS IN CYSTIC FIBROSIS

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Introduction: The lungs of cystic fibrosis (CF) patients become colonised with a variety of bacterial species, commonly with *Pseudomonas aeruginosa*. *Candida* species feature highly in the CF lung microbiome, however their role in disease pathogenesis is not fully understood. Co-existence of *Candida* and *P. aeruginosa* in respiratory tract biofilms poses a significant treatment challenge due to an association with increased clinical resistance to antimicrobials. We have shown that *Candida* spp. can produce significant levels of mutagenic acetaldehyde (ACH) as part of their ethanol metabolism. Potentially, candidal ACH could facilitate the development of antibiotic resistance and *Pseudomonas* transition to hypervirulence in mixed-species biofilms by causing mutations within DNA mis-match repair (MMR) genes (*MutS*, *MutL*) and inducing up-regulation of the error prone DNA polymerase IV (*DinB*). **Aims:** First, to look for clinical evidence of concomitant *Candida* and *P. aeruginosa* colonisation associated with the development of antibiotic resistance retrospectively in a cohort of CF patients. Second, to analyse the effects of ACH exposure on *MutS* and *MutL* sequence integrity and *DinB* gene expression in *P. aeruginosa* biofilms *in vitro*. **Hypothesis:** *Candida* co-colonisation is linked to changes in bacterial resistance patterns and exposure to mutagenic ACH leads to defects in *MutS*, *MutL* and up-regulation of *DinB*. **Methods:** Medical records and microbiological findings of 77 patients followed up at our hospital since 2006 were analysed retrospectively for oral candidiasis, lung *Candida* colonisation and *P. aeruginosa* antibiotic susceptibilities. In addition, the effects of ACH on *P. aeruginosa* biofilm *DinB* expression was analysed by real-time PCR, potential mutations within the MMR genes were investigated by sequencing. Increased mutation rate was investigated by analysis of rifampicin resistance. Biofilm growth and morphology was examined by crystal violet and Live/Dead staining. **Essential results:** Oral candidiasis frequently coincided with *P. aeruginosa* exacerbations and antibiotic resistance changes. *In vitro* ACH exposure impaired cell membrane permeability and biofilm organisation and increased biofilm biomass production of *P. aeruginosa*. *DinB* was upregulated although no mutations within MMR genes were identified. **Conclusions:** These results suggest that *P. aeruginosa*

can respond to mutagenic ACH exposure by increasing its *DinB* gene expression and its alginate production and that candidal ACH could facilitate the transformation to hypervirulence and antibiotic resistance in *P. aeruginosa*.

64A

A SCREEN FOR IDENTIFYING AGENTS THAT CAUSE PATHOGENIC A/ALPHA BIOFILMS TO BECOME PERMEABLE, PENETRABLE AND DRUG SUSCEPTIBLE.

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"Pathogenic" biofilms formed by Candida albicans a/alpha cells on silicone elastomer at 37°C, in air or 20% CO2, in RPMI 1640 medium are impenetrable to leukocytes, impermeable to high and low molecular weight molecules and drug-resistant. We have developed a screen for agents that cause penetrability and drug susceptibility. In this screen, biofilms formed in 12 well plates on elastomer discs are overlaid with cells of a leukocyte line expressing GFP. These cells will not penetrate a biofilm generated by untreated a/alpha cells. Penetration of the leukocytes is assayed by confocal microscopy. The treated biofilms are also analyzed for architecture, for the wild type phenotype which includes a multicellular basal layer (the lower 20% of the biofilm) and an upper layer (80%) composed of vertical hyphae embedded in a dense matrix. We have used the assay to test 44 natural products so far. Eight of the natural products caused penetration. These were then tested for susceptibility to fluconazole. The identity of the active agent in each natural product is now being investigated. These purified agents will then be retested in the in vitro model and then in tissue and animal models, now being developed in which pathogenic a/alpha biofilms are formed. Testing in all models will also be performed with fluconazole to assess whether these agents facilitate drug susceptibility.

65B

TRANSCRIPTIONAL AND METABOLIC RESPONSES OF *CANDIDA ALBICANS* BIOFILMS TO MUTAGENIC LEVELS OF ACETALDEHYDE

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Introduction:*Candida albicans* biofilms are resistant to many antifungals and can produce high (>100µM) levels of acetaldehyde. Acetaldehyde is a mutagenic intermediate of alcohol fermentation associated with the carcinogenicity of persistent candidal infections. ACH is also an important mediator of candidal biofilm formation. **Aim:** To investigate the effects of acetaldehyde exposure on growth, alcohol metabolism and expression of a set of acetaldehyde metabolism,azole resistance and DNA mismatch repair genes in *C. albicans* biofilms. **Hypothesis:***C. albicans*tolerates exposure to acetaldehyde byhigh expression of its MMR genes. In fluconazole-resistant isolates this can be maintained when co-exposed to fluconazole. **Methods:** Twelve isolates of *C. albicans*with differing fluconazole MICs isolated from patients with chronic mucocutaneus candidiasis (n=4), candidalvulvovaginitis (n=4) or candidemia (n=4) were grown on coverslips for 24h at 37°C before being treated with 100 µM and 10 mM acetaldehyde for 2h. Metabolic activity was measured by XTT and acetaldehyde production by gas chromatography. Changes in the expression of DNA mismatch repair genes *MLH1* and *EXO1,* acetaldehyde metabolismgenes *ADH1* and *ALD5*and azole resistance gene*ERG11*were measured by RT-PCR. **Results:** Biofilm metabolic activity of resistant isolates was not affected by exposure to low levels of acetaldehyde but was decreased in all isolates at 10 mM.This exposure also decreased acetaldehyde catabolism and expression of genes responsible for this. At 100 µM acetaldehyde*ALD5* expression was

increased, particularly in resistant isolates. The expression of DNA mismatch repair genes, *MLH1* and *EXO1*, was inherently high and up-regulated in some susceptible isolates after acetaldehyde exposure. *EXO1* expression correlated with XTT.*ERG11* expression level correlated with the metabolic activity of resistant isolates. **Discussion:** The results of this study show that candidal biofilms tolerate high levels of acetaldehyde exposure by up-regulating DNA mismatch repair.

66C

FURTHER PROOF THAT THE TRANSCRIPTION FACTOR BCR1 IS NECESSARY FOR MTL-HETEROZYGOUS, BUT NOT WHITE MTL-HOMOZYGOUS, BIOFILM FORMATION

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Bcr1 appears to be the major downstream regulator of the MTL-heterozygous biofilm matrix, which is responsible for the pathogenic traits these biofilms exhibit. These traits include drug resistance, impenetrability by leucocytes and impermeability toward high molecular weight molecules. Bcr1 is also necessary for the vertical orientation of hyphae in the upper region of a biofilm and biofilm thickness. Bcr1 does not play a major role in MTL-homozygous biofilms, which lack these traits. Activation of Bcr1, leading to its functional state, is mediated by the protein kinase Cbk1, a major component of the RAM network. We tested whether activation of Bcr1 by Cbk1 is necessary for matrix formation and the pathogenic traits of MTL-heterozygous biofilms, by analyzing the biofilms formed by the mutant cbk1/cbk1. MTL-heterozygous cbk1/cbk1 biofilms were defective, exhibiting reduced thickness, penetrability by leukocytes, drug susceptibility and a defective upper region, involving reduced matrix and disorientation of hyphae. Mutant cbk1/cbk1 MTL-homozygous biofilms were indistinguishable from wild type biofilms. They exhibited the same architecture as wild type MTL-homozygous biofilms. These results reinforce the conclusion that Bcr1 is the major regulator of matrix formation in a/α biofilms, but not a/a or α/α biofilms.

67A

INHIBITION OF MEDICAL SILICONE BIOFOULING BY CANDIDA IN MIXED-SPECIES BIOFILMS

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Soft silicones are used for the manufacture of medical devices such as PEG tubes, intra-nasal stomach tubes and voice prostheses. Such devices require frequent replacement due to the build-up of mixed-species biofilms, which obstruct device function. We are investigating the process of biofilm establishment and maturation during long-term (~6 weeks) mixed-species colonization. In particular, we are investigating the ability of microbes to compromise silicone function by ingress and degradation. We are currently trialling a variety of approaches to silicone modification with the aim of extending the useful life of medical devices. We are testing these against a panel of bacterial (*Proteus mirabilis, Klebsiella oxytoca*, and *Micrococcus luteus*) and *Candida* (*albicans, dubliniensis,* and *parapsilosis*) species isolated from a single clinical prosthetic device. Initial screening of various permanent alterations of the silicone surface identified a candidate modification strategy with long-term potential. AMPEG450, a hydrophilic, linear polymer containing a (-O-CH2-CH2-) repeating unit and a terminal vinyl functional group, was attached to an HMS501-modified Nusil (DDU4320) silicone surface, in order to impart a hydrophilic character to an otherwise hydrophobic surface. Contact angle measurements

of AMPEG450-modified silicone confirmed a decrease in hydrophobicity (mean sessile drop advancing water contact angle alteration from 101.78 to 79.00). The initial adhesion of the 3 isolated *Candida* strains was reduced by 90% on the modified silicone, and initial adhesion of bacterial strains was similarly reduced (>70%). 48-hour biofilm formation of *C. albicans* was significantly decreased on AMPEG450-modified silicone compared to unmodified Nusil. Studies involving long-term biofilm experiments will confirm whether the conceptual approach underpinning this silicone modification is suitable for further development.

68B

ENTEROCOCCUS FAECALIS DISRUPTS BIOFILM FORMATION AND HYPHAL MORPHOGENESIS IN CANDIDA ALBICANS

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Diverse populations of bacteria and fungi inhabit various niches in the human body. Found in niches that overlap with *Candida albicans,* the bacterial species *Enterococcus faecalis* is a commensal colonizer of the intestinal tract and an opportunistic pathogen, which can inhabit a variety of different areas in the host including the mouth and urogenital tract. Clinical studies have frequently shown isolation of both organisms from patient samples taken throughout the body and it has been well documented that *C. albicans* and *E. faecalis* co-infections are a leading cause of infection in post-root canal therapy. The mouth is a prevalent microbial habitat in the human body where complex host, bacterial, and fungi interactions occur. While there are clinical incidences of these two species occurring in overlapping niches, such as the human mouth, the details of their inter-kingdom interactions are unknown. We have shown that *E. faecalis* produces a secreted molecule that inhibits hyphal morphogenesis and attenuates virulence in a *Caenorhabditis elegans* infection model. Preliminary characterization of the inhibitory signal is consistent with that of a small cyclic peptide that is heat stable and between 3 to 10 kDa. In addition, the inhibitory activity was found to be partially dependent on the Fsr quorum-sensing system, a major regulator of *E. faecalis* virulence and biofilm formation. In the present study, an *in vitro* dental biofilm model was employed to assess *C. albicans* biofilm formation in the presence of sterilized supernatant from *E. faecalis* cultures. Biofilms were grown in artificial saliva for 24 hours and biofilm biomass was quantified using a crystal violet release assay. A significant reduction in biofilm biomass and hyphal morphogenesis was observed in biofilms grown in the presence of spent supernatant. Hyphal morphogenesis was quantified using a transcriptional fusion of *HWP1-GFP* (Hyphal Wall Protein 1). Expression of *HWP1*, was reduced in biofilms grown in the presence of *E. faecalis* supernatant, indicating that the *E. faecalis* secreted inhibitory signal reduces biofilm biomass and hyphal morphogenesis. A candidate gene regulated by the Fsr regulon was identified, *phdA* (putative hyphal disrupter A) that encodes a putative bacteriocin. A loss of biofilm and hyphal inhibition was observed in *C. albicans* biofilms grown in the presence of supernatant from a Δ*phdA* strain, indicating PhdA as the inhibitory signal. Elucidation of the inter-kingdom interactions between these medically relevant pathogens could pose alternative strategies for treatment and prevention of polymicrobial infections. Bacteriocins in particular have demonstrated a noteworthy potential as therapeutics and bacteriocin-producing probiotic organisms have been considered as beneficial.

69C

MICROBIOME AND HOST IMMUNE RESPONSE SIGNATURES IN THE GUT ENVIRONMENT AND THEIR INFLUENCE ON COLONIZATION WITH *CANDIDA.*

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Motivation: By competing for nutrients and synthesizing inhibitory secondary metabolites and quorum sensing molecules, fungi and bacteria mutually influence each other, thereby maintaining GI homeostasis. In addition, both directly alter the GI immune response via metabolites and host pathogen recognition receptors. The host immune response can, in turn, modulate the composition of the resident bacterial and fungal flora. Unlike humans, mice are not normally colonized with *Candida albicans*, unless the resident GI flora is perturbed by the administration of oral antibiotics. **Hypotheses:** We hypothesized that antibiotic treatment would induce specific changes in the bacterial and fungal microbiome, as well as the GI immune response, that would influence the extent of *C. albicans* colonization. **Methods:** C57BL/6 mice were administered either vancomycin (Van) or a combination of penicillin, streptomycin, and gentamicin (PSG). After 7 days, we administered *C. albicans* by gavage and followed the mice for 14 days. We determined the level of *C. albicans* colonization over time by quantitative culture of fecal pellets. We profiled the microbiome communities in both ileum and fecal pellets before and after *C. albicans* challenge by 454 sequencing. We also measured a panel of cytokines representing the Th1, Th2 and Th17 axes. We built statistical models to identify the microbiome-cytokine signatures with the greatest influence on colonization. **Results:** While mice treated with Van had only transient GI colonization with *C. albicans*, those treated with PSG showed sustained, high level colonization. After 7 d of antibiotics, prior to *C. albicans* challenge, while both Van and PSG suppressed Th17, PSG-treated mice had slightly lower Th17. This differential suppression under PSG was associated with a decrease in fungal genera belonging to *Ascomycota* as well as bacterial genera belonging to *Firmicutes* (e.g. *Lactobacillus*, *Anaeroplasma*) and *Proteobacteria*. PSG also suppressed Th1 much more than Van. This reduction was associated with a decrease in another set of *Firmicutes* (e.g. *Lactococcus*, *Clostridium*), *Proteobacteria* (e.g. *Proteus*, *Cronobacter*), and *Bacteroidetes* (e.g. *Parabacteroides*, *Porphyromonadaceae*), as well as some fungi (e.g. *Candida*). 21 days of PSG treatment and *C. albicans* colonization resulted in a further decrease in Th17 and a strong increase in Th1. This was associated with yet another set of *Proteobacteria* (e.g. *Delftia*) and *Firmicutes* as well as fungi such as *Candida*. *Streptophyta* and *Parasutterella* contributed to these signature changes in both immune axes and in colonization across the entire span of the experiment by their respective increase in PSG and decrease under Van. **Conclusion:** A complex bacterial-fungal interplay and an evolving immune-microbiome signature across time explain the different patterns of GI colonization induced by vancomycin and PSG treatments.

70A

ANALYSIS OF CANDIDA ALBICANS AND CANDIDA GLABRATA BIOFILM FORMATION BY SEM.

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Candida glabrata is not a dimorfic fungus, however express proteins such as the Epa 1 which enables adherence to epithelial cells and

the GPI-CWPs. These suggest that C. glabrata is able to form biofilm. So it is important to know the structure of the biofilm formed by C. glabrata at different times and observe the interactions between conidia that allow biofilm establishment. The determination of the characteristics and structure of the biofilm was carried out by SEM as described by Romo et al. (2006) using acetate films of 2 cm of diameter as a support for the biofilm formation, which were placed on 6-well cell culture plates. Separated suspensions of C. albicans (clinical isolate13094) and C. glabrata (clinical isolate 707) containing 1x106 cells / mL each in RPMI was added into 6-well cell culture plates and incubated at 37°C for 4, 24, 48 and 72 hours. Then, the biofilms were washed with PBS and fixed with glutaraldehyde 2% for 2 hours. The biofilms were washed with PBS and post-fixed with 1% osmium tetroxide for 2 hours. Acetates with the biofilm formed and fixed were dehydrated with ascending ethanol series. Then the acetates were put into a critical point dryer and they were coated with gold. Micrographs were obtained and analyzed at 1000x, 2000x and 5000x magnification. SEM observations showed the different stages of biofilm formation and their characteristic cell morphology between C. albicans and C. glabrata. In the initial phase (4 h), cells of C. albicans stuck and formed microcolonies, while the intermediate development phase (24 h) was characterized by an increased presence of these microcolonies. During the growth and proliferation phase (48 h) it was observed to a greater extent growth of yeast cells and extracellular matrix (ECM) formation that was observed as diffuse material around the cells. During the maturation phase (72 h) we observed a decrease in cell density and hyphal cells were presented surrounded by ECM. In the case of C. glabrata the initial phase of biofilm formation the microcolonies began to form and the biofilm had a poor growth. During the intermediate phase the biofilm started to grow and the ECM was present. In the growth and proliferation phase the biofilm had an abundance of yeast cells stacked one on another, further development and cell proliferation was observed. The ECM increase around the yeast cells also. During maturation phase the cellular proliferation decreased, the microcolonies tent to disappear and the ECM was present. Our results shown similarities to the previous reported for C. albicans biofilm formation. The architecture of C. glabrata biofilm is different because C. glabrata did not to produce hyphal cells, but it can form small extensions at the ends of the conidia. Maybe these extensions are involved in conida-conidia adhesion and together with the EMC allowing the biofilm formation. This is the firs microscopic description of C. glabrata biofilm formation.

71B

A NANOSTRING BASED METHOD TO ASSAY PATHOGEN GENE EXPRESSION DURING INVASIVE INFECTION

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Gene expression has often proven to be an excellent predictor of function. Here we apply that principle in the context of a fungal invasive infection, taking both knowledge-based and discovery-based approaches to deduce the signals and pathways that drive pathogen gene regulation during invasive *Candida albicans* infection. Our knowledge-based approach employs nanoString probes for numerous environmentally responsive genes; our discovery-based approach employs nanoString probes for all predicted *C. albicans* transcription factor genes. Our data suggest that infection is associated with rapid induction of zinc and iron limitation response genes, as well as genes characteristic of invasive hyphal cells. There is a more gradual induction of oxidative stress response genes, which coincides with expression of host inflammatory cell reporters. We identify new virulence regulators among

highly expressed transcription factor genes, and profiling of attenuated mutants during infection reveals a novel zinc acquisition pathway. Finally, we find that genes induced by the antifungal drug caspofungin during infection correspond to genes that are repressed at the onset of infection. The pathogen response circuitry is tailored uniquely during infection, with many relevant regulatory relationships that are not evident during growth in vitro. Notably, repression of a key drug response pathway during infection may reveal the pathogen's Achilles' heel. Our findings support the principle that virulence is an emergent property at the level of gene expression, a property manifested only in the context of host-pathogen interaction.

72C

CLOX CASSETTES FOR RAPID AND EFFICIENT GENE DISRUPTION IN *CANDIDA ALBICANS*

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A basic goal in the study of the pathogenesis of *Candida albicans*, a major opportunistic fungal pathogen of humans, is rapid and efficient gene disruption. This goal has been hampered by the naturally diploid state of *C. albicans*, its non-canonical codon usage, and by virulence defects associated with locus-specific effects on auxotrophic marker expression. In recent years, considerable advances have been made in gene disruption technologies culminating in the creation of recombinable disruption cassettes that utilize an array of auxotrophic and dominant-selectable markers. These systems include FLP-mediated recombinable cassettes and our previously described Cre-*loxP* system. FLP cassette systems have been an invaluable tool for gene disruption, but cassette recycling efficiencies can range from 8-80%. In contrast, Cre-*loxP* cassettes have nearly 100% recycling efficiency in *C. albicans*. However, due to issues with plasmid stability, this system has suffered the disadvantage that the synthetic *cre* recombinase gene had to be integrated in an additional, sequential step after the target alleles were disrupted with *loxP*-flanked auxotrophic markers. To address this issue, we have optimized Cre-*loxP* (CLox) cassettes for rapid gene disruption in *C. albicans*. We improved plasmid stability by re-engineering the *cre* sequence to include a *TUB2* intron, thus preventing Cre expression in *Escherichia coli*. We constructed *URA3*-CLox and *NAT1*-CLox cassettes that can be used with auxotrophic and prototrophic strains, respectively. Both CLox cassettes can support single marker recycling strategies or, in conjunction with our previously described *loxP-HIS1-loxP*, *loxP-URA3-loxP*, and *loxP-ARG4-loxP* cassettes, a multi-marker disruption strategy. The *URA3*-CLox and *NAT1*-CLox systems were validated by *ADE2* disruption using the multi-marker and single marker recycling strategies, respectively. Each disruption strategy required less than two weeks for generation of the homozygous mutant. Furthermore, we have found that the high efficiency of recombination obviates the need for PCR genotyping prior to Cre-*loxP* resolution. In conclusion, we have generated CLox cassettes that exploit the efficiency of Cre-*loxP* recombination for rapid and convenient gene disruption in *C. albicans*.

73A

IN-SILICO DIAGNOSIS OF CANDIDA SPECIES CAUSING OCULAR INFECTIONS IN PAKISTAN

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The unique structure of the human eye as well as exposure of the eye directly to the environment renders it vulnerable to a number of uncommon infectious diseases caused by fungi

and parasites etc. The effective treatment is always based upon rapid diagnosis of the disease and identification of its causative agent, Due to the limitations of classical methods for the detection of systemic fungal infections and the high mortality rates associated with these infections, it is essential to develop a quick, sensitive and specific detection assay. The present study was conducted on fungal pathogen (Candida) causing ocular infections. A polymerase chain reaction (PCR) method was developed that was capable of detecting a wide range of medically important fungi from clinical specimens. The 18S, 5.8S and 28S rRNA gene sequences of different Candida species were obtained from GenBank or EMBL databases, and different set of unique primers were designed based on these conserved regions, using GENEIOUS tool. Specificity of the designed primer pairs was analyzed by AMPLIFX tool, amplifying a fragment into target sequence. To find out the genotype of Candida species, a software "NEBcutter" was used to analyse the linear DNA, followed by using restriction enzymes to cleave the DNA, for species level identification. This technique also identified the number of copies of a gene present in the genome of one individual, as well as the number of gene mutations occurred within a Candida species. In prevailing medical facilities in Pakistan, the prediction of Candida sp. involved in ocular infections following our technique will be a valuable addition of information in the field of medicine.

74B

HOW TO GET RID OF THOSE ANNOYING CTG CODONS

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Candida albicans as well as other pathogenic yeast species of the genus Candida belong to the CTG clade. These yeasts have an exception from the universal codon usage since CUG encodes serine instead of leucine due to a mutated tRNA. The tRNA serine is ambiguous since it translates CUG to serine ≈97% of the time and thus in around ≈3 % of the translations leucine will be incorporated into the protein. Around 66% of the genes in C. albicans have 1-38 CTG codons and many of them encodes proteins important for virulence. To understand the molecular mechanisms of C. albicans virulence in the quest for new treatments of Candidiasis, recombinant protein expression of Candida proteins is of crucial importance. Moreover, to study biological processes in C. albicans, ectopic expression of genes from other organisms are important, when for example tagging proteins with Green Fluorescent Protein (GFP) for localisation studies. Before this kind of studies can be undertaken all the CTG codons has to be changed. Since C. albicans has around 2700 genes with 6 or more CTG codons mutating them one by one is not an option. There are two alternative methods for the introduction of multiple changes in the DNA; enzymatic assembly or overlap-extension PCR. We have improved the overlap-extension PCR method, so it can now efficiently be used to change the CTG codons to TCT for recombinant expression of C. albicans proteins. Here we present data on the generation of large DNA fragments, up to 7.4kb, where at least 13 CTG codons has been changed using a genomic template. The improved method is faster, due to fewer reaction steps, and more accurate due to less PCR cycles, meaning that it can effectively compete with the enzymatic assembly method. The site-directed mutations can be introduced anywhere between 50bp to 1800bp from each other. To test our method we chose three genes encoding important virulence factors, namely the three histidine kinase genes, CHK1, SLN1 and NIK1 from C. albicans and successfully changed in total 27 CTG codons to TCT in these genes (Wäneskog and Bjerling, Anal Biochem 2014). The method is highly reliable and predicted to be applicable also to other types of DNA engineering when the introduction of multiple changes in a DNA sequence is required.

75C

USE OF AN INFRA-RED FLUORESCENT PROTEIN TO MONITOR CANDIDA ALBICANS INFECTIONS

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Candida species are a common cause of bloodstream infection in severely ill patients, causing 11% of all infections, with the majority caused by *Candida albicans*. *Candida* bloodstream infections have a high (~ 44%) mortality rate, due to difficulties in diagnosing infection, delays in initiation of effective antifungal therapy and a limited choice of antifungal drugs. To increase our understanding of systemic *Candida* infection initiation and progression, and to develop and evaluate novel, more effective diagnostics and antifungal therapies, experimental infection models remain essential. At present, to monitor experimental *Candida* infection, we are limited to sampling groups of mice at various time points and determining the fungal burdens in the major target organs, the kidneys. Significant biological variation between mice (5-10%), even in the hands of experienced researchers, requires 3-6 mice to be sampled at each time point. A reporter system allowing repeated imaging of infection in the same animals would significantly reduce animal usage. Previously, attempts have been made to develop reporter systems to visualise fungal cells *in vivo*. These include green fluorescent protein (GFP), firefly luciferase and the gLUC59 luciferase reporter. Each of these systems has drawbacks. In mice, far-red and near-infrared fluorescent proteins show improved detection and sensitivity in mice compared with GFP, due to minimal overlap with the auto-fluorescence of animal tissues, thus permitting detection of fungal cells deep within the mouse. The aim of this pilot study, therefore, is to create an iRFP reporter for use in *C. albicans* and to exploit it for monitoring fungal infection *in vivo*. As a first step, we have created a CTG-optimized iRFP for use in *C. albicans* and have characterized the expression of the iRFP protein under the control of a selection of promoters *in vitro*. Using FACS to evaluate infra-red fluorescent *C. albicans* cells we have initially studied the effect of carbon source on fluorescence, as well as the concentration of available chromophore, biliverdin. By titrating biliverdin levels, we found that a concentration of 100 µM gave the highest levels of fluorescence. Preliminary results indicate the importance of carbon source on expression, reflecting different sites of infection. On glucose the *ACT1*, *FAA4* and *PGA54* promoters display increased fluorescence over a no promoter control and *CAT1*. On lactate fluorescence is negligible for all promoters, whereas growth on oleic acid increases the signal from all promoters. The interim conclusions are that a *C. albicans* strain containing an iRFP construct under control of a selection of promoters will fluoresce under different growth conditions. Further studies to examine expression under stress conditions, as well as fluorescence microscopy and subsequent *in vivo C. albicans* infections, will be presented.

76A

A HIGHLY PARALLEL CONDITIONAL OVEREXPRESSION SCREENING STRATEGY IN CANDIDA ALBICANS

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Background: Gene overexpression is a powerful strategy for mapping pathways and phenotypes. It mimics gain-of-function mutations, complements loss-of-function phenotypes and allows to study the function of both essential and non-essential genes. Because lack of a complete sexual cycle and aneuploidy hamper the use of classical genetics in C. albicans, we hypothesized that combining gene overex-

pression and strain barcoding would help in understanding gene function in C. albicans. **Methods:** We constructed a collection of barcoded C. albicans strains each overexpressing a unique ORF under the control of a doxycycline (dox)-inducible promoter and set up a strategy for measuring the relative abundance of every strain in mixed population experiments using microarrays. Two sets of collections are currently available and include i) 531 strains carrying the pNIM1 system that imparts moderate overexpression and ii) 579 strains carrying the strong pNIMX overexpression system. **Results:** To examine the effect of gene overexpression on cell growth, pools were grown during 18 generations in rich medium in the absence or presence of dox. Using the pNIM1 and pNIMX collections, we respectively found 8 and 25 genes whose overexpression decreased C. albicans fitness in rich medium by at least 2 standard deviations ($p<0.05$), indicating that increasing overexpression levels correlates with increased assay sensitivity. These genes encode (or are predicted to encode) regulators of DNA-damage response or cell-cycle progression (RAD53, RAD51, PIN4, others), filamentation (SFL1, SFL2, BRG1, others) and response to stress. Pools from the pNIM1 collection were also used to screen for genes whose overexpression affects biofilm development in a microfermentor model. We strikingly found that strains overexpressing cell surface Glycosyl-Phosphatidyl Inositol (GPI)-anchored protein-encoding genes (PGA59, PGA19, PGA15, PGA41, PGA32, others) were significantly overrepresented in dox-treated biofilms relative to untreated controls, indicating that overexpressing cell surface proteins promotes the development of multistrain biofilms. We are currently testing our collections in both ex-vivo and in vivo models of C. albicans infection. **Conclusions:** We identified genes whose overexpression affects C. albicans competitive growth under different conditions. Our results constitute a proof-of-principle of the technology which can be used as a tool for competitive fitness profiling both in vitro and in vivo.

77B

COMPREHENSIVE GENOMIC ANALYSIS OF THE RESPONSE OF CANDIDA ALBICANS TO OXYGEN RELATED STRESSES

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To understand how human pathogens infect a host, we must determine how the pathogen responds to the host defenses. During infection, invasive pathogens face varying microenvironments, and interact with the host in different niches. Upon any environmental change, the pathogen responds to the new circumstances by, among other responses, reprogramming the expression of certain sets of genes. There is a vast array of genomic tools that are currently available to characterize changes in the yeast transcriptome, enabling the monitoring of global changes in gene expression to any physiological event. Yet none of these techniques are able to provide insights to the transcriptional regulation that underlies the response. To address this issue, we have developed the BioGRO: a run-on based genomic technique that enables the measurement of several parameters involved in the mRNA life cycle, including transcription rate, degradation rate and mRNA stability. The method labels nascent transcripts readily as they are being generated by RNA polymerases, and is suitable for strand-specific high throughput transcriptome analysis, either by hybridization (tiling microarrays) or by deep sequencing. In this study we have used the method to track changes in the *C. albicans* transcriptome upon the exposure to two environmental stresses that it encounters during systemic infection: hypoxia and oxidative stress. Our analysis reveals differential expression of regions of the genome that had not been previously identified, such as non coding RNAs or antisense transcripts.

78C

MICROARRAY DATA AND VISUALIZATION TOOLS AT CANDIDA GENOME DATABASE

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The *Candida* Genome Database (CGD, www.candidagenome.org) is a web-based compendium of information about the genes and proteins of *Candida albicans* and other *Candida* species. CGD collects and provides access to sequence data and manually curated functional information that includes free-text descriptions, Gene Ontology annotations, and mutant phenotype data. For several years CGD has also been collecting published expression datasets and making them available in our Dataset Archive, from which the data can be downloaded and searched/analyzed by CGD users with their own tools. However, the ultimate goal has been to make the expression data accessible for searching and browsing via a user-friendly interface within the CGD environment. To meet this goal, we have implemented GeneXplorer. The tool allows conversion of variously formatted expression datasets into a common pcl format that can be displayed within a web page. Each publication that has such data associated with it in CGD is linked to a GeneXplorer-generated web page that contains a colorimetric image of the clustered expression profiles. A user is able to zoom out to see an overall view of the clustered data in the genes vs experiments matrix, or to click on any profile to see the expression patterns of the most similarly or dissimilarly expressed genes. The display shows gene names along with their descriptions, thus providing an instant clue about gene functions. Further navigation, via hyperlinked gene names, leads to Locus Summary pages that contain GO annotations, mutant phenotype data, and other information about each gene. The GeneXplorer page also allows searching for a specific gene of interest within a dataset. We are initially focusing our efforts on existing microarray datasets, but we will also incorporate RNA-Seq data. GeneXplorer now provides a link between large-scale expression data and the wealth of functional data available at CGD. CGD staff welcome any feedback and suggestions for further improvements. We can be reached at candida-curator@stanford.edu.

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79A

SEQUENCING AND REANNOTATION OF THE CANDIDA ALBICANS DBC AND GRACE™ STRAIN LIBRARIES.

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The National Research Council of Canada has been mandated by Merck for the maintenance and public distribution of the *Candida albicans* DBC (Double BarCoded haploid gene deletions) and GRACE™ (gene replacement and conditional expression) strain libraries. The 5,467 strains of the DBC library are used in the Genome-Wide Fitness Test, an effective method to identify the mode of action of antifungal compounds (Xu et al. 2007). These were the precursors of the 2,357 GRACE™ mutants which allow for the transcriptional repression of tetracycline promoter-regulated genes (Roemer et al. 2003). Both libraries were produced before the *C. albicans* genome was fully assembled and annotated. In order to validate the inactivated genes and barcode sequences, we isolated and sheared genomic DNA from a pool of DBC strains. We then used adapters and PCR to individually amplify the upstream and downstream barcodes along with genomic DNA from the insertion sites. These were subsequently sequenced in an Ion Torrent PGM. Analysis of more than 7 million

sequence traces confirmed the insertion sites of 4,038 (73.8%) strains. We also noted that 2 distinct insertion cassettes, which differ in the orientation of the *HIS3* gene, were used in strain construction. 271 (5%) strains required corrections in their inactivated gene. Most of these were either fused with a neighbouring ORF in subsequent genome assemblies or were members of a multigene family. Furthermore, we corrected the sequences of 2,371 barcode sequences (21.7%). Barcode errors were usually minor and probably resulted from incomplete reactions during the oligonucleotide synthesis. Finally, the annotations of both libraries were updated and brought up to current standards. These strain libraries will be useful for more than genetic screens and the Fitness Test. For example, we are currently using the GRACE™ strains to produce Reverse Phase Protein Arrays spotted with total protein extracts from *C. albicans* cells grown in the yeast or hyphae morphologies. These RPPAs are a powerful tool to rapidly characterize the targets of our new panel of anti-Candida monoclonal antibodies.

80B

COMPARATIVE GENOMICS AT THE CANDIDA GENOME DATABASE

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The Candida Genome Database (CGD: www.candidagenome.org) is an online resource that provides primary sequences and gene models, search and analysis tools, and literature-derived manual curation of gene function for Candida-related species. CGD provides a number of tools and resources that allow researchers to leverage the power of comparative genomics to study Candida genes, their functions, and the processes in which they are involved. In 2011, CGD expanded to become a multi-species resource, and we now provide up-to-date sequences and gene models, complete literature curation, and individual summary pages for each gene of C. albicans, C. glabrata, C. parapsilosis and C. dubliniensis. We also provide sequences, gene models, orthology- and domain-based functional annotations, and BLAST resources for six additional species - C. guilliermondii, C. lusitaniae, C. orthopsilosis, C. tropicalis, Debaryomyces hansenii and Lodderomyces elongisporus - and a second strain of C. albicans, WO-1. A new resource, the CGD Homology Page, provides links between these Candida-related species through their orthologous gene clusters, defined based on synteny by the Candida Gene Order Browser (CGOB, http://cgob3.ucd.ie/). Comparing and contrasting the members of ortholog clusters can shed light on their biological similarities and differences. In addition to listing orthologous genes and linking to gene-specific information pages, the CGD Homology Page displays a phylogenetic tree showing the evolutionary relationships between orthologs in each cluster, and multiple-sequence alignments for their protein and coding sequences. These can be used to determine conserved sites and regions within genes and proteins of interest (which are often functionally important). Comparisons with well-characterized homologs in more distant organisms can suggest possible functions for uncharacterized Candida sequences, so the CGD Homology Page also provides links to homologs in other fungal species, including S. cerevisiae, A. nidulans, N. crassa, and S. pombe, as well as homologs in even more distant species - dictyostelium, mouse and rat. We believe the CGD Homology Page will greatly aid and simplify phylogenetic analyses and cross-species comparisons for Candida researchers now, and will become essential as we add more tools and analyses, and as additional sequenced genomes from Candida-related species become available. We welcome your feedback and suggestions, which can be sent to candida-curator@stanford.edu. This work was supported by the National Institute of Dental and Craniofacial Research at the US National Institutes of Health [grant no. R01 DE015873]

81C

EFFECT OF DEBARYOMYCES HANSENII KILLER TOXIN ON CANDIDA ALBICANS (SC5314) AND CANDIDA TROPICALIS (10985)

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Candida spp. are opportunistic yeasts of the gastrointestinal tract, skin, and mucous membrane and cause severe candidiasis. Due to increasing resistance to existing drugs, new strategies are important to reduce Candida infection. The production of antimycotically active toxins known as killer toxins is a widespread phenomenon in various yeast genera. Debaryomyces hansenii, a commonly found yeast in cheese, has been found to be able to produce antimycotically active toxin. We examined killer toxin activity of 42 strains of D. hansenii isolated from different types of cheeses against Candida albicans (SC5314) and C. tropicalis (10985) using the streak plate agar bioassay and agar diffusion well bioassay. The highest D. hansenii killer activity was found at lower temperature (25 C) with low pH (4.5), while killer activity was diminished at 35 C and pH 7.0. Killer activity of D. hansenii against C. albicans (SC5314) and C. tropicalis (10985) differs with D. hansenii strains. Killer toxin activity against C. tropicalis (10985) is higher at 25 C and lower at 35 C than against Candida albicans (SC5314). The result confirmed that killer toxin activity of D. hansenii isolated from different cheese differs with strains, temperature and sensitive species.

82A

A *CANDIDA ALBICANS* PEPTIDE ATLAS AS A TOOL FOR TARGETED PROTEOMICS: A PILOT STUDY FOR UNDERSTANDING APOPTOTIC PROCESS

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Candida albicans is the major etiologic agent of invasive candidiasis that is associated with high morbidity and mortality in cancer, post-surgical and intensive care patients. However, its clinical relevance is not reflected in the number of public proteomic data sets, which still have a very limited presence in online repositories. For this reason, we have created a *C. albicans* PeptideAtlas that comprises over 2500 canonical proteins. It represents the most extensive characterization of the *C. albicans* proteome up to the current date (approximately 41% coverage of the open reading frame sequences present in the database used for the searches). This PeptideAtlas provides several useful features, including comprehensive protein and peptide-centered search capabilities and visualization tools. Furthermore, it is a valuable resource for the selection of candidate proteotypic peptides (peptides with high mass spectrometry observability and unique protein mapping) for targeted proteomics. We applied this targeted approach for the detection of a set of *C. albicans* proteins related to apoptosis in an attempt to achieve a better understanding of the molecular mechanisms underlying this process as well as to develop novel antifungal therapies. Programmed cell death was induced in *C. albicans* by different types of environmental stress. The presence of known apoptotic markers was assessed in each apoptosis-inducing condition. This set of targeted proteins was detected across the different environmental stresses by selected reaction monitoring (SRM) mass spectrometry with high sensitivity and reproducibility. Our results demonstrate the utility of SRM-based targeted proteomics to identify systematically *C. albicans* proteins of biological and clinical interest, such as those with a known and suspected role in programmed cell death.

83B

CHARACTERIZATION OF WOR1 FUNCTION THROUGH IDENTIFICATION OF INTERACTING PROTEINS

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Paramount to the yeast *Candida albicans'* ability to survive and thrive in multiple host niches is its morphological plasticity. By shuttling between distinct cell types and lifestyles, *C. albicans* is able to acclimate itself to a plethora of different environments, and it is this ability that makes *C. albicans* such an evasive pathogen. One such transition is the switch between white and opaque phases, which occurs stochastically and at low frequency, but is reversible and heritable. This switch is controlled by the master regulator Wor1, which when expressed induces the transition to the opaque phase. Many studies have focused on regulation of *WOR1* transcription and its stabilization through a positive feedback loop. Our aim was instead to dissect the functional role of Wor1 protein as a transcriptional regulator. To address this question, we looked at global Wor1 protein interactions using tandem affinity purification coupled with mass spectrometry. With this method we were able to identify potential Wor1 interacting proteins and even detect putative post-translational modifications. The top interacting protein identified was the general transcriptional repressor Tup1. Tup1 does not bind DNA directly but instead associates with other sequence-specific DNA binding proteins, allowing for Tup1 to repress many different targets. ChIP experiments using tagged Tup1 in cells of both phases showed that Tup1 binds to the *WOR1* promoter preferentially in opaque cells in a region where Wor1 binds, indicating that Wor1 recruits Tup1 to the *WOR1* promoter in opaque cells. Since Δ*tup1* cells are unable to spontaneously switch to the opaque phase, we hypothesize that Tup1 is recruited by Wor1 and can function as a co-activator, contributing to the ability of Wor1 to function as a positive transcriptional regulator. To assess this hypothesis, we are using a functional 1-hybrid assay as a reporter of Wor1 activity. We will perform this assay in a conditional *TUP1* deletion mutant to determine if Wor1 activity is altered. Using this conditional mutant we will also perform ChIP of known transcriptional co-activators to determine if their presence at known Wor1 binding sites is altered in the absence of Tup1. To conclude, in order to better understand the function of Wor1 as a transcriptional regulator, we have identified Wor1 interacting proteins and through a combination of functional and genetic approaches have attempted to characterize the role of a putative interacting protein on the ability of Wor1 to function as a transcriptional regulator.

84C

T2MR FOR THE RAPID IDENTIFICATION OF CANDIDA AND BACTERIA SPECIES IN MURINE AND HUMAN WHOLE BLOOD

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The T2Candida assay developed using T2 Biosystems' proprietary T2 magnetic resonance platform (T2MR) has demonstrated rapid and sensitive detection of fungal targets in human whole blood as low as 1 CFU/mL. We report limit of detection (LOD) data and describe our methods for establishing the measurement with a quantified spike preparation method where LOD was defined as ≥95% hit rate for N≥16 replicates. LOD values of 1 CFU/mL were observed for C. parapsilosis, 2 CFU/mL for C. krusei and C. glabrata, and 3 CFU/mL for C. albicans and C. tropicalis. We report the performance of the T2Candida assay in patients with symptoms of septicemia using 24 fully blinded whole blood specimens; eight samples were from N=3 candidemic patients, eight from N=8 bacteremic patients, and eight

from N=8 blood culture–negative patients. In addition, we report detection of fungal clearance after administration of an antifungal in 21 clinical specimens collected serially. For serially collected specimens on candidemic patients, 100% agreement between blood culture and T2MR was observed before administration of an antifungal, whereas T2MR data indicated persistent presence of Candida cells for up to 4 days after micafungin administration while blood culture indicated clearance of the infection upon administration. Because of the increasing prevalence of candidemia in immunocompromised patients and the high mortality rate associated with delayed diagnostics, T2Candida may be utilized as a compelling methodology for research, development and clinical trial applications for improved diagnostics and treatments. Here, we demonstrate that the fully automated T2Candida assay can be used for highly sensitive detection of spiked Candida cells in whole blood of mice and humans. K2 EDTA treated mouse and human whole blood samples were spiked with negative and positive levels of Candida and run on the T2Dx instrument. Results, obtained in approximately 3 hours, indicate sensitive detection of candidemia. Likewise, we present evidence for detection of multiple bacterial targets to complement the Candida panel for broad identification of pathogens that may cause sepsis. Preliminary results indicate sensitivity as low as 3 CFU/mL for common bacterial targets in spiked human whole blood, including S. aureus and P. aeruginosa, and K. pneumoniae. With a blood-based pathogen identification panel that does not require a blood culture specimen for analysis, broad matrix application and rapid time to result, the T2Candida assay can aid in improved screening, diagnostics, and enrollment in drug discovery applications from pre-clinical to diagnostic applications.

T2MR, T2Candida, and T2Dx are registered trademarks of T2 Biosystems.

The T2Candida test is currently intended for Investigational Use Only. The performance characteristics of this product have not yet been established.

85A

CANDIDA INTERACTIONS IN PEDIATRIC SMALL BOWEL TRANSPLANT RECIPIENTS

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Systemic bloodstream infections are a major cause of morbidity and mortality among pediatric small bowel transplant (SBT) recipients. While transplantation aims to restore organ function, these patients have a higher susceptibility to invasive Candida bloodstream infections due to the nature of the procedure (e.g. disrupting the gastrointestinal barrier and translocating enteric gut Candida yeasts into the bloodstream; the interplay between host and foreign donor microbiota; and immunosuppressive therapies to prevent organ rejection, consequently reducing the ability to fight infections). Fungal infections are well-recognized complications after organ transplantations; however, little information is known about the ecology of Candida yeasts causing invasive candidiasis post-transplantation. This study aims to investigate the composition and competition of the intestinal microbiota after transplantation as well as the epidemiology of invasive candidiasis in pediatric SBT recipients. Fecal/ileostomy samples are routinely collected during hospitalization of SBT recipients at the University of Nebraska Medical Center. Samples prior to, concurrent with and following infection (positive Candida bloodstream isolates) have been examined by DNA fingerprinting to define the microbial community composition and compare Candida isolates in the samples. Results of this study will allow us to determine the source of the Candida and characterize the role of fungal interactions in SBT patients with high levels of candidiasis. Ultimately, this could inform pre-transplant interventions to the host or donor organ to prevent invasive candidiasis.

86B

IDENTIFICATION, TYPING, ANTIFUNGAL RESISTANCE PROFILE, AND BIOFILM FORMATION OF CANDIDA ALBICANS ISOLATES FROM LEBANESE HOSPITAL PATIENTS.

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As the leading opportunistic fungal pathogen found in immunocompromised nosocomial infections with high rates of morbidity and mortality, identification and epidemiological subtyping of Candida species is crucial. Such proper identification is essential in recognizing outbreaks of infection, detecting cross-transmission, determining the source of the infection, recognizing particularly virulent strains, detecting the emergence of drug resistant strains, or even assessing hospital hygiene. In this study our objective was to compare Candida albicans hospital identification rates and conventional laboratory employed methods such as API, CHROMagar and germ tube formation with more reliable molecular method such as ITS typing. Furthermore, our aim was to assess biofilm-forming capabilities, drug resistance profiles and correlate them with MLST typing of isolates. ITS typing was performed for 85 isolates and the misidentification rate for each method was deemed significant: 15.3% for API and CHROMagar, and 21.2% for the hospital. Moreover, antifungal susceptibility testing to 4 antifungal drugs encompassing different classes was performed, and multidrug resistance to 3 drugs was unexpectedly found to occur amongst 25% of isolates tested raising serious questions concerning the methods of hospital treatment. In addition some strains with significant biofilm forming capabilities correlated well with strains that were multidrug resistant. Such strains grouped closely together in a neighbor-joining tree generated by MLST typing indicating phylogenetic relatedness, microevolution or recurrent infection. In conclusion, this pilot study gives much needed insight concerning Candida albicans isolates circulating in Lebanese hospitals, and is the first study of its kind correlating biofilm formation to antifungal resistance and strain evolutionary relatedness.

87C

THE DEACETYLASE SIR2 FROM THE YEAST CLAVISPORA LUSITANIAE LACKS THE EVOLUTIONARILY CONSERVED CAPACITY TO GENERATE SUBTELOMERIC HETEROCHROMATIN

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Background: Deacetylases of the Sir2 or sirtuin family are thought to regulate life cycle progression and life span in response to nutrient availability. In yeast of the *Saccharomyces* clade, sirtuins repress genes involved in cell type identity, sporulation, and adhesion. Therefore, sirtuins are good candidates to influence morphological transitions in yeast of the *Candida* clade. The sirtuin family has undergone successive rounds of duplication and diversification, enabling the enzymes to perform a wide variety of biological functions. Two evolutionarily conserved functions of yeast Sir2 proteins are the generation of repressive chromatin in subtelomeric domains and the suppression of unbalanced recombination within the tandem rDNA array. **Results:** Here, we describe the function of the Sir2 ortholog ClHst1 in *Clavispora lusitaniae*, a yeast of the *Candida* clade that is an occasional opportunistic pathogen. ClHst1 was localized to the non-transcribed spacer regions of the rDNA repeats and deacetylated histones at these loci, indicating that, like other Sir2 proteins, ClHst1 modulates chromatin structure at the rDNA repeats. However, we found no evidence that ClHst1 associates with subtelomeric regions or impacts gene expression directly. This surpris-

ing observation highlights the plasticity of sirtuin function. **Conclusion:** We conclude that the single Sir2 ortholog in *C. lusitaniae* possesses only a fraction of the functions observed for Sir2/Hst1 orthologs in other fungal species. Related species, including *Candida albicans*, possess an additional Sir2 ortholog. Thus, it is likely that the ancestral *Candida SIR2/HST1* gene was duplicated and subfunctionalized, such that *HST1* retained the capacity to regulate rDNA whereas *SIR2* had other functions, perhaps including the generation of subtelomeric chromatin. After subsequent species diversification, the *SIR2* paralog was apparently lost in the *C. lusitaniae* lineage. Thus, *C. lusitaniae* presents an opportunity to discover how subtelomeric chromatin can be reconfigured.

88A

TRISOMY OF CHROMOSOME R, A NOVEL CHROMOSOMAL ANEUPLOIDY, IS INVOLVED IN AZOLE RESISTANCE IN CANDIDA ALBICANS

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C. albicans genome is unstable and has enough plasticity to support a variety of chromosomal aneuploidies resulting from gain and loss of whole chromosomes or chromosome fragments, and that changes in chromosome copy numbers often arise as a response to stresses. Routine genetic manipulations of C. albicans, such as treatment with short pulses of high temperature or electroporation, and especially treatment with lithium acetate, often results in the acquisition of unwanted chromosomal aneuploidies. In this study, we investigated the mechanism of the resistance to antifungal azoles in variant strains CaLY188, CaLY350, CaLY190 and CaLY191 obtained during our construction of an SRD1 deletion. These four strains showed trisomy of chromosome R (Chr R) by using array based comparative genomic hybridization technology and relative quantitative real-time PCR of genomic DNA, and resistance to azoles confirmed by spot assays, minimal inhibitory concentration and time-kill curve assay. In contrast, serial passage of CaLY188 in drug-free medium restored disomy of Chr R and resulted in loss of the drug resistance. Thus we propose that trisomy of Chr R contributed to azole resistance. Whole genome expression profiling showed that 71 genes on Chr R were up-regulated in CaLY188, including ERG25 of the ergosterol biosynthesis pathway. However, disruption of one copy of ERG25 in the strain CaLY188 did not affect the antifungal drug sensitivity. The transcriptional profiling analysis identified genes on other chromosomes, including ERG2, CSH1, ECM21, PGA7, RBT5 and PHO88, which were differentially expressed in the strain CaLY188, and which might contribute to the increased FLC resistance. Our results therefore suggest that azole resistance in C. albicans could be achieved through formation of trisomic Chr R, which regulates the expression of azole-resistance-related genes on other chromosomes.

89B

HUMAN HEALTH RISK ASSESSMENT OF CANDIDA UTILIS ATCC 9950

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Under the *Canadian Environmental Protection Act, 1999* (CEPA 1999), Health Canada is conducting human health risk assessments of micro-organisms on the Domestic Substances

List (DSL) to determine whether they are harmful to human health. *Candida utilis* ATCC 9950 is one of 68 microbial strains on the DSL, which have potential uses in bioremediation, wastewater treatment, biofuel production, biomass conversion, commercial and household drain cleaner and degreasers, septic tank additives, general cleaning and odor control products, and many more. The human health risk assessment of *C. utilis* ATCC 9950 under CEPA 1999 will take into account the inherent ability of the micro-organism to cause harm (hazard) and the known and predicted conditions of exposure to the micro-organism to determine risk to the general Canadian population. An extensive body of information on *Candida* is available in the scientific literature. However, the absence of data for a specific strain could pose challenges when determining risk. The key elements that will be considered in the hazard assessment of *C. utilis* ATCC 9950 include: its relatedness to known pathogens such as *Candida albicans*; its biological attributes, such as metabolic characteristics, life cycle, environmentally resistant life stages; its ability to survive and grow under specific conditions of temperature, pH, relative humidity; the presence of virulence factors (i.e., toxins, metabolites or structural components); incidence of infections and treatment; and its antifungal resistance profile. The extent of human exposure to *C. utilis* ATCC 9950 from known and potential uses in environmental, industrial, and commercial settings will also be taken into account. The poster is intended to provide an overview of the human health risk assessment of *C. utilis* ATCC 9950, to seek feedback from experts on how to address information gaps, and to solicit their participation as external scientific reviewers of the *C. utilis* ATCC 9950 assessment report.

90C

CANDIDA BLOODSTREAM INFECTIONS CAUSED BY A NOVEL SPECIES *CANDIDA QUERCITRUSA*: EPIDEMIOLOGICALLY-LINKED INFECTION SUGGESTIVE OF AN OUTBREAK AND MYCOLOGICAL CHARACTERISTICS

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Candida quercitrusa is not described as a human pathogen. We encountered three patients of *C. quercitrusa* candidemia in the same hospital in China within 2 months, suggestive of an outbreak. The patients' medical charts were reviewed, and the time-line of infection following hospital admission established. Patient isolates were characterized by phenotypic methods and sequencing of the internal transcribed spacer (ITS) and D1/D2 regions of the ribosomal gene; antifungal susceptibilities were determined. All three patients, previously well but with established risk factors for candidemia, acquired candidemia in the same intensive care unit of the hospital within a 2-month period and were cared by common staff**.** On chromogenic agar, *C. quercitrusa* isolates exhibited either similar color to *Candida albicans* (Brilliance *Candida*) or *Candida tropicalis* (CHROMagar *Candida*). All were misidentified as *Candida pulcherrima* and *Candida lusitaniae* by the VITEK 2 YST and the API 20C AUX systems, respectively, but were correctly identified with 100% sequence similarity to each other by ITS and D1/D2 sequencing. Matrix assisted time-of-flight mass spectrometry profiles were identical although neither genus nor species was assigned. Fluconazole MICs were ≥16 μg/ml; isolates were more susceptible to itraconazole, voriconazole, amphotericin B and caspofungin. One patient who received fluconazole followed by caspofungin then voriconazole for 42 days, and one

who received caspofungin for 6 days were treated successfully. *C. quercitrusa* is a novel cause of candidemia which may be nosocomial. Phenotypic identification methods cannot provide identification. Based on MIC data, it is unlikely to respond to fluconazole therapy.

91A

EPA GENE SIZE POLYMORPHISM IS CORRELATED WITH THE BLOODSTREAM OR DIGESTIVE ORIGIN OF C. GLABRATA STRAINS

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Background: Candida glabrata has emerged as the second etiologic agent, after Candida albicans, of either superficial or invasive candidiasis in adults. Adhesion to epithelial cells has been shown to be mediated by cell-wall protein encoded by the EPA genes. Most of these genes are sub-telomeric and possess repeats of minisatellites regions whose length may be controlled by RIF1 and involved in adhesion properties. Here, we report on length polymorphism of EPA1, 2, 4/5 and 6 in invasive and commensal C. glabrata strains. **Methods:** A panel of 189 strains, previously characterised for their microsatellite genotype, clonal complex (CC) and MAT type, including 111 blood isolates from hospitalized patients and 34 digestive isolates from non-hospitalized patients were studied. EPA1, 2, 4 and 6 genes were amplified for length polymorphism analysis. **Results:** Each EPA gene appeared polymorphic with 5, 6, 4 and 2 different alleles for EPA1, EPA2, EPA4 and EPA6 respectively. Nine strains exhibited two different alleles for EPA2 and 27 strains exhibited two different alleles for EPA4. The combination of the different alleles of EPA1, EPA2, EPA4 and EPA6 generated 114 "EPATypes" (EpaT) in our strain collection. The two most common genotypes, EpaT17 and EpaT11, were shared respectively by 17 and 22 strains. All others were shared by less than 10 strains with 81 genotypes corresponding to a single strain. There was no correlation between CCs and EPA genotypes. However a difference in EPA length polymorphism between CCs was observed. Low polymorphism in CC52 and higher polymorphism in CCs 6 and 77 were observed. While commensal and invasive strains did not cluster according to the EPATypes, we found, as we already seen for microsatellite-based genotypes, a higher degree of polymorphism for commensal digestive strains as compared to blood-isolated strains. **Conclusion:** These data suggest a different evolution of the microsatellites and minisatellites regions in C. glabrata as no correlation of EPA size polymorphism and CCs based on microsatellite length polymorphism was observed. Moreover, they support our previous findings that commensal digestive isolates have higher polymorphism of DNA tandem repeats regions as compared to isolates collected in pathogenic conditions.

92B

MOLECULAR CHARACTERIZATION OF CANDIDA INCONSPICUA AND CANDIDA NORVEGENSIS

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Candida inconspicua (anamorphic stage) and *Candida norvegensis* (teleomorph *Pichia norvegensis)* are rare etiologic agent of invasive infection. They are closely related species

that exhibit a natural reduced susceptibility to azole derivatives. Both belong to the *Pichia cactophila* clade that encompasses in addition, two cactophilic species, namely *P. cactophila* and *Pichia pseudocactophila*. Using 13 clinical isolates and 8 reference strains, the first aim of this work was to investigate the performance of biochemical, proteomic (MALDI-TOF) and molecular approaches for the specific identification of *C. norvegensis* and *C. inconspicua*. Our results further led us to perform multilocus sequence typing (D1/D2 domain of the large subunit and ITS region of the rDNA, EF1), and to look for a possible sexual reproduction of *C. inconspicua* as tested by pure or mixed subculture on potassium acetate agar. Our results demonstrate that *P. cactophila* and *C. inconspicua* could not be distinguished using biochemical, proteomic or molecular approach. *P. norvegensis*, *P. cactophila*-*C. inconspicua* and *P. pseudocactophila* could be easily identified using proteomic and EF1 sequencing, whereas biochemical identification and rDNA sequencing may not be reliable. Multilocus analysis, notably using prior cloning before the sequencing step, revealed that all species of the clade exhibit a particular pattern of rDNA in that the species may have either several loci of rDNA or non identical repeats within the rDNA cluster. Phylogenetic study supports the gathering of *C. inconspicua* with *P. cactophila* within a single group. Finally, all *C. inconspicua* strains tested were able to form in pure culture, ascii which morphology were similar to that of *P. cactophila*. This study (i) confirms the usefulness of MALDI-TOF mass spectrometry for the identification of *C. inconspicua* and *C. norvegensis* (ii) demonstrates the existence of different copies of the rDNA operon in species of the *P. cactophila* clade (iii) supports the evidence that *C. inconspicua* is the anamorph of *P. cactophila*.

93C

FUNCTIONAL CHARACTERIZATION OF SSD1 IN *CANDIDA ALBICANS*

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Ssd1 plays a key role in antimicrobial peptide resistance and virulence in *C. albicans*. However, the mechanism by which Ssd1 mediates antimicrobial peptide resistance was not known yet. *SSD1* is highly conserved among *Sacchyromyces cerevisiae* and other *Candida* species. *C. albicans* Ssd1 has shown the amino acid sequence homology to *S. cerevisiae* Rrp44, which has a ribonuclease activity. To characterize the function of *C. albicans* Ssd1, full-length (Ssd1p f.l.) (residues 1-1275) and two truncated constructs encompassing residues 334-1275 and residues 578-1275 were respectively expressed and purified from *E. coli*. Ssd1 578-1275 lacks the predicted two cold shock domains (CSD1 and CSD2) that is characteristics of Rrp44 orthologs and bacterial RNase II. Ssd1 f.l. was verified with nanoscale liquid chromatography coupled to tandem mass spectrometry (nano-LC-MS/MS). Both Ssd1 f.l. and Ssd1 334-1275 bind to and degrade ssRNA, ssDNA, and dsRNA, whereas Ssd1 578-1275 did not show nuclease activity. This study suggests that *C. albicans* Ssd1exhibits nuclease activity and two cold shock domains are crucial for the nuclease activity of Ssd1.

94A

MECHANISMS CONTROLLING LOSS-OF-HETEROZYGOSITY IN CANDIDA ALBICANS

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Cells have evolved a number of molecular mechanisms to ensure genome integrity through cell generations. For instance, DNA double-strand breaks (DSBs), are repaired via recombination-dependent mechanisms such as gene conversion, break-induced replication and mitotic crossover. Inability to repair correctly a DSB results in the loss or truncation of the damaged chromosome or lethality. In Candida albicans, genome rearrangements known as Loss-Of-Heterozygosity (LOH) events are frequent and the consequence of DSBs. LOH have been observed during C. albicans commensal carriage, superficial infection in humans, systemic infection in mice and contribute to increase antifungal resistance. Various stresses mimicking the conditions encountered by C. albicans within the host during colonization and infection, such as heat shock, oxidative stress and presence of antifungals, result in an increase in LOH frequency. Yet, although LOH play an important role in different aspects of C. albicans biology, little is known about the molecular mechanisms involved in their regulation in this organism. Here, we present new tools for the study of LOH and DSB repair in C. albicans and their application to the identification of novel regulators of LOH. Indeed, we have developed a system that combines fluorescent markers and flow cytometry allowing detection of LOH events at the single cell level. Furthermore, we have developed a DSB induction system that relies on the inducible expression of the Saccharomyces cerevisae I-SceI meganuclease and the presence of its 18bp recognition sequence, conveniently located between the centromere and the fluorescent LOH reporter system. Expression of I-SceI was shown to trigger a site-specific DSB resulting in a 40-fold increase in LOH frequencies. Using this fluorescent-based tool, we have also evaluated the impact on LOH frequency of overexpressing 139 C. albicans genes whose orthologues in S. cerevisiae are involved in DNA repair, replication and recombination. This screen has identified seven genes whose overexpression triggers an increase in LOH frequency: CDC20 and RAD53 (cell cyle regulation), BAS1 and RAD51 (recombination), BIM1, CTF8 and YCG1 (chromosome segregation). Interestingly, some of these genes encode known regulators of genome dynamics involved in cancer progression in humans. Together, these studies provide new insights in the mechanisms that control genome stability in C. albicans and possibly other eukaryotes.

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RAPID IDENTIFICATION OF THE MAIN *CANDIDA* SPECIES ISOLATED FROM BLOOD AND OTHER CLINICALLY SIGNIFICANT CULTURES BY MULTIPLEX REAL-TIME PCR

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The frequency of systemic infections caused by *Candida* has increased considerably in the last few years, especially among hospitalized patients. It has being considered the fourth agent isolated of bloodstream and is associated with significant morbidity and mortality. Although *Candida albicans* still remains as the most common fungal isolate from clinical specimens, longitudinal studies have detected a shift towards non-albicans *Candida* (NAC) species. The non-*albicans Candida* species are associated with the increase of antifungal drug resistance, and hence, species differentiation became very important. The aim of this study was to standardize a molecular technique to rapidly differentiate the most important *Candida* species isolated from the blood cultures. In order to differentiate the *Candida* species, a set of specific primers were designed based on variability in the internal transcribed spacer (ITS) region of ribosomal DNA, whose sequences were aligned using ClustalW. After

sequence alignment, a common sense primer was selected together with specific anti-sense primer for each *Candida* species detected by this system. This molecular technique was initially tested against several know *Candida* isolates and the reactions were confirmed to be specific for each species being detected. The reaction consisted of a PCR mix containing SYBR green as a fluorescent agent, a mixture of primers and fungal DNA in a total volume of 20 [[Unsupported Character - Symbol Font ]]L. Reactions were run on the LightCycler instrument (Roche) and *Candida* identification could be obtained by analyzing the different melting points of the different species. Two separated reactions were used to identify: *C. albicans, C. dubliniensis, C. tropicalis, C. parapsilosis, C. krusei, C. guilliermondii* and *C. glabrata*.The advantage of this methodology is, determine different *Candida* species directly from the blood. With molecular detection directly from the blood, the culture is avoided reducing the releasing time of result. Also, sometimes with the traditional methods used in yeast identification is not possible define property the species. So, it methodology helps to improving the better choice for treatment. Still, this specific method showed good sensibility and specifity in identify the *Candida* species. In conclusion, this is a rapid and possible method to apply in laboratory routine especially that attend hospitalized patients improving the epidemiology characterization and treatment of *Candida*.

96C

DEPOSITION OF H2A.Z BY SWR1 REGULATES EPIGENETIC SWITCHING AND INTERACTS WITH H3K56 ACETYLATION IN *CANDIDA ALBICANS*

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Histone variants and modifications have frequently been implicated in epigenetic regulation of gene expression. In particular, changes in nucleosome stability and chromatin-remodeler-driven histone turnover have become a promising mechanism for which epigenetic states may be formed and inherited. The histone variant H2A.Z, which is enriched adjacent to nucleosome-free regions of promoters of active and inactive genes, has been shown to affect nucleosome mobility and positioning. H2A.Z is deposited by the chromatin remodeling complex SWR1 via exchange with H2A. In multiceulluar organisms, H2A.Z has been associated with epigenetic regulation and reprogramming. Heightened acetylation of Histone 3 Lysine 56 (H3K56) is associated in yeast with histone deposition, as well as reduced incorporation and increased eviction of H2A.Z from nucleosomes. In *C. albicans*, H3K56ac levels have also been shown to regulate the white-opaque epigenetic phenotype, which is significant in understanding the ability of the organism to adapt to host environments. We postulate that incorporation of H2A.Z by SWR1 is a key regulator of epigenetic pathways such as white-opaque switching in *C. albicans* by altering nucleosomal dynamics. In this study, we have reduced H2A.Z incorporation in *C. albicans* through deletion of *SWR1*, the major subunit of the SWR1 complex. The *swr1* mutant displays increased white-to-opaque switching compared to wild type, and enhanced opaque cell stability under conditions normally favorable to white cells. The effect of deleting *SWR1* on switching rate, and its synergy with CO_2-induced switching, are similar to that of N-acetyl-D-glucosamine (GlcNac), suggesting a mechanism for GlcNac to drive white-opaque switching through H2A.Z. In addition, the *swr1* mutant shows misregulation of nucleosome positioning on the promoter of *WOR1*, the master regulator of white-opaque switching. Intriguingly, the *swr1* mutant displays synthetic lethality with nicotinamide, which promotes H3K56 acetylation by inhibiting its HDAC. This lethality demonstrates an interaction between SWR1 and H3K56ac, both of which influence nucleosomal dynamics, functioning upon essential pathways or genome stability. In particular,

this result suggests such a role for H2A.Z, as its incorporation is reduced by both deletion of *SWR1* and elevation of H3K56ac. Together, these results identify SWR1-catalyzed deposition of H2A.Z as a mechanism regulating epigenetic switching in *C. albicans*, and point to far-reaching effects of its interaction with H3K56ac. Further work will elucidate the mechanisms through which this interaction affects epigenetic regulation and genomic stability.

97A

CEK1 PHOSPHORYLATION FACILITATES ANTIFUNGAL ACTIVITY OF HST 5

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Histatin 5 (Hst 5) is a fungicidal salivary peptide active against *Candida albicans* and therefore a potential therapeutic agent for oropharyngeal candidasis (OPC). Fungal Mitogen-Activated Protein Kinase (MAPK) pathways including Hog1 and Cek1 respond to environmental conditions and may modulate *C. albicans'* sensitivity to Hst 5. Msb2 is the head sensor for the Cek1 pathway, and Cek1 phosphorylation (P-Cek1) is initiated by secreted aspartic protease-mediated cleavage of Msb2. We found that Hst 5 itself initiates P-Hog1 in *C. albicans,* making cells less susceptible to Hst 5. However Hog1 is a negative regulator of Cek1, so that cross-talk from the Cek1 pathway may also be responsible for differences in cell sensitivity to Hst 5. **Objective:** To identify whether *C. albicans* P-Cek1 modulates antifungal activity of Hst 5. **Methods:** Candidacidal assays were performed to examine *C. albicans* sensitivity to Hst 5 after pretreatment of cells for 1 h in YNB media with 1.25% N-Acetyl-D-Glucosamine (NAG) at 37C to induce P-Cek1; or in YNB media with 1.25% Glucose at 30C that does not induce P-Cek1. P-Cek1 was measured by Western blots of *C. albicans* lysates. Binding of FITC labeled Hst 5 to *C. albicans* was measured using FACScan, and rate of uptake of FITC-Hst 5 was quantitated using time-lapse confocal microscopy to calculate percentage of propidium Iodide (PI) positive cells/min. **Results:** *C. albicans* incubated with Hst 5 alone did not induce P-Cek1. *C. albicans* cells pre-conditioned to induce P-Cek1 were significantly more sensitive to Hst 5 (76.9% killing) than cells without P-Cek1 (52% killing). The rate of PI uptake in *C. albicans* exposed to Hst 5 was significantly higher (3.7 ± 0.4) when P-Cek1 was induced compared to control (1.3 ± 0.4), indicating faster uptake and killing by Hst 5. *hog1Δ/Δ* knockouts with constitutively high P-Cek1 due to de-repression were more susceptible to Hst 5 (79.7%) when compared to WT cells and had 3.8 fold higher Hst 5 binding and 2.5 fold higher Hst 5 uptake. Conversely, *C. albicans cek1Δ/Δ* and *msb2Δ/Δ* knockouts were less susceptible to Hst 5 killing than WT and *cek1Δ/Δ* cells showed less Hst 5 binding (0.46 fold) and uptake (0.66 fold). Cells pretreated with Pepstatin A, a specific inhibitor of aspartic proteases that blocks cleavage of Msb2 and P-Cek1, were less sensitive to Hst 5 compared to untreated cells. In contrast, *cpp1Δ/Δ* cells lacking Cek1 phosphatase with constitutive P-Cek1 as well as cells pretreated with 10% serum at 37C were significantly more susceptible to Hst 5. **Conclusion:** Cek1 phosphorylation facilitates Hst 5 antifungal activity against *C. albicans* by increasing its binding and/or uptake, likely a result of Cek1 mediated cell wall remodeling. This work was supported by NIH DE10641 from NIDCR.

98B

ANALYSIS OF COMBINATORIAL STRESS RESPONSES IN THE PATHOGENIC FUNGUS CANDIDA ALBICANS APPLYING AN INTEGRATIVE SYSTEMS BIOLOGY APPROACH

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Candida albicans is a major opportunistic fungus that is able to cause infection under certain circumstances [1, 2, 3]. Although others species of Candida have increased their prevalence as nosocomial systemic infections, C. albicans still remains as the major cause of fungemia in hospitals of developed countries. Multiple mechanisms, pathways and responses are involved in C. albicans stress adaptation and contribute to its virulence. To date, numerous studies have shown the strong responses Candida is capable of triggering when reacting to single stresses; but, there is not enough information about how it can respond to combinatorial stress, a situation closer to what happens in its normal environment in the host. Under the CRISP project, a descriptive mathematical model was developed. It was constructed based on the existing data and newly generated experimental data. We are trying to understand and predict how C. albicans responds to different single and combinations of stresses using Integrative Systems Biology approach. Oxidative stress responses in C. albicans include among others Hog1 MAPK signalling and the Cap1 factor transcription [4, 5]. An important number of genes were shown to have altered expression in response to oxidative stress (H2O2) in a Cap1-dependent manner. However, the responses induced under combination of oxidative and cationic stress are delayed. Most of the Cap1-dependent target genes like CAT1 and TRR1 present a delay in their expression under combinatorial stress conditions and this observed delay is in fact due to the increase in the intracellular ROS (Reactive Oxygen Species) and altered mitochondrial membrane potential and apoptosis induction [6]. Considering that immune system cells also generate reactive nitrogen species to kill Candida, we are trying to understand its behaviour including the nitrosative stress alone and in combination with oxidative and cationic challenge using the mathematical model. In general, combinatorial stresses induce more severe damage in Candida cells than individual stress due to cross talks happening between different stress responses pathways.

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99C

DEVELOPMENT OF AN IN VITRO ASSAY FOR THE DETECTION OF FUNGAL PATHOGEN-ASSOCIATED MOLECULAR PATTERNS

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The immune response to Candida spp. is initially mediated by pattern recognition receptors (PRRs) present in cells of the innate immune system as well as in non-immune cells. These receptors recognize pathogen associated molecular patterns (PAMPs) found in the fungal cell wall. Many PRRs participate in this process: Toll-like receptors (TLR) 2 and 4 and C-type lectin receptors like Dectin-2 recognize different forms of mannans and mannose-derived glycoconjugates, while Dectin-1 and

TLRs 2 and 6 recognize β-glucan. The stimulation of these PRRs by their respective ligands triggers a signaling cascade that will ultimately control cytokine and chemokine expression, and by that coordinate the innate immune response. To look in more detail into receptor ligand interactions and the triggered signaling cascades we use reporter cell lines able to analyze individual PRR or PRR-complexes. We currently improve and adapt existing PRR-reporter cell lines, to develop screening assays for analyzing collections of chemical compounds and selected fungal PAMPS for PRR-modulating activity. In addition we are setting up novel reporter assay and simple tissue models as tools to study host-pathogen interaction.

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STRUCTURE OF A NEW SEQUENCE-SPECIFIC DNA BINDING DOMAIN, THE WOPR DOMAIN, WHICH REGULATES PATHOGENESIS IN A WIDE VARIETY OF FUNGAL SPECIES

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WOPR-domain proteins function as master regulators of cell morphology and pathogenesis throughout the fungal kingdom. In Candida albicans and Candida tropicalis, the WOPR protein Wor1 is the master regulator of the white-opaque epigenetic switch. WOPR proteins regulate the yeast-mycelia transition in the dimorphic human pathogen Histoplasma capsulatum and are important for plant pathogenesis in species like Fusarium oxysporum and Botrytis cinerea. Genetic and biochemical experiments have previously demonstrated that these proteins bind specifically to DNA and regulate transcription. However, their primary sequence shows no relationship to any known DNA binding protein and the basis for their ability to bind DNA remained unknown. We have determined the 2.61Å crystal structure of a WOPR domain in complex with its preferred DNA sequence. Surprisingly, the structure reveals that two conserved regions, separated by an unconserved linker, form an interdigitated β-sheet that is tilted into the major groove of DNA. Although the main interaction surface with DNA is in the major groove, the highest affinity interaction occurs in the minor groove through an arginine residue. Further biochemical experiments confirm the importance of the protein-DNA contacts observed in the structure. No previously characterized DNA-binding domain combines these elements in the specific manner seen in the WOPR domain. As such, these findings reveal a new and unexpected mechanism by which sequence-specific DNA-binding proteins can recognize specific DNA targets. WOPR proteins use this unique structure to regulate morphology and pathogenesis in a wide variety of fungal species.

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A NEW TOOL TO QUANTIFY RECEPTOR RECRUITMENT TO CELL CONTACT SITES DURING HOST-PATHOGEN INTERACTION

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Candida species fungal pathogens are responsible for significant morbidity associated with mucocutaneous infections as well as mortality (~40%) caused by bloodstream infections. C-type lectins (CTLs), such as DC-SIGN and

CD206 (mannose receptor), are the receptors involved in forming fungal contact sites and interact with carbohydrates, such as mannan expressed on the cell wall of the yeasts. We have shown that mannan-binding receptors are the primary factor required for capturing and retaining yeasts. We hypothesize that mannan receptors are highly recruited at an early time in contact site formation, occupy a large percentage of the contact site and are retained long term to potentiate their important role in pathogen capture and retention. We further hypothesize that dendritic cells (DCs) can recruit receptors differentially to contacts with different fungal species, which allows them to tailor signaling to subtle differences in the cell wall. We developed a novel analysis algorithm to understand the temporal progression of receptor recruitment and distribution. The contact site analysis algorithm transforms the intensities of rectangular voxels to spherical voxels for analysis of the curved contact site and projects the intensity values onto the surface of the sphere for analysis. We found significant differences in area, intensity, and density of DC-SIGN and CD206 for the three species of fungi investigated. Contacts between DCs and *C. albicans*, *C. parapsilosis* or *S. cerevisiae* all recruited CTLs. *C. albicans* contacts recruited the largest amount of CTLs and occupied the largest percentage of the contact site for both CTLs by the end of the first hour. Both *S. cerevisiae* and *C. parapsilosis* also elicited similar, but significantly weaker, responses. When CTL recruitment and percent area were quantified we found intensely colocalized regions within the contact site. When we compare fungal species to one another, we find that *C. albicans* accumulates ~1.4 fold more colocalized DC-SIGN density than both *S. cerevisiae* and *C. parapsilosis*, but interestingly *C. albicans* accumulates ~3 fold more colocalized CD206 density than *S. cerevisiae* and only ~1.5 fold more colocalized CD206 density than *C. parapsilosis*. This is potentially important because the differences in receptor colocalization densities represent a possible mechanism by which DCs discriminate different yeasts. We also found that the majority of CTL recruitment occurs by the end of the first hour. Finally, we report preliminary results from the expansion of this method to higher degrees of receptor multiplexing in DC-*Candida* contact sites using multispectral confocal imaging and multiple component spectral unmixing analysis. The authors acknowledge NIH P50GM085273, NIH R25CA153825, and a UNM RAC grant (A.N.) as sources of funding instrumental to the conduct of this research.

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SUPER RESOLUTION MICROSCOPY REVEALS THE DIFFERENT EXPOSURE OF β-GLUCAN ON CANDIDA CELL WALL

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Candida albicans is a normal commensal and opportunistic pathogen of mucosal surfaces. *C. albicans* switching between yeast and hyphal forms is thought to be crucial to pathogenesis of invasive Candidiasis. Conventional Fluorescence and Electron microscopy studies have indicated that β-glucans are a component of cell wall and presented as patches on the cell wall surface. Previous studies demonstrated diminished β-glucans immunofluorescence staining on hyphae relative to yeast, and it is thought that *C. albicans* masks immunogenic glucan with mannan in attempt to modulate the host's immune response. Dectin-1, a C-type lectin expressed on immune cells, binds soluble β-glucan as well as purified particulate β-glucan. Interestingly, Dectin-1 signaling is only activated by particulate ligand (≥0.5 υm), not soluble β-glucan. However, we do not currently understand the mechanism of this particle discrimination nor do we understand how cellular signaling processes depend upon the mobility and geometry of glucan presentation. We hypothesize that nanoscale changes in β-glucans structure (size, density or inter-

cluster separation) are altered during hyphal transition. These nanostructural ligand changes may influence Dectin-1 signaling. We used direct Stochastic Optical Reconstruction Microscopy (dSTORM) to study the nanostructure of β-glucan exposed on cell wall. β-glucans were labeled with recombinant Dectin-1 conjugated with Alexa Fluor 647. Getis-Ord analysis was used to quantify β-glucan nanostructure in super resolution localization datasets from *C. albicans* yeast and hyphae. Mann-Whitney (non-parametric) statistical analysis indicated no significant differences of nanodomain diameter and intradomain β-glucan exposure density between yeast and hyphal forms. The median nanodomain diameters were 95 nm for hyphae and 114 nm for yeast. The medians of density were 130 counts/nm^2 for hyphae and 116 counts/nm^2 for yeast. However, the minimum inter-cluster distance of β-glucans nanodomains was significantly higher in yeast (median=86 nm) relative to hyphae (median=61 nm). These data represent the first nanoscale quantification of β-glucans exposure on *C. albicans*. The authors acknowledge NIH P50GM085273 and a UNM RAC grant (A.N) as sources of funding instrumental to the conduct of this research.

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THE MET RECEPTOR TYROSINE KINASE INTERACTS WITH THE EPIDERMAL GROWTH FACTOR RECEPTOR AND E-CADHERIN TO INDUCE EPITHELIAL CELL ENDOCYTOSIS OF CANDIDA ALBICANS

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Background: Invasion of oral epithelial cells is a key step in the pathogenesis of oropharyngeal candidiasis (OPC). Previously, we found that *C. albicans* invades oral epithelial cells by interacting with E-cadherin and a complex composed of the epidermal growth factor receptor (EGFR) and HER2. This interaction causes epithelial cells to endocytose *C. albicans* hyphae. Here we investigated the role of the met receptor tyrosine kinase in triggering the endocytosis of *C. albicans*. **Methods:** The tyrosine phosphorylation of met was assessed by immunoblotting with an anti-phospho-met antibody. The accumulation of EGFR, met, and E-cadherin around *C. albicans* in the OKF6/TERT-2 oral epithelial cell line was determined by indirect immunofluorescence. Epithelial cell invasion of *C. albicans* was quantified using a differential fluorescence assay. The therapeutic efficacy of the EGFR kinase inhibitor, gefitinib, and the met kinase inhibitor, SGX523, was determined using the corticosteroid-treated mouse model of OPC. **Results:** Infection of epithelial cells with *C. albicans* hyphae for 20 min stimulated the tyrosine phosphorylation of met. Also, met accumulated along with both EGFR and E-cadherin around hyphae in the infected epithelial cells. For example, after 20 min of infection, 98% of *C. albicans* hyphae that were surrounded by EGFR were also surrounded by met. Similarly, 78% of hyphae that were surrounded by E-cadherin were also surrounded by met. Almost no hyphae were observed to be surrounded by met without concomitant EGFR or E-cadherin. Furthermore, treatment of epithelial cells with either SGX523 or met siRNA decreased *C. albicans* invasion by approximately 50%. Dual inhibition of met and EGFR resulted in slightly greater reduction of epithelial cell invasion compared to inhibition of either receptor alone, whereas the combined inhibition of met and E-cadherin did not result in additive inhibition. Finally, mice treated with SGX523 had a 20-fold reduction in oral fungal burden after 5 days of infection compared to mice treated with vehicle alone. **Conclusion:** Met functions in the EGFR-E-cadherin pathway and is required for *C. albicans* to invade epithelial cells, both *in vitro* and *in vivo*.

104B

DUAL ROLE OF AN IMPORTIN-ENCODING GENE IN PROMOTING VIRULENCE AND COMMENSALISM IN CANDIDA ALBICANS

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Pathogenic fungi, like bacterial pathogens, must capture iron from host tissues and regulate iron homeostasis to avoid toxicity. In the opportunistic human fungal pathogen *Candida albicans*, we previously discovered that Sef1, a Zn_2Cys_6 zinc knuckle transcriptional factor, regulates iron homeostasis by integrating into a highly conserved regulatory circuit with reciprocal roles in *C. albicans* commensalism and virulence[1]. Post-transcriptional regulation of Sef1, including phosphorylation and nuclear localization, determines its role in pathogenesis[2]. In this study, we present evidence that Nmd5, encoding a putative importin/exportin, acts to transport Sef1 between cytoplasm and nuclei in different iron conditions. Nmd5 physically interacts with Sef1 in both high and low iron conditions. Under low iron condition, deletion of *NMD5* causes a significant increase of Sef1 protein but not RNA, and the relevant strain shows resistance to iron depletion. Moreover, *NMD5* overexpression mislocalizes Sef1 to cytoplasm whereas its deletion does not interfere with Sef1 nuclear localization, indicating that Nmd5 exports Sef1 to cytoplasm under low iron condition. Unexpectedly, in high iron condition we observed a significant decrease of Sef1 protein in the *nmd5*Δ/Δ*sfu1*Δ/Δ double mutant where Sfu1-mediated inhibitory effect on *SEF1* transcription is abolished, suggesting that Nmd5 imports Sef1 to nuclei under high iron condition. Furthermore, *nmd5*Δ/Δ displays significant virulence defects in a mammalian bloodstream infection model and also outcompetes with wild type in a mouse model of gastrointestinal infection. Together, we identified a novel dual role of Nmd5 in transporting Sef1 protein: an exportin to export Sef1 to cytoplasm under low iron condition and an importin to import Sef1 to nuclei under high iron condition, and this specifies its role in promoting both virulence and commensalism, just as Sef1 does. 1. ***Chen, C*.***, Pande, K*., French, S. D., Tuch, B. B. and Noble, S. M. (2011) A unique iron homeostasis regulatory circuit with reciprocal roles in *Candida albicans* commensalism and pathogenesis. ***Cell Host & Microbe***. 10(2): 118-35. (*equal contribution) 2. ***Chen, C.*** and Noble, S. M. (2012) Post-transcriptional regulation of the Sef1 transcription factor controls the virulence of *Candida albicans* in its mammalian host. ***PLOS Pathogens***, 8(11): e1002956.

105C

THE ROLE OF ECE1P PROCESSING IN THE ACTIVATION AND DAMAGE OF EPITHELIAL CELLS BY CANDIDA ALBICANS.

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Candida albicans is the most commonly encountered human fungal pathogen and the third most common hospital-acquired bloodstream infection. Candida infections cause mucosal and life threatening systemic ailments that contribute to high morbidity and mortality worldwide. During disease progression, C. albicans grows as infiltrating pathogenic hyphae that damage and invade host tissue. The ability of the host to respond rapidly to the damage caused by pathogenic infiltration is critical in maintaining tissue homeostasis. Recently, we demonstrated that epithelial cells are able to discriminate between the yeast and hyphal forms of C. albicans through a bi-phasic p38/c-Fos response that is dependent upon hyphal burden, resulting in the induction of a

strong mucosal inflammatory response. During hyphal growth, the ECE1 (Extent of Cell Elongation) gene is highly expressed. ECE1 encodes a 271 amino acid protein (Ece1p) which serves as a substrate for KEX2 protease-mediated cleavage in vitro. Digestion of Ece1p by Kex2p yields eight individual peptide fragments (Ece1 P1-8), seven of which terminate in a conserved lysine-arginine (KR) motif. In this study, we constructed a series of peptides corresponding to Ece1 P1-8 and assessed their ability to damage epithelial cells. We identify Ece1 P3 as the active region of Ece1p responsible for damaging epithelial cells in vitro. To examine the role of the conserved KR motif in the damage process, Ece1 P3 peptides terminating in KA, AA and AR were created. We show that the ability to cause cellular damage is dependent upon an intact terminal KR motif, as substitution of arginine with alanine abolished damage. We next assessed the ability of Ece1 P3 to activate mucosal inflammatory responses. Epithelial cells were exposed to Ece1p peptides and quantified for release of proinflammatory cytokines. Substitution of the Ece1 P3 terminal arginine with alanine resulted in a notable decrease in the production of IL-1 alpha, IL-1 beta and IL-6. Although a substrate for Kex2p in vitro, cleavage of Ece1p by Kex2p in vivo has yet to be demonstrated. Whilst C. albicans kex2 mutants are unable to damage or activate epithelial cells in vitro, disruption of KEX2 exerts many deleterious effects, not all of which can be attributed to a lack of Ece1p processing. To circumvent the problems of kex2 mutant fitness, we used site directed mutagenesis to mutate each of the Kex2p recognition sequences in Ece1p individually and in combination. These mutant strains were quantified for their ability to cause damage, stimulate pro-inflammatory responses and activate the discriminatory pathway. In conclusion, we identify C. albicans Ece1 P3 as the mediator of host damage, the activator of mucosal inflammatory responses, and the target of the epithelial pathogenic discriminatory mechanism.

106A

UNDERSTANDING HOW CANDIDA ALBICANS ESCAPES FROM MACROPHAGES

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Candida albicans evades the innate immune response by escaping from phagocytosis by macrophages. The switching from yeast to hyphal morphology is important for escape, but the mechanism is unclear. The current model is that the highly polarized growth of hyphae results in mechanical destruction of macrophages by causing piercing of the host cell membrane. This model is challenged by recent reports of C. albicans mutants that form hyphae of wild type morphology, but are less able to cause macrophage death. We devised a new live-cell microscopy assay to monitor the interaction of C. albicans with primary bone marrow derived macrophages, and show that C. albicans causes macrophage cell death by at least two mechanisms. In the first 6-8 h post-phagocytosis, C. albicans kills macrophages by triggering pyroptosis, a proinflammatory programmed host cell death pathway. This is followed by a rapid macrophage killing phase that last up to 16 h. This second phase of killing is orechestrated by wild type hyphae, but it does not require the pyroptotic caspases. The gene expression regulator Mediator controls C. albicans hypha formation in macrophages, and the establishment of hyphal surface architecture that is required for triggering pyroptosis. Our data shows that mechanical destruction of macrophages by hyphae plays a small, if any role in early macrophage death following C. albicans infection. Instead, C. albicans hijacks activation of macrophage pyroptosis for escape and immune evasion.

107B

INACTIVATED BLASTOCONIDIA PROTECTS KERATINOCYTES FROM C. ALBICANS GROWTH AND FILAMENTATION BY THE INDUCTION OF HUMAN β-DEFENSINS 2 AND 3

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Candida albicans is microorganism able to transform from commensal (blastoconidia) into a pathogen (hyphae) when local environment changes or in immunosuppressed patients. Keratinocytes are able to discriminate between the both forms of C. albicans, allowing it permanence as a commensal in a homeostatic stage. The role of commensal C. albicans has been poorly explored in keratinocytes. We hypothesized that the commensal form of C. albicans is protective against it invasive form avoiding the immune evasion by hyphae and by the induction of human β-defensins (HBD). Methodology: C. albicans was cultured in YPD and LEE media to produce blastoconidia and hyphae respectively, and later, fungal cells were inactivated by heat. Keratinocytes were cultured to be confluent and stimulated at different times at a multiplicity of infection (MOI) of 1:0.1 with blastoconidia and hyphae at different proportions (100/0%, 75/25%, 50/50%, 25/75% and 0/100%). The cytokine response (IL1-β and IL-10) was measured by ELISA. Later, keratinocytes were infected with alive C. albicans previous stimulation with inactivated blastoconidia versus unstimulated keratinocytes. The HBD-2 and HBD-3 expression was assessed by qPCR. Results: Keratinocytes recognized differentially blastoconidia and hyphae of C. albicans early at 30 min. Hyphae induced a more inflammatory response (IL- β increase) in the first 30 min, but at 24 h an anti-inflammatory balance was observed (IL- β decrease, and IL-10 increase) compared to blastoconidia. The presence of blastoconidia did not prevent the immune evasion by hyphae, independently of the blastoconidia proportion. Inactivated blastoconidia perse induced HBD3 and increased significantly the expression of HBD2 and HBD3 in C. albicans infected keratinocytes at 24 h of C. albicans infection. Blastoconidia stimulated keratinocytes supernatants were able to inhibit filamentation and growth of C. albicans. Conclusions: Although inactivated blastoconidia did not avoid the immune evasion by hyphae they were protective in keratinocytes by the induction of HBD inhibiting C. albicans growth and filamentation. This finding could be relevant to understand the commensal role of C. albicans and for vaccines and/or immunomodulation based therapies development. Project funded by Fondecyt 11110160

108C

ROLE OF HYPHA FORMATION AND ECE1P IN THE COMMENSAL-PATHOGENIC SWITCH OF *CANDIDA ALBICANS*

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The commensal/pathogenic switch of the opportunistic fungus *Candida albicans* is important for understanding its pathogenicity and interactions with mucosal tissues. We previously showed using *C. albicans* strain SC5314 that oral epithelial cells discriminate between the yeast and hyphal forms of *C. albicans* via a biphasic MAPK response. At 2 h, *C. albicans* SC5314 hyphae activate epithelial cells by inducing MKP1 and c-Fos, and at 24 h hyphae induce epithelial cytokines and cause cellular damage. As such, this discriminatory response may be important in identifying when this fungus has become pathogenic. Also in previous work we have identified a clinical 'commensal' *C. albicans* strain, 529L, which

colonised a concurrent oral and vaginal model in high numbers but was unable to efficiently invade or induce damage *in vivo*. Lack of invasion and damage correlated with the lack of activation of MKP1 and c-Fos at 2 h, and cytokines and damage at 24 h. This was in stark contrast to *C. albicans* SC5314, which was unable to colonise the same concurrent model despite activating MKP1, c-Fos, cytokines and damage. We have now identified the hypha-associated protein in *C. albicans* SC5314, Ece1p (Extent of Cell Elongation 1), which activates MAPK signalling, cytokines and damage. Ece1p (271 amino acids) contains seven consensus recognition sites (Lys-Arg: KR) for the protease Kex2p and processing of Ece1p *in vitro* yields eight separate peptide fragments. Analysis of custom-made peptides corresponding to these eight peptide fragments identified that the third N-terminal peptide (Ece1p3; 32 amino acids in length) accounted for signal activation, cytokine induction and cell damage. Given this, we hypothesised that the differences in either *ECE1* gene expression or Ece1p3 activity between *C. albicans* SC5314 and 529L may account for the *in vitro* and *in vivo* phenotypes observed. We found that *ECE1* gene expression was significantly lower in *C. albicans* 529L ('commensal') as compared with SC5314 ('pathogenic'). Also, analysis of additional clinical strains corresponding to the same clades as SC5314 (clade 1) and 529L (clade 16) also reveal lower *ECE1* gene expression profiles from clade 16 strains. Furthermore, amino acid sequence analysis between SC5314 ('pathogenic') and 529L ('commensal') Ece1 protein identified specific differences in the Ece1p3 activating region. Currently we are assessing whether these differences in *C. albicans* SC5314 and 529L Ece1p3 affect epithelial cell activation. Our work identifies *C. albicans* Ece1 is a major pathogenicity factor and immune activator that might be targeted by epithelial cells to identify the commensal-pathogenic switch during mucosal *C. albicans* infections.

109A

REGULATION OF HOST-FUNGUS INTERACTION BY THE MICROBIOTA

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The host immune system is considered one primary obstacle to fungal colonization. In the case of commensals, such as *Candida albicans*, the constant intertwining with the mammalian immune mechanisms would predict a contingency-based system during co-evolution to guarantee persistence in an inflammatory host environment. Recent progress in our understanding of how fungal signaling circuits operate molecularly in sensing environmental factors suggests that this process is much more complex than previously appreciated. It is now clear that a three-way interaction between host, fungi, and microbiota dictates the types of host-fungus relationship. We have discovered that the trpyptophan metabolism pathway is exploited by commensals and the mammalian host to increase fitness in response to fungi via resistance and tolerance mechanisms. Switching from sugar to tryptophan as an energy source−under conditions of unrestricted tryptophan availability such as those resulting from indoleamine 2,3-dioxygenase 1 deficiency in the host or from tryptophan feeding−highly adaptive lactobacilli are expanded and regulate innate IL-22 production. Metabolomics revealed that a variety of indole derivatives, generated through such a conversion acted as endogenous ligands for the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor that mediates IL-22 production. As a result, an optimally balanced mucosal response allows for survival of mixed microbial communities, yet provides colonization resistance to *C.albicans* as well as protection from damage-induced inflammation. Thus, the trpyptophan metabolism pathway is exploited by commensals and the mammalian

host to increase fitness in response to fungi in an immunologically dynamic environment via host's AhR. This might represent a general, important strategy pursued by co-evolutive commensalism for fine-tuning mucosal reactivity. *This work is supported by the SP2-Ideas, ERC-2011-ADG_20110310. FUNMETA, Grant Agreement 293714.*

110B

INACTIVATION OF THE CANDIDA ALBICANS CAP1 TRANSCRIPTIONAL FACTOR FOLLOWING SIMULTANEOUS EXPOSURE TO CATIONIC AND OXIDATIVE STRESSES

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Stress responses are essential for Candida albicans to evade host anti-microbial defence mechanisms, and to survive the rapidly changing microenvironments found within different host niches. Following phagocytosis by innate immune cells, Candida albicans is subjected concurrently to reactive oxygen species and cationic fluxes. This is significant as such combinations of stresses are much more potent at killing this fungal pathogen then the corresponding single stresses. Such combinatorial stress treatments trigger the synergistic killing of C. albicans by significantly increasing intracellular ROS levels which leads to "stress pathway interference". Specifically, we find that the major regulator of anti-oxidant gene expression in C. albicans, the AP-1-like transcription factor Cap1, fails to be activated following combinatorial oxidative and cationic stress treatments. Previously we have shown that activation of Cap1 requires the Gpx3 and Ybp1 mediated oxidation of conserved cysteine residues. This Gpx3 and Ybp1-mediated oxidation masks the nuclear export sequence within Cap1, resulting in the nuclear accumulation of this transcription factor and the induction of Cap1-dependent genes. However, following simultaneous exposure of C. albicans cells to oxidative and cationic stress, Cap1 is hyper-oxidised and fails to accumulate in the nucleus. We find that this combinatorial stress-induced hyper-oxidation of Cap1 requires both Gpx3 and Ybp1 and is transient. Furthermore, we find that a similar hyper-oxidised form of Cap1 is prevalent following exposure of cells to high levels of H2O2, and importantly Cap1 activation is delayed at high compared to low levels of oxidative stress. Based on these findings we predict that the hyper-oxidised form of Cap1 generated in response to high levels of H2O2, or the increased intracellular ROS levels post combinatorial stress treatment, is an essential intermediate in the formation of active oxidised forms of Cap1. Currently we are investigating the oxidation events underlying the formation of hyper-oxidised inactive, and oxidised active forms of Cap1.This will help us understand the mechanism underlying the delay in Cap1 activation following combinatorial stress treatment, which prevents a rapid adaptive response to H2O2 leading to C. albicans killing by innate immune cells.

111C

CONTRIBUTION OF INNATE IMMUNE RECEPTOR TLR2 IN THE LOCAL RESPONSE IN VULVOVAGINAL CANDIDIASIS (VVC)

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Candida albicans is a commensal of the mucosal surface and the most frequent cause of VVC. Vaginal epithelial cells constitute an active barrier that protects the female reproductive tract from different microorganism. These

cells can express pathogen recognition receptor (PRR) that sense the pathogen presence and activate the production of immune mediators. TLR2 (*toll like receptor*) is PRR involved in *C. albicans* recognition and is associated to a protective role during systemic infection. Herein, we aimed to study the contribution of TLR2 innate receptor in the local response against *C. albicans* in a mouse model of VVC. Female C57BL/6 (WT) and TLR2-deficient (KO) mice in estrus phase (estradiol treated) were intravaginally inoculated with 5.10^6 *C. albicans* ATCC 36801 at day (D) 0 (Inf). At D 2,4 and 8 of infection, vaginal lavage (VL) was obtained to evaluate the cell type present, fungal burden (CFU) and IL-6, IL-1β, TNFα and TGFβ levels (ELISA); vaginas were removed for histological studies (HE and PAS stain) and evaluation of inflammation score (IS: 1-5). Estrogenized (E) and Untreated mice were included as controls. VL was characterized by the presence of PMNC and fungal forms infecting epithelial cells. The infection remained along the evaluated days in both strains but fungal burden profile was different; at D2 the CFU in KO-Inf mice was higher than WT-Inf animals ($p<0,05$), meanwhile at D4, the fungal burden in KO-Inf mice was significantly reduced compared to WT-Inf animals ($p<0,05$). In WT-Inf mice, the fungus was present in lumen and stratum corneum and was associated with moderate PMNC infiltrate (IS:2); in KO-Inf mice, the invasive morphotype was clearly visualized (D2) and decreased at D4 and D8, the inflammatory reaction was characterized by frequent intraepithelial abscesses (IS:5). Meanwhile in WT-Inf mice the level of IL-6 did not exhibit changes, the IL-1β was significantly diminished (D4, D8) compared with WT-E group ($p<0,05$). In KO-Inf mice IL-6 (D2, D4, D8) and IL-1β (D4, D8) secretion was enhanced compared to KO-E mice ($p<0,05$). No change in constitutive levels of TNFα and TGFβ were detected. These results illustrate the ability of TLR2 deficient mice to orchestrate an efficient local reaction that controls the vaginal infection by *C. albicans*. While the systemic role of TLR2 is associated with protective mechanisms, intravaginally activation would not be relevant to the control of pathogen.

112A

CHARACTERIZATION OF C. ALBICANS ORF19.4612 DELETION MUTANT, A GENE UPREGULATED IN THE PRESENCE OF ARACHIDONIC ACID

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Background: The pathogenic yeast, *Candida albicans*, can form immunomodulatory prostaglandin E_2 (PGE_2) from host derived arachidonic acid (AA). A study that was conducted to evaluate the effect of AA on the genome wide expression of genes by *C. albicans* revealed several open reading frames that were upregulated in the presence of AA. Further analysis revealed that seven of these orfs may be involved in yeast to hyphal transition as well as biofilm and germ tube development in *C. albicans*. One of these genes, orf19.4612, is currently uncharacterized. Since there is significant overlap in the signaling pathways that regulate yeast to hyphal transition as well as white-opaque switching, we characterized this gene in terms of morphogenic switching (i.e. yeast to hyphal transition and white-opaque switching) in response to PGE_2. **Methods:** The orf19.4612 deletion mutant was constructed using the *SAT1* flipper. Yeast to hyphal transition was assayed using microscopic evaluation of germ tube formation in RPMI medium. White-opaque switching was analyzed on complex and synthetic phloxine B-containing media with different carbon sources (i.e. glucose and *N*-acetyl glucosamine), and visualized with light and electron scanning microscopy. **Results:** It is known that PGE_2 significantly increases germ tube formation in the wild type. This effect was also observed for *Δorf19.4612.*

Interestingly, *Δorf19.4612* opaque phase was maintained at high levels both at 25 °C and 37 °C, with switching frequencies varying on different carbon sources, suggesting that this gene plays an important role in this process. In addition, our preliminary observations also suggest that it may be possible for PGE_2 to induce not only germ tube formation, but also white-opaque switching in both the wild type and the mutant. **Conclusions:** Our data suggest that this gene was upregulated in the presence of AA and/or PGE_2 to enable *C. albicans* to undergo phenotypic switching, which is largely implicated in virulence or to facilitate host invasion.

113B

OVEREXPRESSION OF PUTATIVE ACTIVATORS OF CANDIDA ALBICANS TOR1 REPRESSES NITROGEN STARVATION INDUCED FILAMENTATION AND MEP2 EXPRESSION.

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Target of Rapamycin (Tor1) regulates cell growth and filamentation in response to nutrient availability. This study aims to characterise putative upstream activators of Tor1 and determining if these proteins could be potential antifungal targets. The G-protein complex Gtr1/Gtr2 in S. cerevisiae activates Tor1 in response to amino acid signals via a guanine-nucleotide exchange factor, Vam6. Homologues of these are found in C. albicans. A second activator, the recently characterised G-protein Rhb1 has also been described in C. albicans. Preliminary work involved determining if overexpression of these putative activators resulted in strains with phenotypes characteristic of a constitutive Tor1 activated state. In order to achieve this, the Enolase1 promoter (ENOp) was placed upstream of VAM6, GTR1, RHB1 and TOR1 in C. albicans SC5314 and gene-specific qRT-PCR performed to confirmed overexpression (>10-fold) of each of the genes. Phenotypically, the overexpression strains were indistinguishable from SC5314 on nutrient rich YEPD. However on nitrogen poor solid media (100 µM urea, ammonium sulphate or proline) where SC5314 forms a filamentous fringe, the PENOVAM6, PENOGTR1, PENOTOR1 and PENORHB1 strains exhibited a reduction in the ability to filament. In order to determine if this phenotype was due to constitutive Tor1 activation, the experiment was repeated on media containing rapamycin. Rapamycin treatment reversed the inhibition of filamentous growth in all overexpression strains. Furthermore, analysis of the growth rate of the PENOVAM6, PENOGTR1, PENOTOR1 and PENORHB1 strains in 100 µM urea demonstrated an increase in doubling times compared with WT SC5314, suggesting a constitutive Tor1 active state. In order to determine if these constructs affected Tor1 regulated gene expression, MEP2 expression was analysed. MEP2 is an ammonium permease and regulator of nitrogen starvation-induced filamentation. Previously Taso et al. confirmed a decrease in MEP2 expression in an RHB1 over-expressed strain. Therefore, qRT-PCR was preformed on all PENO strains in order to determine if PENORHB1, as well as the other PENO strains demonstrated this phenotype. Results confirmed a decrease in MEP2 mRNA in all with the largest decrease noted in PENOVAM6. Analysis of filamentous growth in YPD broth with 10% FCS demonstrated that the PENOVAM6, PENOGTR1, PENOTOR1 and PENORHB1 strains produced hyphae and pseudohyphae but exhibited reduced capacity to aggregate, possibly due to altered expression of hypha-specific cell surface proteins. Reduced aggregation could be quantified by filtration of aggregated cultures and enumeration of free planktonic cells. Homozygous knock-out mutants are currently under construction to determine how essential these genes are to growth of C. albicans.

114C

THE PHO4 TRANSCRIPTION FACTOR IS REQUIRED FOR STRESS RESISTANCE AND VIRULENCE IN *CANDIDA ALBICANS*

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Candida albicans is exposed to a range of stresses during phagocytosis by innate immune cells, including reactive oxygen species (ROS), cationic fluxes, and an acidic pH environment. Whilst certain regulatory proteins, including the Hog1 and Rad53 kinases and the Cap1 transcription factor, have been found to respond to ROS or cationic stress, there is still much to learn regarding how this major pathogen senses and responds to physiological relevant stresses. To address this we have performed quantitative fitness analysis to screen available *C. albicans* gene deletion collections for mutants which exhibit impaired growth in response to oxidative stress. This identified genes not previously implicated in stress responses, including the Pho4 transcription factor. Cells lacking *PHO4* were significantly more sensitive to oxidative, cationic, heavy metal and pH stresses compared to wild-type cells, and displayed attenuated virulence in a *Caenorhabditis elegans* model of infection. In addition, we find that Pho4 is robustly phosphorylated in response to the aforementioned stresses, although this occurs independently of the Hog1 stress activated protein kinase. Current studies are directed at delineating the role and regulation of Pho4 in mediating *C. albicans* stress resistance and virulence. Significantly, the Pho4 homologue in *Saccharomyces cerevisiae* is not required for oxidative or osmotic stress resistance, indicating that the function of Pho4 has been reassigned in *C. albicans* to respond to the stressful environments encountered within the host.

115A

THE CANDIDA-INDUCED LOSS OF ENDOTHELIAL BARRIER FUNCTION IS MEDIATED THROUGH THE VEGF SIGNALING PATHWAY.

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Rationale: The opportunistic fungi of the genus *Candida* account for nearly 10% of all nosocomial blood stream infections in the United States and are the fourth leading cause overall, with *C. albicans* isolated most frequently. Mortality rates reach 75%, often despite aggressive antifungal treatment, with costs exceeding $1 billion annually. In recent decades there has been a significant increase in the number of immunocompromised patients and those receiving immunosuppressive therapies (solid organ transplants, etc.) This, in addition to the increased use of broad-spectrum antibiotics, has led to increased frequency of candidemia and other fungal infections of the blood, resulting in substantially higher risk of death, increased hospital stays, and higher costs for treatment. Fungal-induced severe sepsis and septic shock account for increasing morbidity and mortality worldwide, particularly in intensive care units. Recent findings by our group and others strongly suggest that manipulation of the vascular endothelial response can significantly reduce sepsis-related mortality. **Hypothesis:** We hypothesize that *Candida* initiates a unique program of endothelial dysfunction, independent of classical inflammatory mechanisms traditionally implicated in sepsis. **Approach & Results:** We analyzed the endothelial response to *Candida* infection in vitro using qRT-PCR, ELISA, Western blots, cellular assays, and immunocytochemistry. We find that *Candida*-infected endothelial monolayers display a number of extensive biochemical and morphological changes, including widespread apoptosis and the rapid loss of barrier function resulting from the breakdown of adherens junctions. Additionally,

we provide evidence that signaling through the vascular endothelial growth factor (VEGF)-pathway plays a primary role in the breakdown of the endothelial cell-cell junctions that drives *C. albicans*-induced hyperpermeability. **Conclusion:** We propose that the endothelial dysfunction that occurs during the early steps of *C. albicans* infection likely facilitates fungal dissemination and, perhaps more importantly, contributes to many of the life-threatening host responses associated with fungal sepsis. Results from these studies will contribute to the development of novel therapies aimed at reinforcing the endothelial response and delaying the onset of *Candida*-induced hyperpermeability, thereby prolonging the window of opportunity for accurate diagnosis and treatment, and ultimately resulting in increased patient survival.

116B

RELEASE OF ADRENOMEDULLIN FROM VULVO-VAGINAL RECONSTITUTED HUMAN EPITHELIUM (VRHE) DURING CANDIDA INFECTIONS PROMOTES FILAMENTATION IN VITRO

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Whereas extensive knowledge on how Candida species mediate adaptation to host tissues is available on the molecular level, insights into the host response is still in its infancy. Accordingly, our understanding on how human epithelia respond to interactions with various commensals or pathogens is just emerging. In this respect, we analysed vulvo-vaginal reconstituted human epithelia (vRHE) infected with Candida albicans and Candida dubliniensis during early steps of adhesion to find out how vulvo-vaginal A-431 cells responds on the transcriptional level and whether these cells might discriminate between different pathogens on the molecular level. With the advent of next-generation DNA deep sequencing technologies unbiased approaches for the analysis of gene expression from complex metatranscriptomes derived from more than one species became available. Therefore, we applied this approach to simultaneously analyse host-pathogen interactions during infections of vRHE by C. albicans or C. dubliniensis, two Candida species that are closely related with respect to phylogeny, but behave profoundly different regarding colonization efficiency, morphogenesis and virulence. For this purpose, complex RNA from vRHE infected with either of both fungal species was isolated following one or three hours of adhesion and subsequently was analysed using RNA-seq. Remarkably, we found a highly specific set of only 17 genes detected to be differentially regulated in response to infection with C. albicans or C. dubliniensis. There was no significant difference in the transcriptional profiles between host cells infected with one or the other Candida species, indicating that vulvo-vaginal cells do not discriminate between both closely related fungi. Among these genes identified we found adrenomedullin (ADM) that already was strongly upregulated after one hour of infection. ADM is a hypotensive and blood circulating peptide with antimicrobial activity for which a possible role during fungal colonization has not been described yet. Although ADM has been shown to inhibit bacterial growth, we surprisingly found that this peptide promotes hyphal development under certain conditions. Moreover, biofilm formation of C. albicans was also enhanced in the presence of adrenomedullin. Paradoxically, infection of vulvo-vaginal epithelia with C. albicans seems to provoke the release of a host defence factor that promotes colonization of this fungal pathogen.

117C

COMPARATIVE PHENOTYPIC AND TRANSCRIPTIONAL ANALYSIS OF HOMOZYGOUS MED3 AND TLO GENE MUTANTS, ENCODING SUBUNITS OF THE MEDIATOR COMPLEX.

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Candida albicans is signficantly more virulent than the closely related species Candida dubliniensis. Comparative genomic analysis of these two species identified a major disparity in copy number for a family of telomere- associated (TLO) genes that have homology to MED2, which encodes a component of the yeast Mediator complex. The C. albicans genome contains 14 TLO genes whereas C. dubliniensis contains two (CdTLO1 and CdTLO2). Previous studies carried out in our lab revealed that the deletion of the two C. dubliniensis TLO genes affects morphogenesis, growth in galactose- containing media and oxidative stress responses induced by H2O2. We hypothesize that the disparity in gene copy number between C. albicans and C. dubliniensis may contribute to the differential virulence of the two species.
The presence of a conserved putative Med2-like domain in the CaTlo and CdTlo proteins suggests that they are associated with the RNApol II Mediator complex. Previous work carried out by Myers et al. (2012) found that in C. albicans the homozygous deletion of MED3 resulted in phenotypes similar to those observed in the CdtloΔ/Δ double mutant. Given that both the Tlos and Med3 are anchored to the tail region of the Mediator complex, we hypothesized that their similar location may indicate similar roles in transcriptional regulation. In order to investigate this further we generated a homozygous deletion of the gene encoding Med3, a known component of the Mediator complex in C. dubliniensis. We compared the phenotype of the Cdmed3Δ mutant with that if the CdtloΔ/Δ double mutant and found that the two mutants have identical phenotypes. This includes the ability to form true hyphae, poor growth in the presence of alternative carbon sources (i.e. galactose) and increased sensitivity to oxidative stress induced by H2O2. DNA microarray studies have shown similar expression profiles for the tlo$\Delta\Delta$ and med3Δ mutants in relation to galactose metabolism (GAL1, GAL7,GAL10), oxidative stress (SOD5, CAT1) and morphogenesis (HWP1, TEC1, UME6). However, although the expression profiles of the two mutants are similar under nutrient- rich conditions, there are significant differences in the other genes that are up- and down- regulated in the two mutants under hyphal- inducing conditions (10% FBS and H2O), suggesting that the two proteins may differ in the subsets of genes under their respective control under certain environmental conditions

118A

SOD1 AND SOD2 IN THE OXIDATIVE STRESS RESPONSE IN CANDIDA GLABRATA.

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Candida glabrata is an opportunistic fungal pathogen that can cause severe invasive infections. C. glabrata is extremely resistant to oxidative stress and can survive inside the phagocytic cells, probably due to its natural ability to detoxify reactive oxygen species generated by the phagocytic cell. In fact, C. glabrata is highly resistant in vitro to oxidative stress generated by H2O2 relative to that of S. cerevisiae and even C. albicans. This high resistance to H2O2 is mediated by the single catalase Cta1 and regulated by the concerted action of the transcription factors Yap1, Skn7, Msn2 and Msn4. Given that superoxide dismutases (SODs) also contribute to virulence in many pathogenic bacteria and fungi including Candida albicans, we decided to understand the role of SODs during the oxidative stress response in C. glabrata. SODs convert the

highly reactive superoxide radical to hydrogen peroxide (H2O2) and molecular dioxygen. C. glabrata has 2 genes encoding two superoxide dismutases: Cu/Zn-SOD (SOD1) and Mn-SOD (SOD2). SOD1 is the major contributor of the total SOD activity and is localized in the cytoplasm while Sod2 is present in the mitochondria. SOD1 provides protection against superoxide during fermentative growth. In contrast, SOD2 is required to eliminate superoxide during cellular respiration. Consistent with this, the double mutant is more sensitive to oxidants than the single mutants. Interestingly, the sod1Δ sod2Δ mutant is auxotroph for lysine, has a high rate of spontaneous mutation and reduce chronological lifespan. These data suggest that absence of SODs in C. glabrata could increase the levels of superoxide damaging several enzymes involved in lysine biosynthesis, respiration, DNA repair and response to xenobiotics.

119B

THE TELOMERE-ASSOCIATED (TLO) GENES OF CANDIDA DUBLINIENSIS ENCODE SUBUNITS OF THE MEDIATOR COMPLEX THAT EXHIBIT WIDESPREAD LOCALIZATION TO BOTH CODING AND NON-CODING REGIONS OF DNA

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The telomere-associated (*TLO*) genes of *Candida spp*. encode a family of proteins with homology to the Med2 component of the Mediator complex, involved in transcriptional activation. The *Candida albicans* genome contains 14 putative *TLO* genes whereas its less pathogenic relative *Candida dubliniensis* contains only 2. We hypothesized that this disparity in *TLO* copy number contributes to the contrasting virulence between these two closely related species. Tlo1 has also been co-purified with components of the mediator complex and we propose that Tlo1 is the previously uncharacterized ortholog of Med2 in *Candida spp.* Homozygous deletion of both *TLO1* and *TLO2* significantly altered a number of phenotypes, including reduced ability to filament, increased sensitivity to oxidative stress and slowed growth in the presence of alternative carbon sources (e.g. galactose/succinate) and minimal media. Complementation with *C. albicans TLO11* and *TLO12* restored wild-type behavior. Using DNA microarray analysis of Δ*tlo* mutants, we observed changes in expression of important filamentous growth regulators (*RIM101, TEC1, UME6, RAS1*), hyphal-cell wall proteins (*HWP1, RBT5*) and superoxide dismutases. In glucose-poor medium, Δ*tlo* mutant cells exhibit retarded induction of genes essential to galactose metabolism (*GAL1*, *7* & *10*). Restoration of normal expression was observed upon reintroduction of *TLO1. However,* reintroduction of *TLO2* did not fully restore wild-type gene expression or phenotypes. Using HA-tagged Tlo1, chromatin immunoprecipitation (on chip) was performed and we have observed enrichment of Tlo1 protein at a large number of orf's (22%), particularly those of expressed genes, which correlates with its putative role as a transcription factor. In addition, we observed significant enrichment across telomeric and repeat (MRS) regions throughout each chromosome suggesting additional roles of Tlos. Preliminary localization studies using GFP-tagged Tlo proteins in *C. dubliniensis* suggest localization within the nuclear compartment, which is consistent with our proposed role of Tlo proteins in transcriptional regulation as components of the mediator complex. Our studies thus far illustrate that *TLO* genes regulate diverse processes in *C. dubliniensis* and are involved in the ability to exhibit several virulence-associated traits. The presence of a Med2-like domain and recent work from our group suggest that *TLO*s encode a part of the mediator complex required for optimum, high level expression of genes that are induced in response to specific environmental conditions.

120C

SIR3 POLYMORPHISMS IN CANDIDA GLABRATA CLINICAL ISOLATES

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The opportunistic fungal pathogen Candida glabrata adheres tightly to epithelial cells in culture, mainly through the adhesin Epa1. EPA1 is the founding member of a family of up to 23 putative adhesin-encoding genes present in the C. glabrata genome. The majority of the EPA genes are localized close to the telomeres, where they are repressed by subtelomeric silencing that depends on the Sir, Ku, Rif1 and Rap1 proteins. EPA6 and EPA7, also encode functional adhesins that are repressed in vitro. EPA1 expression in vitro is tightly controlled both positively and negatively, and in addition, presents high cell-to-cell heterogeneity, which depends on Sir mediated silencing. We have characterized the ability to adhere to HeLa epithelial cells of a collection of 79 C. glabrata clinical isolates from several hospitals in Mexico and we have found that 11 showed increased adherence to mammalian cells compared to our reference strain. The majority of these isolates displayed over-expression of EPA1 and EPA6 or EPA7, suggesting that a global regulatory mechanism could be affected. Sequencing of the SIR3 gene of several hyper-adherent isolates revealed that all of them contain several polymorphisms with respect to the reference strain. We found that there is a large variability in the expression of EPA genes and adherence to epithelial cells in different C. glabrata clinical isolates. Polymorphisms in the SIR3 gene and the associated phenotypes will be discussed.

121A

PRE-ADAPTATION TO HOST MICROENVIRONMENTS IMPACTS CANDIDA ALBICANS-HOST INTERACTIONS DURING INFECTION

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The human fungal pathogen Candida albicans transitions from a commensal lifestyle in the gut to a pathogenic lifestyle during systemic infection via passage into the bloodstream and tissue penetration. Throughout this process, C. albicans is exposed to glucose-limited microenvironments, necessitating the utilization of alternate carbon sources. Previously, our lab has demonstrated that pre-adaptation to physiologically relevant carbon sources such as lactate and oleic acid has implications for pathogenesis. In vitro work revealed that growth on lactate alters the composition of the C. albicans cell wall, modulates the induction of cytokines by mononuclear blood cells from a Th17 to a Th2 response, and increases stress resistance. Furthermore, pre-growth on alternate carbon sources impacts virulence in the systemic model of infection: lactate-grown cells are more virulent than glucose grown cells, and oleate-grown cells are less virulent than glucose grown cells. In this work, we examine the role of fungal pre-adaptation to different carbon sources during proliferation and pathogenesis both in vitro and in vivo. Using live cell confocal microscopy, we demonstrate that carbon source impacts rates of recognition and uptake by immune cells, including macrophages and neutrophils. Additionally, we assess the mechanisms whereby pre-adaptation to lactate utilization increases C. albicans virulence through the use of mutants with carbon source-dependent defects in stress resistance and virulence. Finally, we begin to dissect the contribution of the host immune response to increased C. albicans virulence. Overall, this

work adds to our understanding of C. albicans adaptation and response to the dynamic microenvironments that comprise the human host.

122B

EXAMINING SIGNALING DOWNSTREAM OF DFI1P IN CANDIDA ALBICANS

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The opportunistic pathogen, Candida albicans, requires morphological shifts to achieve wild type levels of virulence. Oval yeast cells allow colonization, and can shift to elongated hyphal cells, permitting invasion into tissue. This transition, known as filamentation, is a response to many stimuli, including contact with an agar matrix, which may mimic contact with host tissue. Wild type levels of filamentation in this context require signaling by Dfi1p, an integral membrane protein. Dfi1p has previously been shown to signal to Cek1p, a C. albicans mitogen activated protein kinase (MAPK). My project focuses on identifying additional proteins involved with Dfi1p signaling. I hypothesized that the Dfi1p-dependent pathway leading to Cek1p activation would involve some proteins which have been previously shown to play a role in signaling to Cek1p: members of the classical MAPK cascade and the GTPase Ras1p. I examined this by measuring levels of activated Cek1p in strains lacking proteins of interest, in order to identify proteins that contribute to wild type levels of Cek1p activation. Dfi1p-dependent Cek1p activation was induced either by growing cells on agar medium. Strains without either Hst7p or Cst20p, proteins that are part of the classical MAPK cascade, have decreased levels of Dfi1p-dependent Cek1p activation, demonstrating their involvement in the pathway. Additionally, preliminary results suggest the involvement of Ras1p. I have also investigated the role of another GTPase : Rac1p, which is required for filamentation during growth within an agar matrix. Rac1p is not required for Dfi1p-dependent Cek1p activation, but may play a role in the signaling pathway under some conditions. Taken together, my results suggest that Dfi1p signals via previously identified signaling molecules, but there may be a regulatory nuance specific to this pathway.

123C

UNDERSTANDING THE MECHANISM OF FARNESOL INHIBITION OF PROTEIN SYNTHESIS IN *CANDIDA ALBICANS*

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Candida albicans is a polymorphic yeast that can cause life threathening systemic infections in immunocompromised individuals. One key attribute of *C. albicans* that could enhance its pathogenicity is the ability to switch morphologies in response to specific and environmental conditions. Farnesol, a quorum sensing acyclic sesquiterpene alcohol secreted by *C. albicans* in its growth medium has been shown to block the yeast to mycelia transition in vitro. Similar to cellular stress conditions, exposure to this alcohol causes a rapid inhibition of global protein synthesis which likely leads to altered programmes of gene expression. Previous studies have shown that in *Candida albicans* and in the related yeast, *Saccharomyces cerevisiae*, fusel alcohols signal nitrogen scarcity to inhibit protein synthesis by targeting the eukaryotic translation initiation factor, eIF2B, independently of the Gcn2 kinase Using a c-terminal GFP tagged *GCD1* (λ subunit of eIF2B), we studied the impact of farnesol on the localisation and dynamics of eIF2B in both the CAI4 and *gcn2Δ* strains of *C. albicans*. Data obtained shows that in contrast to fusel alcohols, farnesol does not impact upon the eIF2B dynamics or activity as was observed with fusel alcohol. Studies carried out with *GCRE* luciferase reporter and *GCN4* luciferase reporter indicate that farnesol does not alter the levels of *GCN4* mRNA as was observed with fusel alcohols. Formaldehyde crosslink-

ing assays suggest that farnesol impacts upon the interaction of the mRNA with the small ribosomal subunit during translation initiation by affecting the association of the translation initiation 4F complex.This work highlights that the inhibition of translation initiation in response to signalling molecules can lead to a diversity of phenotypic outputs. These may depend on both the mechanism of regulation and the assimilation of this mechanism with the other effects of the signalling molecule. Overall, the integration of these responses may have implications in pathogenicity of this important fungal pathogen.

124A

IRON-RESPONSIVE CHROMATIN REMODELING AND MAPK SIGNALING ENHANCE ADHESION AND BIOFILM FORMATION IN CANDIDA ALBICANS

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Background: Iron is an important nutrient that is toxic in excess; therefore iron homoeostasis is tightly regulated. Recent cumulative data indicates that recruitment of various transcription factors to the chromatin occurs in an iron responsive manner; thus iron has a role in affecting diverse cellular functions in the human pathogenic fungus Candida albicans. However, it is not clearly understood how iron levels affect genes and processes beyond iron homeostasis. **Objective:** To investigate the global effects of high iron on C. albicans using chromatin remodeling analysis. **Methods:** YNB medium with iron chelator bathophenanthrolinedisulfonic acid with our without added iron was used for C. albicans growth at 30°C. Chromatin remodeling at gene promoters was assayed by micrococcal nuclease (MNase) digestion followed by deep sequencing (MNase-seq). Cell adhesion was measured by microscopy and sedimentation assay. Biofilm formation was analyzed in plate assay. Phosphorylation of Mitogen Activated Protein Kinase (MAPK) Cek1 was measured by Western blot of cell lysates. **Results:** MNase-Seq identified groups of iron-responsive genes in C. albicans. Chromatin in the promoter regions of iron uptake and utilization genes showed repressed and active configuration, respectively, under iron replete conditions, in agreement with previously published RNA microarrays. Gene Ontology (GO) Term enrichment analysis of genes with differentially remodeled chromatin in respective promoter locales suggested that genes involved in C. albicans adhesion and biofilm formation are also iron-responsive. Genes involved in these processes showed iron-dependent active chromatin profiles, indicative of potential up-regulation under iron replete conditions. Furthermore, C. albicans was observed to be more self-adherent (2-fold increase) and formed higher biofilm mass (77% increase) in the presence of iron and showed Cek1 MAPK iron-responsive phosphorylation. The transcription factor Cph1, which is activated upon Cek1 phosphorylation and controls adhesion, also showed an active chromatin profile under iron replete conditions. In addition, many of the iron-responsive adhesion genes identified in this study had potential CCAAT motifs that are known to mediate transcriptional repression in the absence of iron for iron-utilization genes; thus allowing de-repression under iron replete conditions by similar mechanisms for both of these gene categories. **Conclusion:** In this study we show how iron influences C. albicans phenotype through chromatin remodeling and MAPK signaling to affect adhesion and biofilm formation. We present here the first iron chromatome for an AIDS-related fungal pathogen and provide evidence for the role of iron in affecting diverse biological functions including important virulence traits in C. albicans.

125B

IDENTIFICATION OF HOST AND PATHOGEN DETERMINANTS OF INFECTION OUTCOME USING RNA-SEQ

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Candida albicans exists as a human commensal of healthy individuals and, in immunocompromised patients, a lethal pathogen. Using isolates sampled serially from the same patients, we have identified an abundance of mutations and changes in ploidy that arise during the course of *C. albicans* infection. Co-occurrence clustering of mutations suggests that *C. albicans* rapidly adapts to not only drug selection, but also the host environment. I hypothesize that over the transition from commensal to pathogen, *C. albicans* evolution is driven by the genotoxic host immune response and exposure to chemotherapeutics, resulting in better evasion of host defenses and exploitation of host resources. Here, we have developed fluorescent reporters of *C. albicans* state and host-pathogen RNA-seq to explore the interface between this ubiquitous opportunistic pathogen and the host. We are using a combination of live-cell microscopy, FACS, and host-pathogen RNA-seq to identify and characterize subpopulations of *C. albicans* upregulating DNA repair pathways, as these subpopulations may be crucial drivers of evolution *in vivo*. Macrophages are a tractable, relevant cell type, as these phagocytes are one of the primary defenders against infection. Ultimately, this study will provide robust quantitative descriptors of the host-pathogen interface, allowing us to test the hypothesis that over the course of the transition from commensal to pathogen, *C. albicans* rapidly evolves to better evade host defenses and exploit the host environment. This work will generate a high-resolution experimental and analytical pipeline capable of being generalized to any host-pathogen interface.

126C

GLCNAC STIMULATES A NOVEL PATHWAY TO PROMOTE CANDIDA ALBICANS HYPHAL GROWTH

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Candida albicans switches to hyphal growth in response to a wide variety of cues, including serum, elevated extracellular pH, and the amino sugar N-acetyl-D-glucosamine (GlcNAc). Our previous studies demonstrated that GlcNAc must be taken up by cells to induce hyphae, although catabolism of GlcNAc by the enzymes Hxk1, Dac1, and Nag1 is not required. However, our recent studies showed that GlcNAc catabolism by wild type cells raises the extracellular pH, presumably due to excretion of excess nitrogen as ammonia, and that this alkalinization synergizes with GlcNAc to stimulate hyphal-specific gene expression. The important role of this synergy between GlcNAc and extracellular pH is supported by studies of mutants lacking the GlcNAc catabolic enzymes, which can be induced to form hyphae without inducing the typical cluster of hyphal-specific genes. These results indicate that GlcNAc can directly activate the hyphal morphogenesis program without stimulating the cAMP pathway that leads to the induction of hyphal-specific genes. In support of this, we found that cells require a normal basal level of cAMP to be competent for hyphal induction by GlcNAc, but no increase in cAMP is required for this sugar to induce hyphal formation. Altogether, these data indicate GlcNAc is capable of activating a novel pathway to promote the changes in cell polarization that effect hyphal morphogenesis.

127A

IMPACT OF ADENOSINE A2A RECEPTORS IN THE HOST CELL RESPONSE TO YEAST INFECTION

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Yeasts, members of human body normal flora and ubiquitous in food environments, are, as known, important agents of infection, namely in individuals with immune diseases or with weakened immune system. In this line of evidence, the natural immunosenescence forthcoming with human ageing leads to a decreased ability to control infections. Thus, yeast infections recognition and clearance by host phagocytic cells, such as macrophages, seems to be a complex procedure involving multiple recognition systems and inflammatory responses. Purines, adenosine in particular, are important endogenous signaling molecules in immunity and inflammation. Adenosinergic signaling contributes to the fine-tuning of inflammatory and immune responses, eliminating threat efficiently and controlling to minimal the inflicted damage. This work aims to explore the hypothesis that adenosine, and their sensing devices may constitute one of the systems exploited by *Candida* spp. to modulate macrophage response, bolstering its pathogenic success. We focused on the study of the adenosine receptors role, especially A_{2A}(A_{2A}R) in the efficiency of phagolysosome towards yeast clearance. Using several approaches it was elucidated the interaction between *Candida albicans, Candida parapsilosis, Candida glabrata* and *Saccharomyces cerevisiae* with RAW 264.7 macrophage cell line. In particular, it was observed that upon infection with those yeasts, adenosine A_{2A} receptors are activated and localize around phagosomes containing yeasts cells, although the gene expression does not increase (in contrast to the observed with LPS treated macrophages). Moreover, drugs, both agonist and antagonist, acting in A_{2A}R, affects macrophage phagocytic efficiency. Extracellular ATP and ecto-nucleotidases activity are other related aspects explored in an attempt to understand how yeasts overcome phagocytosis. Deciphering the A_{2A} trafficking to the phagosomal membrane and the effect of siRNA of A_{2A}R in the immuno-inflammatory response of macrophages from young and aged mice, are also part of the ongoing work. Overall our results show that upon interaction with macrophages, *Candida* is able to change immune and inflammatory responses modulating both the source of adenosine activating A_{2A}R and through re-direction of A_{2A}R. Supported by: PTDC/SAU-MIC/115598/2009; PEst-C/SAU/LA0001/2011 and SFRH/BD/74181/2010.

128B

SECRETION OF QUORUM SENSING MOLECULES BY CANDIDA ALBICANS ALTERS THE HOST INNATE IMMUNE RESPONSE

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The polymorphic commensal fungus, Candida albicans, causes life-threatening disease during bloodstream infections. Although host immune evasion is a common strategy employed by successful human pathogens, including C. albicans, we hypothesize that particular morphologies of C. albicans may also seek immune recognition by host innate immune cells whose defences are incapable of controlling fungal virulence on their own. Distinct C. albicans morphologies elicit different responses from the host immune system. C. albicans white cells secrete a small molecular weight macrophage chemoattractant and are able to

survive within and escape from macrophages. On the other hand, opaque cells do not secrete this chemoattractant. One likely candidate is farnesol because opaque cells, unlike white cells, do not accumulate detectable levels of farnesol. Macrophages are capable of detecting and responding to exogenous farnesol. Earlier our group reported that farnesol stimulates the expression of both pro-inflammatory and regulatory cytokines in mouse macrophage. The production of these warning signals by macrophages is an important indicator of how the body ultimately hopes to clear the infection. Others have shown that farnesol suppresses the anti-Candida activity of macrophages through its cytotoxic effects, thus making it all the more difficult to eliminate the fungus early in infection. Here we report that E,E-farnesol (secreted by only white cells) is a potent macrophage chemoattractant and that its activity as a chemoattractant is enhanced by yeast cell wall components and aromatic alcohols. Modulation of farnesol secretion to stimulate host immune recognition by ineffective phagocytes may help explain why this commensal is such a successful pathogen.

129C

QUORUM SENSING CONTROLS HYPHAL INITIATION IN *CANDIDA ALBICANS* THROUGH UBR1-MEDIATED PROTEIN DEGRADATION

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Candida albicans is the most common cause of invasive fungal infections in humans. Its ability to undergo the morphological transition from yeast to hyphal growth forms is critical for its pathogenesis. Hyphal initiation requires the activation of the cAMP-protein kinase A (PKA) pathway, which down-regulates the expression of *NRG1*, the major repressor of hyphal development. Hyphal initiation also requires inoculation of a small amount of *C. albicans* cells from overnight culture to fresh medium. This releases the inhibition from farnesol, a quorum-sensing molecule of *C. albicans*, accumulated in the spent medium. Here, we show that farnesol inhibits hyphal initiation mainly through blocking the protein degradation of Nrg1. Through screening a kinase mutant library, we identified Sok1 as the kinase required for Nrg1 degradation during inoculation. *SOK1* expression is transiently activated upon inoculation during hyphal initiation, and overexpression of *SOK1* overcomes the farnesol-mediated inhibition of hyphal initiation. Screening a collection of transcription factor mutants, the homeodomain-containing transcription repressor Cup9 is found to be responsible for the repression of *SOK1* expression in response to farnesol inhibition. Interestingly, farnesol inhibits Cup9 degradation mediated by the N-end rule E3 ubiquitin ligase, Ubr1. Therefore, hyphal initiation requires both the cAMP-PKA pathway dependent transcriptional down-regulation of *NRG1* and Sok1-mediated degradation of Nrg1 protein. The latter is triggered by the release from farnesol inhibition of Cup9 degradation and consequently, derepression of *SOK1* transcription. Neither pathway alone is sufficient for hyphal initiation.

130A

A RIM101-INDEPENDENT PH-SENSING PATHWAY IS IMPLICATED IN HYPHAL INITIATION

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Candida albicans is a dimorphic organism capable of switching between a yeast and hyphal morphology, a characteristic that is important for its virulence. Hyphal development is initiated by various external environmental stimuli, one of which is a change in pH. A Wild-type strain fails to grow germ tubes at acidic pH 4.0 but grow germ tubes at neutral pH 7.0, sug-

gesting that the change in pH severely retards hyphal initiation. Rim101 pathway is a key pH response pathway, which is important for hyphal growth. Switching from acidic to neutral pH promotes the removal of an inhibitory C-terminal region of Rim101 and activates it, allowing the expression of hyphal genes. Studies have shown that *rim101Δ* strains cannot form hyphae at any pH. However we find that a constitutively active Rim101ΔC (deletion of the Rim101 inhibitory C-Terminus) fails to rescue germ tube formation at acidic pH (4.0). Therefore, a pH-sensing pathway independent of the RIM pathway is implicated in hyphal initiation. A key step in hyphal initiation is the degradation of the transcriptional repressor Nrg1. We find that pH 4.0 inhibits Nrg1 degradation, but Rim101 is not required for Nrg1 degradation. Nrg1 degradation is mediated by the kinase Sok1 (see Poster by Lu *et al.*). We are testing if acid pH inhibits Nrg1 degradation through rgulating Sok1 expression or activity. We conclude that a novel pH-sensing pathway works in parallel with the Rim101 pathway to prevent hyphal initiation in acidic pH environment. Future experiments will address how this new pH-response pathway regulates Nrg1 degradation, and identify components of the pathway responsible for pH sensing.

131B

IDENTIFICATION OF CELL SURFACE SENSORS FOR N-ACETYLGLUCOSAMINE IN *CANDIDA ALBICANS*

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The monosaccharide *N*-acetylglucosamine (GlcNAc) is a major component of microbial cell walls and is ubiquitous in human GI tract. It is a potent inducer of hyphal growth in *Candida albicans*, which is a commensal organism that colonizes the mammalian gut and causes disease in the setting of host immunodeficiency. GlcNAc stimulates the yeast-to-hypha transition by activating the cAMP pathway through unknown sensors/receptors, and GlcNAc metabolism is not necessary for signaling. *C. albicans* has 6 GAP (general amino acid permease) genes, but only Gap2 and Gap6 are similar to *S. cerevisiae* Gap1 in ligand recognition. Interestingly, expression of *GAP1* is found to be induced by GlcNAc. To explore potential roles of GAPs in GlcNAc sensing, we expressed *C. albicans* GAPs in the *S. cerevisiae gap1* mutant, and found that Gap1 and Gap4 can sense the GlcNAc signal and cause a 2-3 fold increase in the activity of trehalase, a downstream target of PKA. Deletion of either *GAP1* or *GAP4* in *C. albicans* has no effect on GlcNAc-induced hyphal initiation. However, the *gap1 gap4* double mutant exhibits significant defects in hyphal induction in GlcNAc-containing medium, suggesting that redundancy exists between Gap1 and Gap4 in sensing GlcNAc. Unlike Ngt1, a specific GlcNAc transporter, Gap1 and Gap4 are not involved in the metabolism of GlcNAc, and the *gap1 gap4* double mutant grows normally in GlcNAc-containing medium. Chitin (GlcNAc polymer that can't be taken up by *C. albicans)* can induce germ-tube formation in wild-type *C. albicans*, but not the *gap1 gap4* double mutant. Our results suggest that Gap1 and Gap4 are involved in sensing GlcNAc on the cell surface to activate the cAMP pathway for hyphal initiation in *C. albicans*. Therefore, the GAP gene family is expanded from one in *S. cerevisiae* to six in *C. albicans.* This expansion is associated with the development of new functions and ligand recognition that are important for sensing host signals as well as fungal pathogenesis, suggesting adaptations associate with virulence.

132C

RISK FACTORS ASSOCIATE WITH A HIGH PREVALENCE AND ANTIFUNGAL PROFILE OF VAGINAL CANDIDIASIS AMONG FEMALE UNIVERSITY STUDENTS IN CAMEROON.

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Background: The incidence of vaginal candidiasis (VC) is significantly modified by dressing patterns and aberrant health-care practices. Over one-half of contemporary young women especially among university students in Cameroon have a preference for trousers or tight under wears which also coincides with a rise in easy access to medication and the phenomenon of auto-medication in our communities. These could result in increased prevalence of vaginal candidiasis and antifungal drug resistant yeast strains. **Method:** A cross-sectional study involving 150 female students (aged 17-29 years) of the University of Buea was conducted between March 2011 and August 2011. Information on socio-demographics, risk factors and clinical symptoms were gotten through a questionnaire. Vaginal smears were collected from each participant and; culture, identification and antifungal susceptibility testing performed following standard microbiological procedures. **Results:** Of the 150 participants who submitted vaginal swabs, yeasts was isolated in 98 (65.3%). Of the 98 yeasts isolates, 73.5% were Candida species, mainly C. albicans (65.3%) while 26.5% were other yeasts species. The prevalence of VC was not significantly different between symptomatic and asymptomatic subjects. Previous episodes of vaginal infection were significantly associated with VC. Although not statistically significant ($p > 0.05$), VC was higher among subjects who wear tight under wears, trousers, use oral contraceptives and in those who acknowledge recent use of antibiotics. Antifungal susceptibility results showed a high resistance to fluconazole (82.0%), nystatin (80.0%) and ketoconazole (72.0%), while clotrimazole (50.0%) was the most susceptible antifungal drug. **Conclusion:** There was a high prevalence of VC in this study population with previous vaginal infection being an important risk factor for reoccurrence. C. albicans was the most frequently isolated species while clotrimazole was the drug of choice in the treatment of VC in this population. Our findings will serve as important information of public health importance especially as VC may increase a woman's risk of contracting other sexually transmitted diseases.

133A

TIME-KILL ASSAY AND ETEST® EVALUATION FOR SYNERGY WITH POLYMYXIN B AND FLUCONAZOLE AGAINST *CANDIDA GLABRATA*

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Background: Fluconazole-resistant *Candida glabrata* is an emerging pathogen causing fungemia. Polymyxin B (PO), a last resort antibiotic used to treat multi-drug resistant Gram-negative bacterial infections, has been found to possess *in vitro* fungicidal activity and showed synergy with fluconazole (FL) against a single strain of *C. glabrata* (Zhai et al., 2010, *JAC*, 65:931-8). Since both agents may be used simultaneously in ICU patients, we performed synergy testing with this combination against 35 *C. glabrata* blood isolates using 2 methods: time-kill assay (TKA) and an experimental MIC:MIC Etest method. **Methods:** Thirty-five genetically-unique *C. glabrata* bloodstream isolates were collected during 2009-2011 from individual patients. Isolates were identified using the API 20C yeast identification system and genotyped by rep-PCR. MICs (µg/ml) were determined by Etest: PO, 64 - 1024; FL, 12 to >256 (32% R-CLSI 2012). Synergy testing was performed by a modified bacterial Etest synergy method (Pankey et al., 2005, *AAC*, 49:2959-64) and TKA (Klepser et al., 1998, *AAC*, 42:1207-12) using concentrations of ½ MIC PO + 1× MIC FL. The Etest MIC:MIC synergy method was performed in triplicate. Final results were read at 48h and ∑FIC (summation fractional inhibitory concentration) calculated: synergy ≤ 0.5; indifference >0.5 - 4; antagonism >4. TKA synergy was defined as a $\geq 2 \log_{10}$ decrease in CFU/ml after 48h by the combination compared with that by the most active single agent alone. TKA results that were discordant

to the Etest method were confirmed by a repeat TKA. **Results:** Etest method showed synergy in 24/35 (69%) isolates (∑FIC 0.2-0.5), including 8/11 (73%) FL-resistant isolates. TKA showed synergy in 21/35 (60%) isolates (-2.0 to -4.0 $\log_{10}$ change), including 10/11 (91%) FL-resistant isolates. Isolates not showing synergy (by either method) were indifferent. No antagonism was found. Concordance between methods occurred in 26/35 (74%) isolates. **Conclusions:** *In vitro* synergy with polymyxin B and fluconazole against the majority of *C. glabrata* isolates was demonstrated by both methods. The bacterial Etest synergy method adapted well when used with *C. glabrata*. It may be more efficient than TKA to evaluate synergy. Further studies with additional *C. glabrata* isolates are needed. *In vitro* synergy may or may not correlate clinically.

134B

SURVEY OF COMPARATIVE EXPRESSION CDR1 ,CDR2 AND MDR1 GENES IN RESISTANCE AND SENSITIVE CANDIDA ALBICANS BY RT REAL_TIME PCR

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Introduction and Objectives: Widespread using of Azoles in comparison with Candida albicans yeast, show resistance to this drug in indisposed that are suffering from Candidasis such as: AIDS patients, diabetic, cancers and organ-transporters .One of the major reason for resistance to fluconazol in Candida albicans is CDR1, CDR2, MDR1 genes expression in resistant and sensitive Candida albicans. **Materials and Methods:** In this research isolated Candida albicans from vaginal and oral Candidiosis patients are used. MIC test was done for these isolates by MICRODILLUTIO BROTH method. Standard sample of resistance to fluconazol Candida albicans (SC5314) was used for PCR reaction control. We performed on resistance and sensitive isolate RT REAL-TIME PCR reaction and CDR1, CDR2, MDR1 genes expression in INVITRO condition and compared together. **Results:** From 46 isolated Candida albicans: 20 sensetive, 12 sensitive dose dependent and 14 resistance to fluconzole. after RT REAL-TIME PCR reaction doing , the three genes expression CDR1,CDR2,MDR1 in sensitive and resistance Candida albicans isolates To fluconazol showed that these genes had a middle expression in sensitive Candida albicans isolates to fluconazole in absence of drug, as these genes expression were so few or zero in resistant isolates. In being or absent of drug in media, don't expression of these genes in resistance isolates be showed and got same results as in sensitive isolates in absent of drug . **Conclusion:** Difference between CDR1, CDR2, MDR1 genes expression in sensitive and resistant Candida albicans showed that those genes role in resistance to fluconazol. Apparently don't being expression of these genes in resistance and expression of these genes in sensitive to fluconazole is effective. **Keywords:** Candida albicans, resistance, sensitive, fluconazole, CDR1, CDR2, MDR1 genes expression, RT REAL_TIME PCR

135C

NEW SCREENING SYSTEM FOR IDENTIFICATION OF INHIBITORS OF *C. GLABRATA* CDR1 MULTIDRUG RESISTANCE PUMP

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The prophylactic use of fungistatic fluconazole has been associated with an increased

frequency of drug resistance in clinical isolates and the selection of non-*albicans Candida*: *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*. Especially *C. glabrata* has become a serious medical problem due to its intrinsic high resistance to the common antifungal drugs currently used in clinics. The high drug resistance of *C. glabrata* is frequently ensured by overexpression of the *Cg*Cdr1 and *Cg*Cdr2 ATPases, which both contribute to multidrug resistance (MDR) phenotype by exporting drugs from *C. glabrata* cells. Thus finding of their potent inhibitors may contribute to problem solving, as the administration of inhibitors together with conventional drugs will help to combat yeast infections. To effectively test the potential inhibitors of *C. glabrata* MDR pumps and to study the activity and structure of the *Cg*Cdr1, we constructed a series of plasmids allowing the expression of *Cg*Cdr1 in *S. cerevisiae*, its visualization in yeast cells and its isolation. We use *S. cerevisiae* AD1-8 mutant lacking most of its MDR pumps [1] and a combination of biological tests and diS-C_3(3) fluorescence assay. This fluorescence method was originally developed for the measurement of plasma-membrane potential in *S. cerevisiae* [2] and later used for the characterization of activity of *Sc*Pdr5 and *Sc*Snq2 MDR pumps [3]. We newly extended the applicability of diS-C_3(3) assay from *S. cerevisiae* to pathogenic yeast species. We found out that diS-C_3(3) is a substrate of Cdr1, but not Cdr2 of *C. glabrata*. We show that this potentiometric probe is a suitable tool for 1) identification of new inhibitors of *Cg*Cdr1, and 2) detection of inhibitors' possible side effects on the membrane potential or integrity of the plasma membrane. Similarly to other current yeast screening systems for identifying new inhibitors, the method is based on the changes in accumulation of the fluorescent probe (whose uptake is driven by the plasma-membrane potential and efflux is mediated via MDR pumps) in the presence/absence of potential MDR-pump inhibitors. We verified the applicability of diS-C_3(3) assay both with *C. glabrata* strains and with *S. cerevisiae* AD1-8 cells expressing *Cg*Cdr1p and with several drugs such as FK506 or enniatin B. **Acknowledgement** The work was supported by GA CR P302/12/1151 and The Center of Biomedical Research (CZ.1.07/2.3.00/30.0025) co-funded by the European Social Fund and the state budget of the Czech Republic. **References** 1. Decottignies A, et al.. 1998. *J Biol Chem* 273: 12612-22 2. Gaskova D, et al. 1998. *Yeast* 14: 1189-97 3. Hendrych T,et al. *Biochim Biophys Acta* 1788: 712-23

136A

DISSECTING THE ROLE OF CALCINEURIN AND PROTEIN KINASE C SIGNALLING IN HSP90-DEPENDENT CASPOFUNGIN TOLERANCE

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Candida albicans is the major etiological agent of fungal infections in humans. Although antifungal drugs are routinely used to treat *Candida* infections, their therapeutic efficacy is limited by the basal drug tolerance of fungal pathogens, which can enable the evolution of drug resistance. The essential molecular chaperone heat shock protein 90 (Hsp90) is a key modulator of drug tolerance and resistance in *C. albicans.* Recent studies suggest that Hsp90 stimulates responses to drug-induced cell wall stress through its client protein calcineurin and the cell wall integrity (CWI) pathway mediated by protein kinase C (PKC). Here, we focus on elucidating the Hsp90-dependent regulatory circuitry that controls tolerance to echinocandins, which target the fungal cell wall and are the only new class of antifungal to reach the clinic in decades. Consistent with previous findings that deletion of *PKC1* phenocopies inhibition of Hsp90 function, we found that Hsp90 regulates CWI signalling by stabilizing Pkc1. We confirmed that Pkc1 is an Hsp90 client in a co-immunoprecipitation experiment where Hsp90 co-purifies with Pkc1. Genetic

epistasis analyses indicate that the recently identified transcription factor and regulator of echinocandin tolerance, Cas5, functions downstream of the Hsp90 clients Pkc1 and calcineurin. Quantitative RT-PCR analysis of transcript levels demonstrated that Cas5 regulates the expression of CWI gene *PGA13,* as well as calcineurin-dependent transcript *ECM331* in response to caspofungin. We found that Cas5 protein stability is subjected to complex regulation by post-translational modifications under both basal and caspofungin treatment conditions. Pkc1 is required for the maintenance of Cas5 stability under basal conditions and calcineurin regulates Cas5 stability in response to drug-induced cell wall stress suggesting that Cas5 stability may be contingent upon dynamic changes in phosphorylation status. We are currently deciphering the Pkc1- and calcineurin-dependent post-translational modifications that regulate Cas5 stability by mass spectrometry and site directed mutagenesis. Our work illuminates novel cellular circuitry through which Hsp90 regulates drug tolerance and implicates Cas5, a fungal-specific protein downstream of Pkc1 and calcineurin, as a potential therapeutic target for the treatment of life-threatening fungal diseases.

137B

DISSECTING THE ROLE OF METAL DEPLETION IN *C. ALBICANS* MORPHOGENESIS AND ECHINOCANDIN RESISTANCE

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Candida albicans prevails as one of the leading causes of invasive fungal infections worldwide, causing severe systemic infections in immunocompromised individuals. The only new class of antifungal drugs to reach the clinic in the past decade is the echinocandins, which inhibit synthesis of a structural polysaccharide in the fungal cell wall. Resistance to this newest class of antifungal has already emerged in fungal pathogens, with the main resistance mechanism being mutations in the drug target gene, *FKS1*. Additionally, cellular stress responses are required to mediate resistance acquired by drug target mutations, namely signaling through the protein phosphatase calcineurin and the cell wall integrity Protein Kinase C (PKC) pathway. However, analysis of resistance phenotypes and known resistance mechanisms suggests that additional mediators of echinocandin resistance remain to be discovered. Here, we utilize a pharmacological screen to identify novel circuitry that regulates resistance to echinocandins. We screened a library of 1280 pharmacologically active compounds, and identified four that reduce echinocandin resistance of a clinical isolate. To further characterize these compounds, we tested their influence on *C. albicans* morphogenesis, as the ability to transition between yeast and filamentous states is a key virulence trait governed by many well-defined pathways. We focused on a chelator, DTPA, which we found to both abrogate echinocandin resistance and induce filamentation. By altering metal concentrations in synthetic defined medium, we identified zinc depletion to be the key filament-inducing cue, and magnesium depletion to be a major contributor to abrogating echinocandin resistance. To characterize the mechanism by which DTPA induces filamentation, we screened a *C. albicans* library of transcription factor deletion mutants to identify those that are unable to filament in response to DTPA, and identified *BRG1* and *ROB1*. We are currently exploring the circuitry through which Brg1 and Rob1 influence responses to DTPA and zinc depletion. Further, to characterize the mechanism by which DTPA abrogates echinocandin resistance, we will take hypothesis-based approaches to examine its impact on key stress response pathways, and will take an unbiased approach based on genome sequencing of resistant mutants. This work is poised to reveal novel mechanisms of *C. albicans* morphogenesis and echinocandin resistance, with broad therapeutic potential.

138C

ANALYZING THE ROLE OF G-PROTEIN-COUPLED RECEPTOR-LIKE PROTEINS, RTA2 AND RTA4 IN CANDIDA ALBICANS

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Candida albicans is a major opportunistic pathogen of immunocompromised hosts and is the leading cause of bloodstream infections. Current antifungal therapies are limited to azole, amphotericin B and echinocandins, which come with their own limitations. Therefore, there is a need to look for new antifungal targets. In this context, our laboratory is interested in analyzing the roles of GPCR-like 7-transmembrane proteins, referred to as the Rta1 (Resistance To Aminocholesterol)-like family in S. cerevisiae. C. albicans has three such GPCR-like genes, RTA2, RTA3 and RTA4, which are unique to the fungal kingdom. These proteins do not show an overall sequence conservation with the yeast GPCRs, hence referred to as GPCR-like or non-GPCRs. RTA2 and RTA4 are downstream effectors of the calcineurin pathway, while RTA3 is a putative phospholipid translocase co-regulated with efflux pumps, CDR1 and CDR2, via a single transcription factor, Tac1p. In this study, we investigate the functional relevance of RTA2 and RTA4, which are downstream effector molecules of the calcineurin pathway. Despite the importance of this pathway in antifungal therapy, only a few of its components such as CRZ1 and CRZ2 have been identified as mediators of calcineurin-dependent transcriptional response. However, effector molecules downstream to CRZ1 have not yet been functionally characterized. The relevance of RTA2 in modulating azole susceptibility and sphingoid base release has been shown (Jia et al., 2008, Jia et al., 2009), while the role of RTA4 has not been addressed. Here, we shed light on the regulation of RTA2 and RTA4 and associate the deletion of these genes to an increased susceptibility to 7-aminocholesterol (7AC), tunicamycin (TM) and SDS. We present evidence to show that both RTA2 and RTA4 are regulated by the calcineurin pathway and their expression is induced with azole antifungals and cell wall damaging agents. The sensitivity of these mutants to TM and 7AC and transcriptional induction of RTA2 and RTA4 by these compounds is dependent on the components of the calcineurin pathway. Noteworthy is the ability of the rta2Δ/Δand rta4Δ/Δ cells to flocculate more extensively, compared to the wild type. Considering the increased susceptibility of rta2Δ/Δ and rta4Δ/Δ to cell wall inhibitors (SDS and TM) and their increased flocculation, we predict their role in modulating cellular response to cell wall damage. The absence of RTA2 also results in alterations in the cell wall composition, reinforcing the link between RTA2 and cell wall damage. Similar phenotypic analysis of rta4Δ/Δ mutant is underway in our laboratory. Keeping in view the role of 7 TMD topology proteins in initiating various signalling pathways, it is possible that RTA2 and RTA4 are important in regulating responses to damages inflicted on the cell membrane/wall via the calcineurin pathway in C. albicans.

139A

IN VITRO SUSCEPTIBILITY TO FLUCONAZOLE TESTING OF CANDIDA SPP OF ESOPHAGEAL CANDIDOSIS PATIENTS IN SAINT-PETERSBURG, RUSSIA.

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Aim: was to evaluate in vitro susceptibility to fluconazol testing of esophageal candidosis patients. **Materials and methods:** Prospective study was performed in 2008 -2013. Isolates

of *Candida spp* cultured from 130 patients (36 males and 94 females, age 3 to 80 years median 39.3) with esophageal candidosis. Identification of *Candida spp* was made by test-system AUXACOLOR2 (BioRad, USA). Sensitivity to fluconazole was determined by disk diffusion test (CLSI M44-A). Paper disks 6 mm in diameter imbued with fluconazole 25 μg (Oxoid) were applied. Minimal inhibiting concentration was determined by the use of microbiological analyzer BIOMIC Vision (Giles Scientific, USA). The sensitivity of *Candida spp* was evaluated according to the CLSI M44-A interpretation criteria. **Results:** In general 96,2% esophageal candidosis patients was susceptible to fluconasole, resistance or dose depended sensitivity - in 3,8%. In 123 (94,62%) of cultures the growth of *Candida albicans* sensitive to fluconazole was identified. Also susceptible to fluconazole were two species *C. tropicalis* (1,54%) and one *C. kefyr* (0,77%). Fluconazole-resistant *C. albicans* was identified at two patients (1,54%). SDD sensitivity had *C. glabrata* (2,30%). Notably that one patient had change of species during the year - from fluconazole-sensitive *C. tropicalis* to intermediate sensitive *C. glabrata*. At one patient intermediate sensitive *C. glabrata* totally lost in vitro sensitivity to fluconazole during 2 months. Fluconazole-resistant *C.albicans* strains were isolated at patient with long-lasting recurrent esophageal candidosis. **Summary:** The 96,2% of etiology patogens of esophageal candidosis in Saint-Petersburg, Russia, have susceptibility to fluconazole .

140B

FLUCONAZOLE DECREASES THE EXPRESSION OF ALCOHOL METABOLISM AND UP-REGULATES AZOLE RESISTANCE GENES IN CANDIDA ALBICANS BIOFILMS

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Introduction: Fluconazole (FLC) resistance of planktonically-grown *Candida albicans* is associated with point mutations and up-regulation of drug efflux pump genes. In addition, the biofilm lifestyle of *C. albicans* results in loss of fluconazole efficacy. Biofilm formation is enhanced in high glucose growth conditions. However, physiological tissue concentrations are significantly lower than those commonly used in *in vitro* experiments. Our group has shown previously that *C. albicans* produces significant levels of mutagenic acetaldehyde (ACH) whilst fermenting glucose into ethanol. Also, expression of the alcohol dehydrogenase gene *ADH1*, which converts ethanol into ACH, inversely correlates with the expression of *CDR1* and *CDR2* in planktonically-grown *C. albicans*. Little is known about the growth, metabolism and resistance gene expression of *C. albicans* planktonic cells and biofilms when grown in physiological glucose concentrations and exposed to fluconazole. **Aims:** To determine the impact of FLC exposure on the expression of genes associated with FLC resistance and alcohol fermentation in *C. albicans* biofilms grown in physiological levels of glucose. **Hypothesis:** Exposure to FLC decreases the expression of ACH metabolism genes and increases the expression of drug efflux pumps in *C. albicans* biofilms grown in physiological levels of glucose. **Methods:** A reference isolate susceptible to FLC, and 4 paired patient isolates, with differing MICs to FLC were used. Planktonic cells were grown in shake flasks and biofilms on Thermanox coverslips in RPMI containing 0.72 (physiological) or 2.0 g/L glucose at 37°C for 24h, and then exposed to 20 mg/L FLC for 24h. Planktonic cell numbers were measured by haemocytometer counts and the metabolic activity of biofilms was assessed by XTT, biomass by crystal violet staining and double stranded DNA concentration by PicoGreen assay. Specific primers for multi-drug efflux pumps, ergosterol biosynthesis, and ethanol metabolism were used for RT-PCR assays to compare gene expression in the presence or absence of FLC. **Essential Results:** Growth in physiological glucose

resulted in decreased metabolic activity and expression of target genes compared to growth in 2 g/L despite no significant differences in biofilm biomass. Interestingly, resistant isolates formed significantly less biomass with or without FLC exposure. Expression of *MDR1*, *ERG11* and *ACS2* was significantly increased but *ADH1* and *ADH2* was decreased after FLC exposure. Interestingly, expression of the pyruvate bypass pathway genes, particularly *ACS1*, *ACS2* and *ALD5*, was generally increased in resistant but not susceptible isolates. **Conclusion:** The effects of glucose concentration have important implications for the clinical relevance of *in vitro* results. Increased expression of pyruvate bypass genes in resistant isolates may result in accumulation of mutagenic ACH in these conditions.

141C

FK520 IS A COMPETITIVE INHIBITOR OF ABC TRANSPORTERS WHICH INTERACTS WITH DISCRETE AMINO ACID RESIDUES IN THE DRUG BINDING POCKET OF CDR1 PROTEIN AND CHEMOSENSITIZES YEAST CELLS BY CALCINEURIN INDEPENDENT PATHWAY.

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The presence of 28 putative ABC transporters and 95 putative MFS transporters in the *C. albicans* genome (Candida Genome Database), only Cdr1p and Cdr2p, and the MFS transporter Mdr1p are major determinants of azole resistance. Therefore, the reversal of azole resistance is considered an attractive strategy to desensitize the multidrug resistant clinical isolates of *C.albicans.* Among many modulators and inhibitors of MDR transporters, FK506 exert immunosuppressive and antifungal effect by calcineurin, however, *C. albicans* is resistant to it, however if given in combination with fungistatic azoles, it shows synergistic fungicidal activity. Interestingly, there are evidences which speculate that FK506 dependent synergistic activity is also affected by Cdr1 protein. This study establishes the molecular basis of Cdr1 protein based synergy of FK 520, a homologue of FK 506. For this, we did kinetic analysis of FK 520 effect on drug transport to show that it competitively inhibits R6G and NR transport. By using in-house library of CDR1 protein of 252 mutant variants where each of the helix residues of TMDs was replaced with alanine, we could identify the critical residues within drug binding pocket of protein which if replaced with alanine, abrogate FK520 dependent competitive inhibition and synergy with azoles. Spot and checkerboard assays revealed that FK520 is a selective modulator of drug substrates at their non- toxic concentrations, thereby rendering susceptibility to the fungal cells overexpressing Cdr1p. The spot assays of the entire mutant library revealed host of residues when replaced with alanine, abrogated the modulatory effect of FK520, thereby indicating the putative blocking sites within transporter protein. The residues showing abrogated modulator effect of FK520 towards azoles indicated a pathway that is calcineurin independent. Taken together, our results provide the first evidence that FK520 modulates only ABC multidrug transporters and could be exploited in combination with certain conventional antifungal drugs to reverse efflux pump dependent multidrug resistance in *Candida* cells.

142A

PRELIMINARY STUDIES OF ANTIMICROBIAL ACTIVITIES OF *FLABELLARIA PANICULATA* ON *CANDIDA* SPECIES ISOLATED FROM ORAL CAVITY

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Naturally, Candida species are human's normal microflora residents of mucous membrane but, there is an accumulating evidence that chronic hyperplastic form of candidiasis conditions are reached due to immunosuppression and

immunocompromise thus, this may lead to the likelihood of predisposing the oral mucosa of some persons to malignant transformation. Candidiasis is faced with the rise of resistant microbial strain development to available and established synthetic drugs. This research is therefore based on the anticandidal activity and phytochemical constituents of *Flabellaria paniculata* crude plants extracts on five *Candida* species. The antibiotic disc diffusion method was employed in order to determine the anticandidal sensitivity of leaves, roots and whole plant of *Flabellaria paniculata* while the phytochemical studies includes assays for tannins, anthraquinones, phenols, saponins and flavones. Minimum Inhibition Concentration was also determined by the use of Agar Well Diffusion method.Leaf extracts of *F. paniculata* showed highest zone of inhibition (ZI) against *C. albicans* (15.5 ±1.10mm) with an *f*-value of 5.196. In the root extract, highest and lowest ZI are 14.7 ±0.80mm and 12.7 ±0.80mm against *C. glabrata* and *C. krusei* respectively. The result in the ZI of leaf extracts showed that the response of *C. albicans* and the remaining *Candida spp* (*C. torulopsis, C. stellatoidea, C. glabrata and C. krusei*) with *f*-value of 5.196 may be of two different responses. The phytochemical constituents of the whole plant reveals the presence of saponins, anthraquinones, flavonoids and tannins. This research suggests that the *Flabellaria paniculata* maybe exploited for commercial purposes in controlling candidiaisis when fully developed. **Key words:** *Candida* species, *Flabellaria paniculata*, phytochemicals and oral mucosa.

143B

COMBINATORIAL CELLULAR PERTURBATIONS TO ABROGATE ANTIFUNGAL DRUG RESISTANCE

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Fungal pathogens are a major cause of human disease, with Candida albicans reigning as one of the leading human fungal pathogens. The emergence of drug resistance in pathogen populations outpaces the development of new antifungals, emphasizing the importance of developing new strategies to block the emergence of drug resistance and enhance the efficacy of existing antifungal drugs. Our goal is to elucidate key cellular processes that can be targeted to enhance the efficacy of azoles, the most widely deployed class of antifungal in the clinic. Here, we report on mechanisms that underlie the potent synergy observed between azoles and a microbial natural product, beauvericin (BEA), a depsipeptide of the enniatin family. BEA was identified in a screen for natural products synergistic with azoles, though the mechanisms of antifungal action remain enigmatic. We demonstrate that BEA has little antifungal activity on its own, but that it profoundly enhances the efficacy of azoles against the model yeast Saccharomyces cerevisiae and C. albicans. By monitoring accumulation of radiolabelled azole we observed that BEA treatment increases intracellular azole accumulation, providing a mechanism of drug synergy. To identify the target of BEA we implemented an unbiased genomic approach based on selection of resistant mutants. Although BEA has little antifungal activity against wild-type strains, an S. cerevisiae mutant lacking 16 ATP-binding cassette (ABC) transporter genes is hypersensitive to BEA. Dozens of BEA-resistant mutants were obtained and whole genome sequencing identified mutations in genes encoding the regulatory subunits of protein kinase CK2, suggesting that it may be the drug target. Expression of the CK2 alleles in the parental strain confirmed that they confer BEA

resistance. Affinity purification experiments with a biotinylated version of beauvericin are currently underway. Further analysis will evaluate whether the target is conserved in C. albicans, where BEA affects not only azole resistance but also filamentation. We discovered that BEA blocks filamentation in response to various filament-inducing cues, a key virulence trait of C. albicans. To determine whether BEA blocks filamentation by the same mechanism through which it exerts synergy with azoles, or by a distinct pathway, we utilized a BEA analog. The BEA analog has comparable antifungal activity but does not block filamentation of C. albicans, suggesting that BEA may influence these traits via different targets. We will implement a functional genomic approach, and screen existing C. albicans libraries for mutants that are resistant to the repressive effect of BEA on filamentation induced by elevated temperature. Our study will reveal the mechanisms by which BEA enhances the efficacy of azoles and blocks filamentation, enabling development of new therapeutic strategies to treat life-threatening fungal infections.

144C

ANTIFUNGAL DRUG RESISTANCE IN CANDIDA GLABRATA MEDIATED BY THE FORGOTTEN DRUG:H+ ANTIPORTER FAMILY

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The emergence of antifungal drug resistance among fungal pathogens poses a severe clinical problem. Drug resistance often results from the action of drug efflux pumps from the ATP-Binding Cassette and Major Facilitator Superfamilies (MFS). However, the role of the putative drug:H+ antiporters (DHA) from the MFS in fungal pathogens has largely escaped characterization. The systematic characterization of the DHA transporters from Candida glabrata is being undertaken and most of its 10 uncharacterized DHA1 transporters were indeed found to be implicated in multiple drug resistance. For example, the expression of CgQDR2 in C. glabrata was found to confer resistance to imidazole antifungal drugs. CgQdr2 was found to decrease the intracellular accumulation of 3H-clotrimazole in C. glabrata, and to play a role in the extrusion of this antifungal from pre-loaded cells. CgQDR2 transcript levels were further seen to be up-regulated in C. glabrata cells challenged with clotrimazole and quinidine, in the direct dependency of the CgPdr1 transcription factor [1]. As a second example, the Drug:H+ Antiporter CgAqr1 was identified as a determinant of resistance to the antifungal agents flucytosine and, less significantly, clotrimazole. These antifungals were found to act synergistically with acetic acid against this pathogen. Significantly, CgAqr1 expression was found to reduce the intracellular accumulation of 3H-flucytosine and, to a moderate extent, of 3H-clotrimazole, consistent with a direct role in antifungal drug efflux [2]. Finally, CgTpo3 was found to confer resistance to imidazole and triazole antifungal drugs, catalyzing their extrusion from within preloaded C. glabrata cells. Interestingly, CgAqr1 and CgTpo3 were found to confer resistance to acetic acid and polyamines, respectively. Since these compounds easily accumulate to inhibitory levels in the female and male genital tracts, respectively, it is hypothesized that these transporters may play a role in C. glabrata persistent colonization in these specific niches. Altogether, the results obtained during the ongoing systematic characterization of the forgotten DHA transporter family in C. glabrata are expected to improve current understanding of multidrug resistance in fungal pathogens and to guide de design of new tools for the diagnosis and treatment of Candida infections. [1] Costa et al, 2013, Antimicrob Ag Chemother, 57, 3159; [2] Costa et al, 2013, Front Microbiol, 4, 170.

145A

IN VITRO SYNERGY OF FLUCONAZOLE PLUS DOXYCYCLINE AGAINST *CANDIDA GLABRATA*

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Background: *Candida glabrata* is a pathogen of increasing clinical relevance. Resistance of *C. glabrata* to fluconazole is becoming more prevalent. Echinocandin resistance has begun to develop as well. Fiori et al (2012, *AAC*, 56:3785-96) found the combination of doxycycline and fluconazole to be synergistic against a fluconazole-resistant reference strain of *Candida albicans*. Doxycycline appeared to convert fluconazole activity from fungistatic to fungicidal in that isolate. If confirmed, this may help prevent fluconazole resistance. The goal of our study was to test patients' *C. glabrata* blood isolates for synergy with the combination of fluconazole plus doxycycline using an Etest® method. **Methods**: Twenty unique clinical blood isolates of *C. glabrata* collected 2009 - 2011 from Ochsner Health System patients were screened for fluconazole plus doxycycline synergy. Isolates were identified with the API 20C yeast identification system and genetically fingerprinted by rep-PCR. Etest MICs (µg/ml) revealed 7 fluconazole-susceptible-dose dependent isolates (≤ 32), (0.19, 0.25, 2, 16, 32, 32, 32), and 13 fluconazole-resistant (>32), range 48 to >256. Susceptibilities were interpreted according to 2012 CLSI guidelines. All isolates had doxycycline MICs >256 µg/ml. A MIC:MIC Etest protocol for bacteria, described and validated by Pankey et al [2013, *DMID*, 77(3):220-6], was modified for *Candida* synergy testing. All tests were done in triplicate (mean value used) and read initially at 24 hrs with final results at 48 hrs. A ∑FIC (summation fractional inhibitory concentration) was calculated for each isolate: synergy ≤ 0.5; additivity >0.5-1; indifference >1-4; antagonism >4. **Results**: With the doxycycline plus fluconazole combination, 12/20 (60%) of *C. glabrata* isolates showed synergy 5/20 (25%) or additivity 7/20 (35%), including 9/13 (69%) of fluconazole-resistant isolates. The remaining 8/20 (40%) isolates were indifferent. No antagonism was noted. **Conclusion**: Using an Etest synergy method modified for *Candida*, *in vitro* synergy or additivity with a MIC:MIC fluconazole plus doxycycline combination was demonstrated in 12/20 (60%) of *C. glabrata* clinical blood isolates. The mechanism of this *in vitro* synergy is unknown, and further studies are warranted. *In vitro* findings may or may not be useful in treating patients.

146B

THE INVESTIGATION OF IN VITRO INTERACTIONS OF AMPHOTERISIN B, FLUCONAZOLE AND VORICONAZOLE WITH FARNESOL AGAINST CLINICAL ISOLATES OF *CANDIDA* SPP.

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Nowadays, an increase of incidence of both community-acquired and nosocomial infections is observed. Because of widespread use of broad-spectrum antibiotics, interventional diagnosis and treatments, immunsuppressive diseases and treatment methods, there is an increase of frequency of infections by means of opportunistic pathogens including fungi. The most common fungal agents are *Candida* species. Although, studies to improve more recent and broad-spectrum antibiotics, their eukaryotic cell structure complicates the studies. Therefore, when these constraints are considered, use of additive materials to antifungals are coming up. Especially, the efficiency of farnesol, a quorum sensing molecule produced by *Candida* species as well, on antifungals was point of interest, particularly there are several studies about *Candida* biofilms. In our study,

the efficiency of farnesol on amphotericin B, fluconazole and voriconazole for 20 *Candida* was studied by checkerboard and time-kill methods. MIC values of farnesol, amphoterisin B, fluconazole and voriconazole for *Candida* species are 150->1200 µg /ml, 0,5-2 µg/ml, 0,125-4 µg /ml, and <0,03-0,5 µg/ml, respectively. In combination studies, although, farnesol with especially amphotericin B represents a synergistic interaction for some concentrations, a significant contribution of farnesol on antifungals could not be detected. However, to determine an accurate effect of farnesol on treatment, it is considered that there is a need for similar studies including more fungal specimens from limited fungal species and other antifungals used in antifungal therapy.

147C

DYNAMICS OF PHOSPHATIDYLINOSITOL-(4,5)-BISPHOSPHATE RESPONSE DURING CANDIDA ALBICANS EXPOSURE TO CASPOFUNGIN

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Candida albicans Irs4p and Inp51p were previously demonstrated to physically interact and regulate the levels of an important signaling phosphoinositide, phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P2). Knock-out of either genes led to accumulation of PI(4,5)P2, overactivation of the cell wall integrity pathway, hypersusceptibility to caspofungin, elimination of paradoxical growth at high caspofungin concentrations, impaired hyphal growth, and attenuated virulence in the murine disseminated candidiasis model. In the present study we are investigating the dynamics of PI(4,5) P2 response to caspofungin exposure. We have performed 3-hour live cell imaging using a C. albicans wild-type strain expressing the PH domain, which specifically binds PI(4,5) P2 in vivo, fused to CaGFP. Upon exposure to caspofungin, PI(4,5)P2 levels increased over time. This increase in PI(4,5)P2 levels was dose-dependent; it intensified with the increase of caspofungin concentration up to 4xMIC. Above this concentration, PI(4,5)P2 levels faded progressively but remained higher than at 1xMIC. Thus, PI(4,5)P2 mirrored the paradoxical effect of higher concentrations of caspofungin on cell growth (as we verified by CFU counts), and could be an effector in the molecular mechanisms leading to the paradoxical effect. We observed several other defects caused by the exposure to caspofungin. (i) The abnormal intracellular PI(4,5)P2 patches, which we previously described and which relate to a defect in PI(4,5)P2 distribution, were very dynamic, appearing and disappearing in many locations at the inside surface of the plasma membrane. (ii) A defect during cell division was observed. Indeed, the bud site looked abnormally wide, which made cytokinesis look more like a fission than budding yeast. This again implicates PI(4,5) P2 in cytokinesis events and particularly the bud neck structure. We also studied the effects of two other marketed drugs (Amphotericin B and fluconazole), and two stress agents (hydrogen peroxide (oxidative agent) and Calcofluor white (cell wall agent)). None of these drugs or agents had similar effects to those of caspofungin, and each had a distinctive effect. Overall, our results show the specificity of caspofungin effect seen through the dynamics in levels and distribution of PI(4,5)P2, as well as the extent of this effect, which goes beyond inhibition of (1,3)-β-D-glucan synthase.

148A

THE ROLE OF FUNGAL CELL WALL COMPOSITION AND REMODELLING IN ECHINOCANDIN DRUG TOLERANCE AND HOST INTERACTIONS

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Echinocandins are antifungal agents, which affect fungal cell wall integrity by inhibiting

β(1,3)-glucan synthesis. The assembly of the fungal cell wall is a dynamic process. Echinocandin treatment below the MIC activates compensatory mechanisms that upregulate chitin biosynthesis and results in altered cross-linking and changes in the cell wall proteome, in an attempt to reinforce the cell wall. Proteomic analysis and transcript profiling have identified cell wall proteins (CWPs) and genes that are more abundant and have increased expression in response to treatment with sub-MIC caspofungin. Real-time PCR analysis of fungal cells extracted from infected mouse kidneys demonstrated that expression of specific GPI-anchored proteins is also elevated in response to caspofungin treatment, *in vivo*. These include transglycosidases and cross-linking proteins involved in modifying cell wall polysaccharides (Utr2, Crh11, Phr1, Phr2), as well as novel proteins (Pga31). Treatment with caspofungin also increased the non-lytic expulsion of *C. albicans* cells from macrophages. This was dependent on the specific CWPs that accumulated in the cell wall in response to caspofungin treatment. Changes in cell wall architecture and composition can alter a cell's sensitivity to the echinocandins. Using a tetracycline inducible promoter the genes encoding CWPs, which were upregulated in response to caspofungin treatment, were overexpressed to determine whether this altered susceptibility to caspofungin or interactions with the host. Overexpression of *CHT2* and *SSR1* lead to reduced susceptibility and elevating expression of *CRH11*, *UTR2* and *PHR1* resulted in increased sensitivity to caspofungin. Increased expression of CWPs on the cell surface also resulted in altered interactions with macrophages. In the majority of cases overexpression of CWPs resulted in increased phagocytosis by macrophages, which in turn resulted in increased killing of macrophages by *C. albicans*. An exception to this was the *UTR2* overexpression strain which had reduced phagocytosis by macrophages due to the expulsion of cells from macrophages once they began germinating. To determine the effect of overexpression of *UTR2* during infection *Galleria mellonella* larvae were used as a preliminary screening model. The ability of the *UTR2* overexpression strain to escape from macrophages facilitated survival *in vivo* and as a result overexpression of *UTR2* resulted in increased virulence. Therefore, overexpression of fungal cell surface proteins can alter the susceptibility of *C. albicans* to caspofungin *in vitro* and *in vivo* and can affect interactions within the host.

149B

CHARACTERIZATION OF AZOLE RESISTANCE MECHANISMS, IN CHILEAN ISOLATES OF CANDIDA ALBICANS.

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Candida albicans is a commensal yeast causing opportunistic infections in susceptible hosts. In Chile, the most widely used antifungals are azoles. In a previous study in our institution, we detected a 3.7% of resistance azoles resistant C. albicans isolated from hospitalized and ambulatory patients. The aim of this study was to characterize the resistance mechanisms involved in the azole resistance in clinical isolates, detecting the most prevalent. Methodology: Ten resistant and 20 susceptible isolates (including dose-dependent susceptible) were checked for the occurrence of mutations in the hot spot 408-488 of ERG11 by sequencing. The gene expression of ERG11 and efflux pumps CDR1, CDR2 and MDR1, was measured by q-PCR. The functionality of efflux pumps was estimated through a rhodamine-6G efflux assay, reading the pellet absorbance at 527 nm. The ATCC strain C. albicans SC5614, was used as a control. Results: No mutations associated to resistance, were found in ERG11 and the overexpression of this gene was only present in 1/10 of the resistant strains. The half of resistant strains (5/10)

overexpressed the efflux pumps CDR1, CDR2 and MDR1 ($p<0.05$) which correlated with a lesser intracellular accumulation of rhodamine 6-G in growing C. albicans. Conclusion: The most prevalent resistance mechanism to azoles in C. albicans isolated from our institution was the overexpression of efflux pumps. The use of q-PCR to quantify the efflux pumps expression could be a valid alternative for early detection of clinical azole-resistant C. albicans. This preliminary study should be complemented with a higher number of strains to make these results more representative.

150C

UNDERSTANDING THE MOLECULAR MECHANISMS OF HETERO-RESISTANCE IN *CANDIDA GLABRATA*

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Candida glabrata is the second most frequently isolated *Candida* species, causing 15-20% of all *Candida* infections in US hospitals. It is becoming increasingly prevalent due to its natural adaptation to antifungal drugs, yet mechanisms that make *C. glabrata* more drug-resistant and/or drug-tolerant are not entirely clear. Heteroresistance, the ability of a fraction of a susceptible microbial population to tolerate and survive antifungal drug exposure, has been previously described in many species of bacteria including *Staphylococcus aureus* and *Acinetobacter baumannii*, as well as in fungi such as *Cryptococcus neoformans*. Here we compared heteroresistant (HetR) and non-heteroresistant (non-HetR) clinical isolates of *C. glabrata*. We determined the whole genome sequence of 6 HetR and 3 non-HetR isolates. All sequenced strains carried the 3 identical translocations that are present in lab strain BG2 but absent in the sequenced reference strain CBS138. Many of the strains have copy number variations, but none of the CNVs are specific only to the HetR strains and many of them involve genes in the subtelomeric regions. Several SNPs are common only to the HetR isolates. The possible role of these SNPs in the phenomenon of *C. glabrata* heteroresistance is currently being examined. Taken together, the results detected no major genomic differences common to all HetR strains, although we do not rule out the possibility of changes arising only in the tolerant subpopulations of HetR strains.

151A

INTERACTION OF CASPOFUNGIN WITH β-GLUCAN IN *CANDIDA ALBICANS* BIOFILMS AND CELL WALLS

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The ability of *C. albicans* to form biofilms has a profound effect on the ability of the organism to cause disease. This is due, in part, to the fact that biofilms are very resistant to antifungal therapy. Nett et al. have reported that *C. albicans* cell wall and biofilm glucan play a prominent role in resistance of biofilms to fluconazole and amphotericin B (*Antimicrob. Agents Chemother.* 51:510, 2007). However, little is known about the structure of *C. albicans* biofilm glucan or its interaction with antifungal drugs. To address these deficits in our knowledge, we isolated glucan from *C. albicans* biofilms. The biofilm isolate was identified as (1→3,1→6)-β-glucan by 1D and 2D NMR. The ability of biofilm glucan to interact with antifungal drugs was examined by labeling caspofungin with a fluorescent tag, i.e. AlexaFluor 488. We examined the binding interactions of caspofungin-488 with *C. albicans* biofilm glucan, a reference β-glucan, UV killed *C. albicans* and heat killed *C. albicans* using a flow cytometric analysis. Casofungin-488 interacted with *C. albicans* biofilm glucan with characteristics of a single specific binding site and half maximal concentration of ~20 μg/ml. Caspofungin-488 also interacted with the reference *S. cerevisiae* glucan, but

with lower binding affinity. Interestingly, caspofungin bound non-specifically to UV killed *C. albicans* yeast, but it bound specifically to heat killed *C. albicans* yeast. Heat killed *C. albicans* show increased exposure of cell wall β-glucan when compared to UV killed *C. albicans*. Thus, our data suggest that increased exposure of glucan in the *C. albicans* cell wall facilitates caspofungin binding. In conclusion, our data confirm the presence of β-glucan in *C. albicans* biofilms. Of greater significance, our data indicate that glucan isolated from *C. albicans* biofilms as well as glucan exposed in the *C. albicans* cell wall specifically interact with caspofungin-488. We speculate that the ability of *C. albicans* biofilm and cell wall glucan to interact with caspofungin may alter the efficacy of this antifungal drug.

152B

UPC2A IS REQUIRED FOR HIGH-LEVEL AZOLE ANTIFUNGAL RESISTANCE IN *CANDIDA GLABRATA*

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Candida glabrata, the second most common cause of *Candida* infections, is associated with high rates of mortality, and often exhibits resistance to the azole class of antifungal agents. Upc2 and Ecm22 in *Saccharomyces cerevisiae* and Upc2 in *Candida albicans* are the transcriptional regulators of *ERG11*, the gene encoding the target of azoles in the ergosterol biosynthesis pathway. Recently two homologs for these transcription factors, *UPC2A* and *UPC2B,* were identified in *C. glabrata*. One of these, *UPC2A,* was shown to influence azole susceptibility. We hypothesized that due to the global role for Upc2 in sterol biosynthesis in *S. cerevisiae* and *C. albicans*, disruption of *UPC2A* would enhance the activity of fluconazole in both azole-susceptible and -resistant *C. glabrata* clinical isolates. To test this hypothesis, we constructed mutants disrupted for *UPC2A* and *UPC2B* alone and in combination in a matched pair of clinical azole-susceptible and -resistant isolates. Disruption of *UPC2A* resulted in increased susceptibility to all azoles tested, as well as additional sterol biosynthesis inhibitors, lovastatin and terbinafine, in both the susceptible and resistant mutants, while the *UPC2B* mutant exhibited no difference. In addition, the strains disrupted for *UPC2A* also demonstrated a lower fluconazole minimum fungicidal concentration and greater inhibition of growth measured by time kill analysis. Loss of *UPC2A* had no effect on baseline expression of genes known to be involved in azole resistance in *C. glabrata* – transcription factor, *PDR1*, and ABC transporters, *CDR1*, *PDH1*, and *SNQ2*. However, in the susceptible isolate disrupted for *UPC2A* the upregulation of these genes in response to fluconazole treatment was attenuated. Ergosterol levels in both the susceptible and resistant isolates were decreased in the *UPC2A* mutants. Both baseline and inducible expression of a panel of genes involved in the sterol biosynthesis pathway was reduced in the *UPC2A* disruptants compared to the parent isolates. Our results indicate that Upc2A is a key regulator of ergosterol biosynthesis and is essential for resistance to sterol biosynthesis inhibitors in *C. glabrata*. As such, the *UPC2A* pathway may represent a potential co-therapeutic target for enhancing azole activity against this organism.

153C

MOLECULAR MECHANISMS OF MULTIDRUG RESISTANCE IN CANDIDA PARAPSILOSIS

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While C. albicans is considered to be the principal causative agent of invasive candidiasis, there are an increasing number of studies reporting a shift in species distribution to non-albicans Candida species. Of these, C. parapsilosis is often reported as the 2nd most commonly isolated Candida, is known for persisting and spreading through hospitals by hand carriage, and is the predominant fungal pathogen recovered from neonatal ICUs. The mechanisms by which Candida species develop resistance to antifungal drugs has been well studied in C. albicans but little is known surrounding such resistance in C. parapsilosis. The aim of this study is to explore the basis of multidrug resistance in a matched antifungal-resistant and -susceptible C. parapsilosis clinical isolate pair. The isolates, collected from a patient with prosthetic valve endocarditis, were previously confirmed to be identical strain types through electrophoretic karyotyping. We first confirmed susceptibilities and found the initial isolate to be susceptible to both fluconazole and caspofungin while the subsequent isolate was resistant. We then performed RNA sequencing and compared the expression profiles to identify genes differentially expressed in the resistant strain. Furthermore, we performed qRT-PCR to measure relative expression of select genes for which the homolog in C. albicans is known to contribute to antifungal resistance. Interestingly, two ergosterol biosynthesis genes - ERG2 and ERG6 - showed an increased expression of at least 3-fold between the initial and subsequent isolates. No other genes involved in ergosterol biosynthesis were upregulated. There was also no upregulation of the common drug efflux genes CpMDR1 or CpCDR1. We also performed sequence analysis of select genes for which mutations have been linked to antifungal resistance. Only two genes analyzed contained a mutation in the resistant isolate leading to a predicted amino acid substitution and therefore may contribute to azole and echinocandin resistance respectively, ERG3 and FKS3. These data are the first such analysis of multidrug resistance in C. parapsilosis and indicate that mechanisms similar to those observed in C. albicans may also mediate resistance in this emerging species.

154A

ROLE OF N-LINKED GLYCANS IN CELL WALL INTEGRITY AND HOST IMMUNE RESPONSE AGAINST CANDIDA PARAPSILOSIS

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Candida parapsilosis is an opportunistic fungal pathogen commonly associated with nosocomial infections in newborns and immunocompromised patients. The fungal cell wall is the first point of contact with the host cells; and thus, this structure strongly influences the fungus-host interaction, and plays an important role in the pathogen recognition by the host immune system. Little is known about *C. parapsilosis* cell wall; therefore, in this work we aimed to characterize the cell wall composition and organization, and to assess the relevance of its components during the recognition by peripheral blood mononuclear cells (PBMCs). Fungal cells were allowed to interact with PBMCs during 24 h and then, supernatants were recovered to quantify pro-inflammatory (TNF-alpha, IL-6 and IL-1beta) and anti-inflammatory (IL-10) cytokines by ELISA. We found that *C. parapsilosis* cell wall is mainly composed by proteins and polysaccharides of glucose, mannose and glucosamine, distributed in a similar way as it has been reported in *C. albicans*. Nevertheless, there are significant differences in the cell wall porosity between these two *Candida* species. Relative porosity to polycations showed to be higher in *C. parapsilosis*, suggesting that the β-glucan layer might be more exposed at the wall surface, probably due to a small size of mannans. In agreement, during Alcian Blue binding assays, *C. parapsilosis* showed

lower levels of cell wall phosphomannan. In order to investigate the importance of the *N*-linked mannans during the *C. parapsilosis* immune recognition, we generated an *och1*Δ null mutant, which has truncated *N*-linked mannans at the cell wall. The characterization of the mutant showed increased levels of cell wall chitin and glucan, together with higher porosity to polycations. A reduced phagocytic index by murine macrophages and a different profile of secreted cytokines by PBMCs were evident, indicating a key role of *N*-linked mannans for the immune sensing of *C. parapsilosis* by both macrophages and PBMCs. Taking together, these results provide new information about the immune response elicited against *Candida* non-*albicans* species, showing remarkable differences in the pro-inflammatory cytokine profile compared to that reported in *C. albicans,* despite the similarity in the cell wall composition of these two species. Furthermore, the results state the importance of *N-linked* mannans during the triggering of this protective response against *C. parapsilosis* and for the phagocytic clearance by murine macrophages.

155B

IDENTIFICATION AND CHARACTERIZATION OF TISSUE-ASSOCIATED VIRULENCE GENES OF *CANDIDA ALBICANS*

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Candida albicans is able to infect virtually all organs during systemic infections. Both, fungal colonization and host immune response contribute to pathogenesis and organ damage and can ultimately lead to sepsis with high mortality rates.However, in mice the outcome of infection is organ specific. While *C. albicans* is gradually cleared from liver and spleen, infection progresses in the kidneys due to a delayed, but prolonged influx of neutrophils and accompanied by a strong cytokine response. The mechanisms underlying these organ-specific differential host responses are not known. We hypothesized that both organ-specific host determinants and fungal factors contribute to the organ specific outcome.To identify *C. albicans* factors or activities which contribute to the protective or non-protective host responses we used 1800 *C. albicans* mutants, provided by the fungal genetic stock center (FGSC). The potential of these mutants to cause damage and to induce cytokine / chemokine secretion was analyzed on a wide range of host cell lines, including hepatocytes, kidney-, oral and intestinal epithelial cells, and macrophages. In this initial screen we identified several *C. albicans* mutants which induced altered cytokine responses without showing differences to the wild type in regard to filamentation, growth rates and cytotoxicity. Among these genes of interest were genes with unknown function as well as genes known to contribute to virulence, such as *IFF11*, demonstrating the suitability of this approach.In a second approach we use in vivo transcriptional profiling of *C. albicans* genes expressed in mouse kidneys and liver to identify niche-specific expression of genes. An optimized RNA isolation protocol was established to isolate good quality RNA in sufficient amounts to perform microarray analysis.The combination of these two approaches will allow us to detect genes which are both infection-associated and niche-specific and which contribute to the different and organ-specific pathogenesis. Detailed analysis of candidate genes of unknown

function using large sets of established *in vitro* and *in vivo* protocols to define their biological role are currently under way.

156C

INTERFERON 1 PATHWAY COMPONENT MDA5 (IFIH1) IS HIGHLY UPREGULATED AFTER *CANDIDA* STIMULATION AND GENETIC VARIANTS ARE ASSOCIATED WITH SYSTEMIC *CANDIDA* INFECTIONS

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Background and aim: The dimorphic and commensal fungus *Candida albicans* is found in about 80% of healthy individuals without causing any symptoms or signs of discomfort. Nevertheless, when the host immune system is diminished, *Candida* species in general and *Candida albicans* in particular can cause severe infections such as systemic candidiasis. Although environmental factors have been associated with an increased susceptibility to systemic *Candida* infections, genetic factors are also believed to play an important role for the susceptibility and/or severity of the infection. We initiated a complementary transcriptomic and genetic approach to assess genes important for susceptibility to *Candida* infections. **Results:** By assessing microarrays of macrophages stimulated with *Candida*, stimulation of a specific MDA5 pathway seemed to be induced by the fungus. In line with a role of MDA5 for antifungal defense, we identified two coding single nucleotide polymorphism (rs3747517, rs1990760) in this gene that were significantly associated with systemic blood infections with *C. albicans*. Furthermore these SNPs resulted in a upregulated cytokine response of IL-10 and in a reduced cytokine response of the anti-fungal cytokine IL-17. Additional in vitro studies have shown that MDA5 expression is defective in immune cells from patients suffering from chronic mucocutaneous infections (CMC). In an IFIH1 (MDA5) knock out mouse model we show that the cytokine response after *Candida* stimulation was specifically reduced compared to control mice. **Conclusions:** Taken together, these observations point out towards a major role of MDA5 within the type I IFN pathway in the anti-*Candida* response. Genetic variations in this gene most likely lead to imbalances in the cytokine response against this fungus. These new insights may lead to a better understanding of the of the immune response against *C. albicans*.

157A

T CELL FUNCTION IS AFFECTED BY *CANDIDA ALBICANS* SECRETED ASPARTIC PROTEASE EXPRESSION IN MURINE PERITONITIS

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Secreted aspartic proteases (Saps) produced by *Candida albicans* have been considered viru-

lence-associated factors. In particular, *SAP4 to SAP6* were shown to significantly contribute to *C. albicans* virulence in murine peritonitis. Here, we assessed the importance of *SAP1* to *SAP6* expression in the immune response induced in BALB/c mice intraperitoneally infected with *C. albicans* wild-type (WT) strain SC5314 and its derived *SAT1*-flipping mutants Δ*sap123* and Δ*sap456*. No major differences were observed in the early inflammatory response elicited in the peritoneal cavity of the different infected mice groups. However, splenic and mesenteric lymph nodes of WT-infected mice contained higher proportions of T regulatory cells ($Foxp3^+$) comparatively to control and *sap*-null mutant-infected mice. In addition, splenic $CD4^+CD25^+$ T cells of WT-infected mice suppressed more efficiently the proliferative response of $CD4^+CD25^-$ T cells than those obtained from mice of the other experimental groups. $CD4^+CD25^+$ T cells of Δ*sap456*-infected mice were the least suppressive. Moreover, anti-CD3-stimulated $CD4^+$ T cells from WT-infected mice produced higher levels of IL-10 than the corresponding cells from mice infected with the mutant strains. Interestingly, Δ*sap456*-infected mice presented less $Foxp3^+$ cells in kidney lesions than the other infected counterparts and also lower *C. albicans* CFU counts in this organ. Altogether, these results implicate Sap expression in the modulation of the host immune response to *C. albicans*, and thus provide additional evidence for the role of these secreted proteins as virulence determinants in *Candida* peritonitis. This work was supported by FCT Project POCTI/SAU-IMI/58014/2004, SFRH/BD/31354/2006 fellowship and Pest-C/SAU/LA0002/2011.

158B

LIVE CELL IMAGING IDENTIFIES *CANDIDA ALBICANS* VIABILITY, GLYCOSYLATION-STATUS AND MORPHOLOGY AS PLAYING A KEY ROLE IN THE MATURATION OF MACROPHAGE PHAGOSOMES AND INFLUENCES HOST-PATHOGEN INTERACTIONS.

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Control of infection relies upon professional phagocytes which recognise, bind, phagocytose and destroy pathogens. Newly formed phagosomes mature by dynamic interactions with endocytic and lysosmal vesicles to generate a microbicidal lumen characterised by acidic pH, reactive oxygen species and an abundance of lytic enzymes. *Candida albicans* is able to escape phagosomal destruction by at least two mechanisms; non-lytic exocytosis and lysis of phagocytic cells by hyphal extension. Sophisticated live imaging has shown how fungal surface glycosylation promotes migration and uptake by macrophages. The current study utilised confocal and spinning disk microscope visualisation of macrophage-*Candida* interactions in real time, with fluorescent markers of maturation including Lysotracker Red (acidity), Rab5 (early phagosomes), Rab7, LAMP1 and Cathepsin B (late phagosomes). Live *C. albicans* markedly delayed acquisition of acidity and hydrolytic enzymes relative to dead yeast (from 30min-180min, 5-20% of phagosomes were positive for markers whereas dead yeast resulted in over 75-95% of phagosomes becoming labelled), highlighting that fungal viability influences phagosome maturation. Furthermore we observed that phagosomes containing the cell wall mannosylation mutant Δ*mnt1*/Δ*mnt2* matured more rapidly than those containing wildtype *C. albicans* (10-35% of phagosomes labelled) suggesting that *O*-mannan deficiency inhibits *C. albicans* ability to interfere with phagosome

maturation. Greater exposure of β-glucan on the Δ*mnt1*/Δ*mnt2* cell surface was detected by Fc-Dectin-1, implying a putative mechanism via Dectin-1 for processing of cell wall glycosylation deficient mutants within macrophage phagosomes. Furthermore, our data show that *C. albicans* morphology at the time of uptake impacts on the rate of phagosome maturation. Phagosomes containing hyphal cargo mature at a slower rate (i.e. co-localisation of Rab7 to CAI4 yeast and hyphal cargo took 30min and 60min, respectively. Interestingly, hyphal-dependent delay of Rab7 recruitment was not observed in the Δ*mnt1*/Δ*mnt2* mutant strain. The differential maturation of phagosomes containing *O*-mannan mutant cells was further exemplified by analysing hyphal development in individual phagosomes. Hyphal extension rates of Δ*mnt1*/Δ*mnt2* cells inside phagosomes were significantly reduced by 29% but extension rates of CAI4 hyphe and extracellular hyphae of both strains increased by 9-10%. Diminished intraphagosomal hyphal growth of Δ*mnt1*/Δ*mnt2* impacted lytic capacity and this was as confirmed in live imaging macrophage killing assays. In summary, we have assessed pathogen-phagocyte interactions at the level of individual phagosomes in real time and have established that fungal viability, glycosylation and morphology play a key role in phagosome maturation, impacting the fungal-pathogen interaction.

159C

CANDIDA ALBICANS AND CANDIDA PARAPSILOSIS INDUCE DIFFERENTIAL IL-1B PRODUCTION IN HUMAN MONOCYTES

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Candida parapsilosis is the third most common species causing invasive candidiasis worldwide. However, little is known about the molecular background of immune responses induced by this pathogen. In this study, our aim was to investigate the host responses induced by live C. albicans and C. parapsilosis using two different in vitro model systems: human peripheral blood mononuclear cells (PBMCs) and PMA-induced THP-1 monocytes. We have previously shown that heat killed C. parapsilosis induces similar TNFa and IL-6, but slightly lower IL-1b production in human PBMCs compared to C. albicans. Furthermore, we have shown that C. parapsilosis induces significantly lower T helper 17 (Th17) differentiation in comparison to C. albicans. In this study, first we compared the pro-inflammatory cytokine responses evoked by C. albicans and C. parapsilosis in PBMCs using live microorganisms. PBMCs were stimulated with C. albicans or C. parapsilosis for 24 h and the cytokine production was measured by ELISA. We found that PBMCs stimulated with live C. parapsilosis produced similar quantities of TNFα and IL-6, and much lower amounts of IL-1β, compared to C. albicans-stimulated cells. As IL-1b has been shown to be important for Th17 differentiation, we further examined the details of IL-1b production upon C. albicans and C. parapsilosis infection using PMA-induced THP-1 monocytes (the prototypic cell line for IL-1b production and inflammasome activation studies). We found that while C. albicans induced the release of IL-1b after 24 hours already at an MOI of 1:100 (Candida : THP-1), a 100-times higher dose of C. parapsilosis cells (MOI 1:1) was needed for the induction of IL-1b secretion. Moreover, using different isolates of C. albicans and C. parapsilosis, we proved that this difference was species rather than strain specific. Furthermore, our first data suggest that the difference in secreted IL-1b levels originates from the differential processing of IL-1b protein rather than from different transcriptional or translational regulation of IL-1b synthesis. Additionally, we examined the production of IL-1b in THP-1 cells following

the inhibition of Caspase-1 and Caspase-8, as well as the pattern recognition receptor Dectin-1 and the tyrosine kinase Syk. In conclusion, our results show that there is a marked difference in the production of IL-1b induced by C. albicans and C. parapsilosis, possibly contributing to the differential Th-responses induced by the two species. Importantly, these results implicate that the activation of inflammasome, the protein complex responsible for IL-1b processing, may be less effective upon infection with C. parapsilosis. Our findings highlight the importance of studies focusing on different Candida species rather than C. albicans alone when investigating the immunity against these pathogens.

160A

CANDIDAL VAGINAL COLONIZATION DRIVES A PRO-INFLAMMATORY RESPONSE AND IMPAIRS MEDIATORS FAVORING REGULATORY T CELLS

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Introduction: Th17 cells are important for defense against systemic and vaginal candidiasis, and expansion of regulatory T cells (Tregs) is critical to sustain pregnancy. In three clinical trials, vaginal antifungal suppositories reduced the incidence of preterm birth by 25-40%. We hypothesized that vaginal colonization with *Candida albicans* elicits an environment favoring Th17 cells at the expense of Tregs. **Methods:** VK2/E6E7 vaginal epithelial monolayers and full thickness EpiVaginal™ tissue were colonized with 10^6 *C. albicans* for 24 hrs at 37°C. Cytokines released into culture supernatants were measured by ELISA or multiplex bead technology. Consequences of inflammasome activation were assessed with inhibitors. Cleavage of pro-IL-1 beta was analyzed by Western blot. **Results:** *C. albicans* significantly increased the release of pro-inflammatory cytokines IL-1 beta, TNF-alpha, IL-17A, IL-6, IL-22 and IL-23 from VK2/E6E7 monolayers and EpiVaginal tissue ($p<0.01$), while impairing production of Treg-inducing mediators including CCL20, TGF-beta, and human beta defensin-2 ($p<0.019$). Concentrations of Th17-inducing cytokines elicited from EpiVaginal tissue were 20-100 fold greater than from VK2/E6E7 monolayers. Three mediators of preterm labor---IL-1 beta, TNF-alpha, and PGE2---were dramatically induced by *C. albicans* ($p<0.001$). Complement C5a was significantly increased in supernatants from EpiVaginal tissue colonized with *C. albicans* ($p<0.0001$), but not from VK2/E6E7 monolayers. Both pro- and activated IL-1 beta were detected in cellular supernatants. Glybenclamide and parthenolide, which target the Nlrp3 pathway, led to a dose-dependent decrease in activated IL-1 beta in supernatants of VK2/E6E7 cells colonized with *C. albicans* ($p<0.006$). Strains with disruptions in the genes encoding Int1 or the *Candida* prostaglandin synthesis proteins Ole2 and Fet3 elicited lower amounts of IL-1 beta and PGE2, respectively ($p<0.0001$). Colonization by strains with disruptions in the genes encoding Pmr1 and Mnn4 reduced release of IL-1 beta ($p<0.0001$), and the *mnn4* double disruptant reduced release of TNF-alpha as well ($p<0.0001$). Treatment of colonized VK2/E6E7 cells and EpiVaginal tissue with heparin (500 or 1000 U/ml) significantly decreased the release of Th17-inducing cytokines IL-1 beta, IL-6, and IL-23 ($p<0.001$) and increased the Treg-inducing mediators CCL20 and human beta defensin-2 ($p<0.004$). **Conclusion:** *In vitro*, colonization of vaginal epithelium with *C. albicans* skews the inflammatory response in favor of Th17-inducing cytokines at the expense of Treg-inducing mediators. Because heparin reverses these effects, vaginal heparin may be a therapeutic adjunct to sustain pregnancy in women with candidal vaginal colonization.

161B

EXPERIMENTAL AND CLINICAL EVIDENCES REVEALING NEW INSIGHTS ON ANTI-CANDIDA ALBICANS MANNAN ANTIBODY SPECIFICITY AND PATHOPHYSIOLOGICAL AND DIAGNOSTIC RELEVANCE

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C. albicans mannan (Mn) comprises a large repertoire of oligomannosides (OM) with different types of mannose linkages. Among these, some have been shown to be protective in experimental models of *Candida* infection. Although anti-*C. albicans* Mn antibody levels are sometimes determined for diagnostic purposes nothing is known about the qualitative distribution of these antibodies in terms of epitope specificity. A library of synthetic OM with a versatile biotine sulfone tag allowing surface plasmonic resonance (SPR) of molecular interactions or multiple analysis profiling (MAP) of antibody specificity was designed. These tools were used to analyse the binding of antibodies either monoclonals (murines) or polyclonals (patients with candidiasis) and mannose binding lectin (MBL). Biotine sulfone tagged OM consisted in β-1,2 linked mannobiose (2b) to mannotetraose (4b) and α-1,2 linked mannobiose (2a) and mannotetraose (4a). IgM selected Mabs consisted in anti-β-1,2 mannosides (5B2, B6.1) and anti-α-1,2 mannoside (EB-CA1). Mab EB-A2 reacting with a Gal*f* epitope was used as a control. Phospholipomannans (PLMs) from *C. albicans* serotype B and serotype A Δ *bmt5* mutant expressing a β-1,2 mannotriose (3b) were used. SPR analysis confirmed Mabs specificities although binding activities were observed for neighbouring degrees of polymerization (DPs) for Mabs B6.1 and CA1. These data were confirmed by MAP as well as the unexpected reactivity of Mab CA1 with 3b described as the "protective epitope" identified by Mab B6.1. This was also confirmed by western blotting on PLMs from *C. albicans* serotype B and *C. albicans* serotype A Δ *bmt5* mutant. When using patients sera in MAP, a significant correlation was observed between detection of antibodies against (Mn) composed of a mixture of α and β. Man epitopes and detection of antibodies against 3b suggesting that this epitope was indeed reacting with human antibodies of both α[[Unsupported Character - Symbol Font ]]and β specificities. Synthetic OM binding to rMBL confirmed that in contrast to 2b and 4b, 3b binding was similar to that observed for 4a and a trend for saturated binding was only observed for 2a. Altogether our data show the unique ability of 3b for a dual interaction with mediators of innate and adaptive immune responses and may contribute to understand the pathophysiology of candidiasis.

162C

THE CONSTRUCTION OF NOVEL CYTOKINE SECRETING STRAINS OF C. ALBICANS AND VALIDATION OF THEIR ABILITY TO MODULATE THE OUTCOME OF A HEMATOGENOUSLY DISSEMINATED INFECTION.

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Candida albicans is a normal commensal of humans, typically found growing on the skin or mucosal surfaces of the mouth, vagina

and gastrointestinal tract. Unfortunately, the fungus is also capable of causing a wide array of diseases ranging from unpleasant, but generally treatable, superficial infections such as oro-pharyngeal (OPC) and vulvo-vaginal (VVC) candidiasis to severe, life-threatening diseases should the fungus disseminate to the deep organs. Although these diseases may have different etiologies, a common theme in the transition of the fungus from the commensal to the pathogenic state is a disturbance in the normal host immune response. In disseminated candidiasis, the greatest risk factor is surgical intervention - insertion of an intravenous catheter, stent, shunt, heart valve or other medical device - and concomitant immunosuppressive therapy. With regards mucosal infections, the most common underlying condition for OPC is HIV infection indicating a major role for CD4+ T cells in directing host responses at this site. The observation that HIV infected women do not have a higher incidence of VVC than non-infected individuals suggests that the manner in which the host responds to the fungus differs by location. Reinforcing this assumption are several recent studies which reveal that ablation of IL-17 mediated immune responses can lead to either increased susceptibility or resistance to candidiasis depending on the site of infection. Rather than use gene deleted mouse strains which lack a particular host response throughout the animal, we have developed plasmid vectors which facilitate the construction of cytokine secreting strains of C. albicans that enable examination of the consequences of modulating host immune responses directly at the site of infection. In this study, we detail the construction of the vectors and the production of various strains that secrete either mouse IL-6 or IL-17A using them. Characterization of the IL-17A secreting strains provided data supporting the recent study which suggested that this cytokine impacts the growth rate of C. albicans. Moreover, by integrating the IL-17A construct into our tet-NRG1 strain, we were able to confirm that this effect was specific to the hyphal form of the fungus. Finally, we examined the ability of the IL-17A secreting strain to modulate the outcome of a hematogenously disseminated infection by conducting an animal experiment in which one group of mice were infected with cells producing the cytokine and the other group of mice were challenged with fungal cells in which the mouse gene was switched off. The median survival time of the former group was 9 days whereas the survival of the latter group was 3 days confirming the ability of the cytokine to alter the course of the infection. We believe that these new tools will prove to be a useful addition to the armory in our quest to unravel the complexities of the Candida - host interaction.

163A

IN VIVO STUDIES IN MICE AND *GALLERIA MELONELLA* OF *CANDIDA ALBICANS* STRAINS FROM SYSTEMIC AND MUCOSAL INFECTIONS

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We have previously reported on a comparative *in vitro* assessment of virulence characteristics of strains isolated from human systemic *Candida albicans* infections (20 S strains) and strains from mucosal infections (22 M-strains) indicating a tendency for higher expression of virulence attributes among the S strains. MLST genotyping revealed a specific genotype found only among the S strains. The aim of the present study was to evaluate *in vivo* the virulence of selected S and M strains, based on the virulence characteristics assessed *in vitro*. Our *in vivo* study included two experimental models: (1). murine systemic candidiasis in naïve and immunocompromised mice and (2). an infection in *Galleria melonella*. Naïve or immono - compromised (by cyclophosphamide treatment) ICR female mice were inoculated intravenously with *C. albicans* suspension of six S or M strains and the *C. albicans* type-species strain CBS 562. A 30-day follow-up

for assessment of mortality and morbidity was carried out, evaluating survival rate, survival time and Candida burden in the kidneys by CFU enumeration and by histopathology. The same strains were also inoculated into the hemocoel of Galleria melonella .Caterpillars were incubated at 37°C for 9 days and larva survival was monitored daily considering death of the larvae when they did not respond to physical pressure. The experiments in both models showed that the control strain CBS 562 was the most virulent, resulting in 0% survival of mice within 10 days and 2 days in *Galleria melonella*. Survival analysis by the Kaplan-Meier test indicated for one of the S strains a similar pattern to CBS. In general the analysis demonstrated earlier lethality of S strains as compared to M strains. A correlation with the Candida burden in the kidneys and survival rate was noted. In summary, the *in vivo* observations are compatible with the *in vitro* data showing a tendency for higher expression of virulence among the S strains, although also among M strains virulent strains are noted. More extensive experiments, which are planned, are needed to strengthen these observations.

164B

A NOVEL IN *VITRO CANDIDA* VIRULENCE ASSAY

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Systemic candidiasis is most commonly studied in animal models, particularly in the murine intravenous (IV) challenge model, where infection with a virulent *Candida albicans* strain leads to increasing fungal kidney burdens and increasing proinflammatory cytokine levels. Early renal levels of the chemokine KC correlate with infection outcome (MacCallum *et al*., 2009). We have developed a new *in vitro* model, based on renal M-1 cortical collecting duct epithelial cells, to evaluate virulence of *C. albicans* isolates and mutants, in attempts to reduce the number of mice used in virulence studies (Szabo & MacCallum, 2014). We demonstrate that these epithelial cells respond only to live fungal cells, unlike immune cells. Renal epithelial cell responses reflect early events in the mouse model, with KC and MIP-2 produced in response to virulent *C. albicans* strains or mutants. This chemokine production correlates with *C. albicans* damage to epithelial cells (Szabo & MacCallum, 2014). Our data suggest that the ability to switch between yeast and hyphal growth forms is important for full virulence in our *in vitro* model, similar to results found in the mouse model, as morphological mutants exhibited attenuated virulence. We also demonstrate that non-*albicans Candida* species, which are attenuated in the mouse model, are unable to elicit chemokine responses from mouse kidney epithelial cells, despite increasing the inoculum. Using this new *in vitro* model we have confirmed that renal epithelial cells are able to discriminate between virulent and attenuated strains of *C. albicans,* allowing this model to be used as an initial screen for altered virulence.

165C

EVALUATION OF THE WHITE-OPAQUE PHENOTYPIC SWITCH IN INTERACTIONS WITH THE HOST

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Candida species include human commensals and opportunistic pathogens that can adapt to environmental cues by rapid changes in phenotype and morphology. The best-characterized phenotypic switch is that between white and opaque states in *C. albicans* and *C. tropicalis*. Despite extensive research into the mechanism of white-opaque switching and the *in vitro* characteristics of white/opaque cells, relatively little is known about how these states differ in their interactions with the host. In phagocytosis assays using *Drosophila* S2 cells, we found

that the percent phagocytosis and phagocytic index of the white cells of both *C. albicans* and *C. tropicalis* were significantly higher than cells in the opaque state. Furthermore, yeast cells were phagocytosed more efficiently than hyphal forms of either white or opaque cells from both *Candida* species. In spite of these differences, white and opaque yeast cells show a similar distribution of β-glucan on the cell surface and similar staining with soluble Dectin-1, the host receptor for β-glucan. To further elucidate differences in white and opaque cells of *Candida*, we utilized the zebrafish hindbrain model of infection. We show that white and opaque *C. albicans* cells can infect and disseminate within the fish, and that both phenotypic forms are induced to undergo filamentation. This is, to our knowledge, the first report of opaque cells undergoing filamentous growth *in vivo*. We also assessed infection across a broad range of temperatures to determine the virulence of *C. albicans* white and opaque cells under these conditions. We demonstrate that white and opaque cells exhibit similar virulence properties at 25°C, with both cell states leading to fish mortality. However, white cells were significantly more virulent than opaque cells at elevated temperatures (30°C and 33°C). This is in spite of the fact that white and opaque cells showed indistinguishable growth rates when cultured in RPMI, YPD, or SCD media at each these temperatures. Future experiments will utilize transgenic zebrafish with fluorescently labeled macrophages or neutrophils to further define differences between white/opaque cells and their interactions with the host immune system.

166A

FUNCTIONAL CHARACTERIZATION OF ZCF15 - A TRANSCRIPTION FACTOR EXPANDED IN PATHOGENIC FUNGI AND IMPORTANT FOR INFECTION

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Virtually all humans are colonized with *Candida albicans* but, under certain circumstances, this benign commensal organism becomes a serious, life-threatening pathogen. The basic mechanisms that regulate this transition and lead to pathogenesis are still poorly understood. We show that a class of fungal specific Zn(II)2Cys6 transcription Factors (Zcfs) are found in multiple copies in fungi capable of a pathogenic lifestyle but not in non-pathogenic fungi. Some of these Zcfs accumulate mutations during human infections and previous studies have shown that *C. albicans zcf1-/-, zcf13-/-,zcf18-/-, zcf29-/-* and *zcf34-/-* have a reduced virulence in live animal models. Our data suggest that *C. albicans zcf15-/-* is more susceptible to macrophage killing compared to wild type and has a reduced virulence in a nematode host. Our data also suggest that *ZCF15* plays an important role in the pathogen's ability to withstands host generated reactive oxygen species. Together these studies underscore the importance of these structurally and functionally related transcription factors during infection. We are currently leveraging genome-wide expression and location profiling, two technologies that have complementary strengths and limitations, to shed some light on the functions of these important transcription factors. Our preliminary data suggest that the duplicated *ZCF* genes have evolved to mediate pathogen's adaptation to the host environment and/or pathogen's defensive mechanisms against host innate immunity.

167B

MEASURING THE RELATIVE FITNESS OF *C. ALBICANS* AND *C. GLABRATA* IN VARIOUS IN VITRO AND IN VIVO ENVIRONMENTS.

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Background: *C. albicans-C. glabrata* (Ca-Cg) co-infections are common in patients, particularly during intra-abdominal candidiasis (IAC), but not well studied in the laboratory. **Methods:** We evaluated Ca-Cg co-infections *in vitro* and in mouse models of IAC and hematogenous disseminated candidiasis (DC). **Results:** We incubated CaSC5314 and CgBG2 alone or in combination in YPD or YNB without amino acids at 30°C. In YPD, there were no differences in Ca *vs* Cg CFU or single *vs* mixed cultures over 50d. In YNB single cultures, there was no difference in Ca *vs* Cg CFU until d40, when Cg drop-off was significantly greater than Ca. In YNB mixed culture, Ca and Cg growth rates were similar for 24h, then Cg drop-off was significantly greater; by d24, Cg was no longer viable while Ca concentrations were similar to single culture. Next, we infected mice intra-peritoneally (IP) with SC5314 or BG2 ($1x10^7$ CFU) alone or in combination (single or mixed infection). All inocula included sterile stool. In peritoneal fluid (PF), Cg achieved higher burdens than Ca at d1and d7 in single and mixed infections (Cg: 5.54±0.1 and 4.91±0.11 $\log_{10}$ CFU/mL for single and mixed on d1; 2.8±0.24 and 1.7±0.33 on d7; Ca: 5.02±0.3 and 3.99±0.17 on d1; 1.83 ± 0.29 and 0 on d7; all $p<0.02$). Burdens of both Cg and Ca were lower in mixed than single infections (all $p<0.05$). Cg also achieved higher burdens than Ca within abscesses (IAA) on d3, d7 and d14 in single and mixed infections (all $p\leq0.002$; e.g., Cg: 6.77±0.17 and 6.40±0.39 for single and mixed on d7; Ca: 5.08±0.47 and 4.26±0.28 on d7). Ca burdens were significantly lower in mixed than single infections (all $p<0.05$). Cg burdens were similar in mixed and single infections. Lastly, we infected mice IV ($1x10^7$ CFU) in single or mixed infections. The mortality rate was 100% by d7 for mice infected with Ca or Ca-Cg, and 0% for Cg. On d1, Ca achieved higher burdens than Cg in kidneys, liver, pancreas, brain and eyes during both single and mixed infections (all $p\leq0.01$). Ca burdens were significantly lower in liver, pancreas, brain and eyes during mixed than single infections (all $p\leq0.04$). Cg burdens were similar in mixed and single infections. **Conclusions:** The relative fitness of Ca and Cg differs in various *in vitro* and *in vivo* environments. Under nutrient-limited conditions *in vitro*, Ca persists longer than Cg and out-competes Cg after 24h. During IAC, however, Cg persists longer in PF and IAA and out-competes Ca beginning at early time points. Futhermore, Ca viability within IAA is reduced in the presence of Cg. Ca out-competes Cg within targets organs during the first 24h of DC, but Ca viability in most organs is reduced in the presence of Cg. Therefore, Cg modifies a number of *in vivo* environments in a manner that is deleterious to Ca, even at tissue sites in which Ca is intrinsically more fit. It is possible that Cg out-competes Ca for certain nutrients, alters immune responses, or produces byproducts or toxins that are harmful to Ca.

168C

INTRAVITAL IMAGING OF MUCOSAL CANDIDIASIS IN THE TRANSPARENT ZEBRAFISH

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Mucosal candidiasis is characterized by the breaching of the epithelial barrier by filaments, compromising epithelial integrity and inducing an inflammatory reaction. This tissue damage can lead to disseminated candidiasis as well as secondary infections. The interaction of C. albicans with epithelial cells and the immune system is difficult to image in vivo with current models and the mechanisms responsible for maintaining and protecting the epithelial integrity during mucosal candidiasis are not yet

well defined in vivo. We have developed an in vivo juvenile zebrafish model where transparency and fluorescently encoded innate immune cells allow spatio-temporal visualization of this interaction in an intact vertebrate host. Upon infection of the swimbladder with C. albicans yeast cells, a morphological switch to filaments takes place within a few hours. Epithelial cells constituting the swimbladder mucosal lining are able to detect the presence of the pathogen, responding by activating the NF-κB pathway. This activation is not only observed in the cells in direct contact with the fungi but also in the surrounding epithelial cells. High-level infection induces a widespread activation of this pathway throughout the epithelium. However, at low-level infection, phagocytes can prevent the activation of NF-κB in epithelial cells by engulfing C. albicans yeast cells. In high-level infection, an inflammatory response is also elicited, with several pro-inflammatory cytokines and mediators highly upregulated. The induction of these cytokines is dependent upon the CARD9 signaling pathway, as CARD9 knockdown by morpholino drastically downregulates the expression of pro-inflammatory cytokines such as IL-6, TNFα, IL-1β and SAA, as well as the mucosal cytokines IL-23, IL-22 and IL-17. Similar to the mammalian infection, neutrophils are rapidly recruited to the site of infection and can extravasate to interact directly with C. albicans in the luminal space of the swimbladder. Dexamethasone treatment renders the fish highly susceptible to mucosal candidiasis, with filaments breaching the epithelial barrier, recapitulating susceptibility in patients under such treatment regiments. The advantages of this juvenile zebrafish model of mucosal candidiasis, such as transparency, genetic pliability and the reliance on innate immune components provide a new window for viewing the interaction of C. albicans with its host.

169A

IN THE MOUSE OROPHARYNGEAL CANDIDIASIS MODEL, OPAQUE CELLS DO NOT MATE AND HAVE ATTENUATED VIRULENCE

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During oropharyngeal candidiasis (OPC), Candida albicans forms a biofilm on the mucosal surface. We have found previously that in vitro, a and α cells form biofilms with matrix-based characteristics different from a/α biofilms and can mate in mixed white and opaque cell biofilms. Here, we used the corticosteroid mouse OPC model to test the virulence of white and opaque cells, as well as mating. In this model, C. albicans forms a biofilm on the dorsal surface of the tongue and invades the superficial epithelium. C. albicans mating was monitored using a and α opaque cells expressing GFP and CaHygB, the latter conferring resistance to hygromycin B, and mCherry and CaSAT1, the latter conferring resistance to ClonNAT, respectively. The outcome of infection was compared among mice inoculated with a majority (90%) of a and α white cells plus a minority (10%) of a and α opaque cells, 90% white a/α cells plus 10% a and α opaque cells, 100% a and α white cells, 100% a and α opaque cells, and 100% a/α cells. One half of each tongue was homogenized and quantitatively cultured to determine the total oral fungal burden and the colony phenotypes. A portion of the homogenates was plated on agar containing hygromicin B and ClonNAT to determine whether mating had occurred. The remaining halves of the tongues were processed for histopathology. We found no evidence of C. albicans mating in the OPC model. The median oral fungal burden of mice infected with white cells was 4.8 log CFU/g, whereas no viable C. albicans cells were

detectable after 5 days in mice infected with opaque cells. In mixed infections, we found that opaque cells significantly inhibited the virulence of white a/α cells, but had possibly a slight enhancement in virulence of white a and α cells. We are currently investigating these interactions in an in vitro oral epithelial cell model. In conclusion, C. albicans does not undergo mating in the mouse OPC model. Possibly, opaque cells play a role in virulence of white a and α cells. Also, opaque cells have highly attenuated virulence during OPC. This last result contrasts with those obtained during C. albicans colonization of the gastrointestinal tract, which induces MTL-homozygous white cells to become opaque (Ramirez-Zavala et al., 2008), and MTL-heterozygous cells to express an opaque-like phenotype (Pande et al., 2013), which suggest that the opaque phenotype may play a more important role in the colonization of the gastrointestinal tract.

170B

THE CCAAT-BINDING-FACTOR-DEPENDENT REGULATION OF THE OXIDATIVE STRESS RESPONSE IN CANDIDA ALBICANS

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The success of Candida albicans as an opportunistic human pathogen has been attributed to several factors. Two such factors include the ability to survive in limiting iron environments and the ability to evade the respiratory burst of human macrophages and neutrophils. The goal of this research is to elucidate the role of the CCAAT-binding factor in the oxidative stress response of C. albicans. Prior whole genome microarray studies performed in our lab compared the gene expression of a wild type C. albicans strain versus a hap5Δ/ hap5Δ strain under iron-limiting growth conditions. Among the differentially regulated genes, it was found that CTA1, encoding catalase, had a four-fold higher level of expression in the hap5Δ homozygous mutant under iron limitation. The sensitivity of the hap5Δ homozygote to oxidative damage was also assessed by growing the strains in iron replete or iron-limiting conditions and exposing them to various concentrations of hydrogen peroxide. The data obtained from these studies supported our hypothesis that the CCAAT-binding factor was involved in the regulation of oxidative stress genes under iron-limiting growth conditions. To further explore the role of the CCAAT-binding factor in the regulation of CTA1, wild type and hap5Δ homozygote were grown in iron replete and iron-limiting conditions, and the total RNA was isolated and Northern blots performed. Consistent with the microarray data, Northern blot analysis indicated a CCAAT-binding factor-dependent regulation of CTA1 under iron limitation. The oxidative stress response in most organisms, including C. albicans, is due to the concerted actions of many gene products. Therefore, we examined the mRNA levels of genes such as superoxide dismutase, thioredoxin and glutaredoxin, which are known to confer resistance to oxidative stress. Northern blotting studies were performed with both wild type and hap5Δ homozygous strains grown under both iron replete and iron-limiting conditions. Together our data indicates that the CCAAT-binding factor in C. albicans regulates the mRNA expression level of different genes involved in the oxidative stress response. Such a concerted regulatory pattern is advantageous in increasing the tolerance of C. albicans to different hostile yet dynamic microenvironments in the human body.

171C

WIDE SCALE GENETIC SCREENS REVEAL EXTENSIVE CARBON AND TEMPERATURE EFFECTS ON CANDIDA ALBICANS STRESS ADAPTATION SCENE.

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Of the circa 5.1 million estimated fungal species, less than 600 are pathogenic to humans.

Amongst these, Candida albicans is a common cause of mucosal infections and life-threatening systemic disease in immune compromised populations. One major factor that allows C. albicans to cause disease is its ability to adapt quickly to dynamic host niches that impose changes in nutrient availability, temperature, and alternating ionic and osmolarity gradients (Brown et. al., 2007; Brock et.al, 2009; Horn et.al., 2012). Thus far, the responses of C. albicans to single stressors have been extensively studied. However, the effects of combinatorial environmental challenges like those encountered within the host remain largely unexplored. Through high-throughput robotics screens we have examined phenotypes for mutants representing approximately 16% of C. albicans genes. The mutants were examined in respect to oxidative, osmotic and nitrosative stress sensitivity, on different carbon sources and at varying ambient temperatures. Our screens have revealed extensive temperature- and carbon- conditional stress susceptibility phenotypes, which would be relevant to neutrophil induced stress. Our future work will seek to dissect the novel molecular hubs that promote cross talk between stress response pathways during growth on different carbon sources. The identification and molecular dissection of novel regulatory hubs will enhance our understanding of C. albicans pathobiology and possibly reveal new leads in this arms race against this major fungal pathogen.

172A

THE INTERPLAY BETWEEN HOST-IMPOSED COPPER OVERLOAD, NUTRITIONAL IMMUNITY AND MICRONUTRIENT ADAPTATION IN CANDIDA ALBICANS DURING THE DEVELOPMENT OF SYSTEMIC CANDIDIASIS

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Micronutrient availability is a key factor that affects microbial proliferation within the host. The host strives to limit microbial outgrowth by withholding of essential elements, while the microbe utilises varied strategies to overcome this nutritional immunity. We have recently shown (PLoS Pathog 9(10): e1003676) that disseminated Candida albicans infections in mice affect host iron homeostasis at a systemic level. These changes result in dramatic iron redistributions in the kidney, with progressively increasing kidney iron loading during the course of infection. Simultaneously, nutritional immunity deprives the fungus of iron in situ. During disseminated candidiasis, the role of copper, another essential transition metal, is less clear. We applied laser ablation inductively coupled plasma mass spectrometry (LA-ICP MS) to construct 2D copper (63Cu) maps across the kidneys of healthy and infected animals. In contrast to renal iron loading which increases progressively as infections progress, there appears to be a transient increase and redistribution of renal copper. This increase is associated with elevated transcription of the hepatic gene encoding the major plasma cuproprotein ceruloplasmin, as assessed by qRT-PCR. Meanwhile, the C. albicans CRP1 copper transporter gene, but not the CRD2 copper metallothionein gene, is up-regulated early in the infection. Then, late in the infection, the C. albicans high affinity copper importer (CTR1) is up-regulated, indicating possible copper starvation. We hypothesise both copper and iron limitation affect the development of fungal lesions in the kidney. Our findings add to the complex micronutritional cross-talk between C. albicans and its mammalian host.

173B

CANDIDA ALBICANS SAP6 IS A MAJOR CONTRIBUTOR TO PATHOGENESIS DURING OROPHARYNGEAL CANDIDASIS

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Secreted aspartyl proteases (Saps), a gene family of 10 members, have been recognized as

virulence factors for mucosal candidiasis. The presence of related but independently regulated and functionally distinct Saps provide elasticity to survive and proliferate at different host niche sites. Previous studies have shown the role of *SAP5* and *SAP6* in oropharyngeal candidasis (OPC) but there is a lack of direct evidence for involvement of both of these genes. **Objective**: To define the role of *C. albicans SAP5* and *SAP6* as virulence factors for OPC. **Methods:** The virulence capacity of *SAP* deletion and *SAP5* and *SAP6* over-expression (O/E) strains mutants was compared with parent strain CAI4 using a murine model of OPC by assessing CFUs/g/tongue tissue. Real Time PCR was performed to detect gene expression of Saps *in vivo* in *Candida* cells collected from mouse tongues and *in vitro* in hyphae-inducing (37°C, N-acetyl glucosamine) and solid agar surface growth conditions. Germination and biofilm formation, invasion to agar surfaces, proteases secretion and epithelial cell damage were also determined. **Results:** Among the 10 SAPs, CAI4 cells harvested from tongue plaques had significantly increased expression only of *SAP6* (15-fold) and *SAP5* (8-fold), when compared to planktonic cells grown in spider media at 37°C. Interestingly, gene deletion of *SAP8* (*sap8*Δ/Δ) resulted in a 3-fold increased expression of *SAP6* grown on solid agar surface, a 10-fold increase in both *SAP5* and *SAP6* expression under hyphae-inducing conditions, and higher secreted protease activity. The *sap8*Δ/Δ was hyper-virulent in murine OPC (4 log-fold higher CFU) compared to CAI4, and cells collected from tongue plaques had elevated expression of *SAP6* (3-fold) and *SAP5* (2 fold). The *SAP6O/E* strain phenocopied the hyper-virulence of the *sap8*Δ/Δ strain; however the *SAP5O/E* strain had equivalent infection as the parental CAI4. Deletion of Saps 4, 5 and 6 (Δ*sap4/5/6*) significantly reduced virulence (4 log-fold lower CFU). The *sap8*Δ/Δ and *SAP6O/E* infected tongue tissues showed a significant increase in TUNEL positive apoptotic cells compared to CAI4. There was increased filament length and higher Cek1 phosphorylation in *sap8*Δ/Δ cells harvested from tongue plaques compared to CAI4. **Conclusion:** *C. albicans sap8*Δ/Δ and *SAP6O/E* are novel hyper-virulent strains with similar phenotypes showing a crucial role for Sap6, but not for Sap5 in oral candidiasis. Higher protease activity, increased filament length and enhanced invasiveness are likely to contribute towards this hyper-virulent phenotype in OPC. This work was supported by R01DE010641 and DE022720 from the National Institute of Dental and Craniofacial Research

174C

MEASURING *C. ALBICANS* AND HOST GENE EXPRESSION DURING INTRA-ABDOMINAL CANDIDIASIS OF HUMANS AND MICE WITH RNA-SEQ

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Background: Transcriptome analysis during invasive candidiasis (IC) may provide insights into pathogenesis, but it is technically challenging during disseminated or oral candidiasis. Intra-abdominal candidiasis (IAC) is an under-studied manifestation of IC that is suited to studies of *in vivo* gene expression. **Methods:** We performed RNA-Seq on peritoneal fluid (PF) from a patient with *C. albicans* IAC. Expression of 145 *C. albicans* genes was compared to nanoString data from peritoneal fluid (PF) of 3 mice with IAC. The role of the highly-expressed gene *ALS1* in virulence during IAC was tested in mice. **Results:** PF was collected from intra-abdominal sites (right and left upper quadrants) in a 63 year old man with *C. albicans* IAC 48 hrs after biliary leak. PF from mice was collected 48 hrs after IP injection of *C. albicans* SC5314+sterile stool. pH of all samples was 8.0. Illumina MiSeq run on human samples generated ~6 million reads; ~3.8 million reads (64%) were

mapped to *C. albicans* coding sequences, representing 93% of ORFs. The 181 most highly expressed genes (>1000 mean RPKM) were assessed for GO term enrichment. Cell wall ($p=6e^{-25}$) and host interaction ($p=4e^{-8}$), mitochondrion ($p=4e^{-4}$) and energy derivation ($p=2e^{-3}$) were over-represented. RNA-Seq data correlated well with nanoString data from mice ($R^2=0.72$). Among the most strongly expressed genes in both patient and mice were *ALS1*, *RIM101*, *ENA2*, *ENA21*, *GPD2, SSB1*, *CAT1*, *TRR1* and *TRX1,* involved in processes like adhesion and alkaline pH, osmotic and oxidative stress/neutrophil (PMN) responses. Hyphal genes *HWP1* and *HYR1* were poorly expressed. Disruption of *C. albicans ALS1*, which encodes an adhesin/invasin, did not impact peritonitis or tissue invasion during mouse IAC, but significantly attenuated persistence in abscesses compared to wild-type and complemented strains ($p<0.001$). The *als1* mutant was more susceptible to phagocytosis and killing by PMNs *in vitro* ($p<0.001$). Overexpression of *C. albicans ALS1* in *C. glabrata* BG2 resulted in greater persistence within abscesses ($p<0.001$), and resistance to PMN phagocytosis and killing *in vitro* ($p<0.001$). RNA-Seq reads during mouse IAC mapped exclusively to mouse genes. The most highly expressed genes were over-represented for antigen presentation/immunoglobulin ($p=3e^{-4}$), chemotaxis ($p=3e^{-4}$), and regulation of adaptive immunity ($p=5e^{-3}$). **Conclusions:** RNA-Seq is a powerful tool for studying *C. albicans* and host gene expression *in vivo*. Transcriptome data from PF afford insights into *C. albicans* biologic processes involved in pathogenesis of IAC, and host immune responses. While a number of highly-expressed *C. albicans* genes in PF encode known virulence determinants, their specific contributions to IAC may be unanticipated. For example, *C. albicans* Als1 does not increase adhesion to abdominal organs, but facilitates resistance to phagocytosis and survival within abscesses.

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INTERACTION OF BLOOD COAGULATION FACTOR XII WITH ADHESINS AND MOONLIGHTING PROTEINS OF THE CANDIDA ALBICANS CELL WALL

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Coagulation factor XII (FXII), also known as Hageman factor, is a serine proteinase precursor (zymogen) which activates upon a contact with negatively charged surfaces to trigger two major regulatory proteolytic cascades of the blood, the hemostatic intrinsic coagulation pathway and the inflammatory kallikrein-kinin system. Physiologic FXII-activating surfaces include blood vessel walls and blood cells. The adsorption and activation of FXII also occur on bacterial cell surfaces, with implications on microbial pathogenicity. Our recent report of the tight binding of Hageman factor to the cell wall of Candida albicans has opened a hypothesis that similar phenomena can also contribute to fungal virulence. The current work aimed at the identification of FXII-binding proteins of C. albicans cell surface, the isolation of the tightest binders and the characterization of their interactions with FXII in terms of thermodynamic and kinetic binding constants. The cell wall proteins (CWP) of hyphal forms of C. albicans were extracted with beta-1,3-glucanase and mercaptoethanol. Particular FXII-binding CWP were identified by adsorption on agarose-coupled FXII and mass spectrometric (MS) analysis with bioinformatic (Mascot) identification of the SDS-eluted material. Alternatively, the FXII-binding CWP were chemically cross-linked to biotinylated FXII with thiol-cleavable reagents, isolated on streptavidin-containing beads, thiol-released and identified by MS. With these two approaches, rather than the main GPI-anchored adhesins (except Als3 pro-

tein), multiple "moonlighting" surface proteins such as enolase (Eno1p), phosphoglycerate mutase (Gpm1p), elongation factor 2 (Ef2p), proteasome component C5 and triosephosphate isomerase (Tpi1p) were identified with the highest Mascot score as putative FXII binders. Three FXII-binding CWP, including Als3p and two moonlighting proteins, Tpi1p and Eno1p, were isolated in non-denatured, electrophoretically pure forms, by the separation of crude CWP extracts by ion-exchange and size-exclusion chromatography, with the fraction selection on the basis of FXII-binding activity, determined by a microplate ligand-binding assay. These purified CWP were immobilized on CM5 chips of the BIACORE 3000 system and analyzed for FXII binding by the measurement of surface plasmon resonance in this instrument. In terms of the determined dissociation constants, Tpi1p bound FXII with the highest affinity (74 nM) as compared to Eno1p (110 nM) and Als3p (174 nM). In summary, the current work emphasized the major contribution of moonlighting proteins, loosely associated with the fungal surface, to the binding of FXII by C. albicans cells.

176B

ADHESIN-MEDIATED CANDIDA GLABRATA INTERACTIONS WITH HIV-1 INFECTED MACROPHAGES

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Candida glabrata is one of the leading causes of candidiasis in immunocompromised patients. One of the unique features of C. glabrata is that it contains an exceptionally large number of GPI-modified adhesin-like wall proteins. Interactions of Candida spp. with phagocytes, such as macrophages, occupy a large portion of the disease cycle, whether in the blood, or at mucosal surfaces. Macrophages are the primary target of HIV and are known to play a significant role in development of HIV induced pathogenesis. We hypothesize that the high incidence of candidiasis in HIV/AIDS patients is associated with an increased viral replication and due to hyper-adherence phenotypes of pathogenic C. glabrata strains. To test this hypothesis we performed experiments to determine the role of C. glabrata adhesins on viral replication. Our preliminary results clearly indicate a significant increase in HIV replication in human monocyte-derived macrophages (MDMs) in the presence of hyper-adherent C. glabrata strains compared to the wild type control (P=0.005). Importantly, a statistically significant decrease in viral replication was observed in MDMs infected with the C. glabrata aed1Δ (AED1 encodes a putative endothelial adhesin in C. glabrata) null mutant after 7 day post infection (P=0.039), suggesting an important role for adhesins in viral replication. Taken together, our results support an important role for C. glabrata adhesins in HIV replication in macrophages and offer novel insights which may be an important mechanism in the establishment of candidiasis in HIV infected patients.

177C

INACTIVATION OF ANTIFUNGAL AND IMMUNOMODULATORY FUNCTIONS OF LL-37 BY ASPARTIC PROTEASES OF CANDIDA ALBICANS - A WAY TO SURVIVE

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Background: LL37, formed by the cleavage of human cathelicidin (hCAP-18), presented in the specific granules of neutrophils and expressed in many types of immune and epithelial cells, plays multiple roles in the modulation of cellular responses to microbial

attack. The main antibacterial function of LL-37 relies on the disruption of microbial cell membrane by covering the cell surface which results in cytoplasm leakage. In the infections caused by Candida albicans, LL-37 can additionally reduce the infectivity by inhibition of yeast adhesion to host cells or biofilm formation. **Objective:** The aim of the current study was to analyze the action of secreted aspartic proteases (SAPs), the main virulence factors of C. albicans, on LL-37 with the impact on its antifungal and immunomodulatory activities. **Methods:** Ten C. albicans SAPs , purified to homogeneity after over-expression in Pichia pastoris, were used in this study. LL-37 degradation products were identified by reversed phase high performance liquid chromatography (RP-HPLC) and electrospray-ionization mass spectrometry (ESI/MS). With all identified peptides the antifungal tests were performed. The production of interleukin (IL)-8 and release of reactive oxygen species (ROS) by neutrophils isolated from human peripheral blood and treated with LL37-derived peptides were analyzed by enzyme-linked immunosorbent assay (ELISA) and chemiluminometric method, respectively. Migration of neutrophils was determined with microchemotaxis chamber technique and their viability was characterized by caspase activity assay. **Results:** The LL-37 degradation patterns in both time- and concentration-dependent manners were determined for all C. albicans SAPs (SAP1-SAP10). A very efficient cleavage of LL-37 by SAP1, SAP2, SAP3 and SAP9 was observed while SAP4 and SAP8 were much less effective. The cleavage of LL-37 correlated with a loss of antifungal properties by LL-37 degradation products. However, at the first phase of the cleavage by the most effective SAPs, a peptide LL-25 was temporarily formed which was still antifungal although quickly lost this activity upon further degradation. To determine whether LL-25 maintained some of the regulatory activity of LL-37 in inflammatory responses, we analyzed production of IL-8 and ROS by neutrophils as well as their chemoattraction and viability. We found that LL-25 did not affect the production of ROS by neutrophils upon PMA treatment as LL-37 did. Also, the stimulation of neutrophils by LL-25, as compared to that by LL-37, showed no IL-8 generation. The chemotaxis of neutrophils and their viability in the presence of LL-25 were also significantly lowered. **Conclusions:** At the place of fungal infection, the profile and level of SAP expression can counteract the functionality of host defense and allow the pathogens to survive or further disseminate.

178A

TARGETED GENE DISRUPTION IN CANDIDA PARAPSILOSIS DEMONSTRATES A ROLE OF CPAR2_404800 IN ADHESION TO HUMAN BUCCAL EPITHELIAL CELLS

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Candida parapsilosis is an important nosocomial pathogen and it represents the second/third most common cause of invasive candidiasis worldwide. However, the molecular mechanisms underlying its virulence properties are only beginning to be characterized. The aim of this study was to investigate the molecular mechanisms of *C. parapsilosis* adhesion to host surfaces, a virulence trait that plays a key role during the early stages of infection. *In silico* analysis of the genome sequence of *C. parapsilosis* indicates the existence of 5 potential homologues of *C. albicans* agglutinin like sequence proteins (Als), known to be involved in the adhesion process. The role played by each of these five genes in *C. parapsilosis* adhesion to biotic surfaces remains to be fully understood. In light of this, CPAR2_404800 (further indicated as *CpALS3*) was selected for site specific mutation on the basis of homology studies with *C. albicans ALS* gene family. The deletion cassette was based on the use of the dominant nourseothricin resistance marker (*CaSAT1*) and its subsequent deletion

by FLP-mediated site-specific recombination. Two independent lines of heterozygous and null mutants were obtained in *C. parapsilosis* ATCC 22019 genetic background. Wild type strain (WT), heterozygous strains (Cp*ALS3/Δals3*a, Cp*ALS3/Δals3*b, HET) and null mutants (Cp*Δals3/Δals3*a, Cp*Δals3/Δals3*b, KO) were compared for their ability to grow under different culture conditions and in the presence of cell wall perturbing agents. WT, HET and KO strains were able to grow in conventional culture media and on YPD agar supplemented with SDS, while preliminary results indicate a different sensitivity of mutants to Congo Red, in comparison to WT. All strains were able to form pseudohypae when incubated in YPD supplemented with 10% fetal bovine serum. However, when strains were tested for their ability to adhere to human buccal epithelial cells (HBECs) obtained from a healthy donor, both null mutants exhibited a significant reduction in the adhesion ability to HBECs ($P<0.001$). HET strains showed adhesive properties to buccal cells comparable to WT ones. Reintroduction of one copy of *CpALS3* gene in the null background restored the ability to adhere to HBECs. We also investigated the impact of *CpALS3* mutation on *C. parapsilosis* pathogenicity in an intra-haemocoelic infection model of *Galleria mellonella* larvae. No significant difference was observed between mortality rate of *G. mellonella* larvae infected with WT, HET, KO, or complemented strains. Collectively, these results indicate that *CpALS3* deletion does not impair physiological growth of *C. parapsilosis* on common media, nor is it primarily involved in *G. mellonella* host invasion and damage. However, *CpALS3* plays an important role in the adhesion process to human buccal cells. Further studies are currently being undertaken to better characterize *CpALS3* mutant phenotypes in vitro and in vivo.

179B

ROLE OF CANDIDA ALBICANS MAPK PATHWAYS IN COLONIZATION OF THE MOUSE GASTROINTESTINAL TRACT.

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Candida albicans is frequently found in the human gastrointestinal tract as part of the fungal microbiota. In this niche, it needs to adapt to the different micro environments that challenge its survival within the host. In order to determine the factors involved in gut adaptation of this opportunistic pathogen, we have used a gastrointestinal model of commensal colonization in mouse to trace the behaviour of fungal cells. We have developed a genetic labelling system based on the complementary spectral properties of fluorescent proteins, GFP and a new C. albicans codon-adapted RFP (dTOM2), that allow a precise quantification of two fungal populations in the gut via standard in vitro cultures or by flow cytometry. This methodology has allowed us to determine the role of three MAP kinase pathways of C. albicans (mediated by the MAPK Mkc1, Cek1 or Hog1) in mouse gut colonization via competitive assays with MAPK pathway mutants and the parental strain. In this way, we have determined that signalling through HOG pathway is a critical factor influencing the establishment and the maintenance of C. albicans in the mouse gut. Less pronounced defects were found in mkc1 or cek1 mutants, only evident after 2-3 weeks of sustained colonization. Among the factors that would affect fungal gut colonization we have seen that hog1 mutants present a partial defect in ex vivo adhesion to the gut mucosa and are more sensitive to bile salts and other detergents.

180C

PROTEOMIC CHARACTERIZATION OF HUMAN PROINFLAMMATORY M1 AND ANTI-INFLAMMATORY M2 MACROPHAGES AND THEIR RESPONSE TO *CANDIDA ALBICANS*.

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Candida albicans is an opportunistic pathogen causing symptomatic infections especially in patients with compromised immune functions. Macrophages are phagocytic cells that play an essential role in the primary response to pathogens, in maintenance of tissue homeostasis, in the promotion and resolution of inflammation, and in tissue repair. Macrophages exhibit a considerable phenotypic diversity and functional plasticity that confer them the ability to efficiently respond to tissue injuries. In general, two main subtypes of polarized macrophages have been defined: M1 and M2. Upon stimulation, M1 macrophages are characterized by a proinflammatory cytokine response, reactive oxygen and nitrogen intermediates generation, promotion of T helper 1 response, and strong microbicidal and tumoricidal activities. By contrast, M2 macrophages are primarily immunosuppressive and exhibit an overall less efficient microbicidal capacity.

The study of human macrophage response to *Candida* is a powerful tool for the analysis of the host response to fungal infections, and should allow the identification of novel antifungal strategies and the mechanisms by which differentially polarized macrophages can contribute to these responses. In the present work, we have analysed for the first time the differential protein expression profile between M1 and M2 macrophages, and determined their differential response to *C. albicans*. The use of 2D-DIGE technology has allowed us to identify 51 differentially expressed proteins between control M1 and M2 macrophages, as well as the identification of 30 proteins whose expression differs between both types of macrophages in response to *C. albicans*. This proteomic approach allowed us to identify metabolic routes and cytoskeletal rearrangement components that are the most relevant differences between M1 and M2 macrophages. The analysis has revealed Fructose 1,6-bisphosphatase (Fbp1), a critical enzyme in gluconeogenesis, up-regulated in M1, as a novel protein marker for macrophage polarization. Regarding the response to *C. albicans*, an M1-to-M2 switch in polarization was observed. This M1-to-M2 switch might contribute to *Candida* pathogenicity by decreasing the generation of specific immune responses, thus enhancing fungal survival and colonization, or instead, may be part of the host attempt to reduce the inflammation and limit the damage of the infection.

181A

ATO5P, A PUTATIVE AMMONIA/ ACETATE TRANSPORTER, MODULATES INTERACTIONS WITH MACROPHAGES BY INHIBITING ALKALINIZATION.

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The innate immune system functions to limit the niches in the human body in which *Candida albicans* can persist and macrophages are vital components of this process. To further understanding of the interactions of *C. albicans* with macrophages a microarray analysis was performed, revealing a transcriptional response very similar to carbon starvation. Part of this

starvation response is an up-regulation genes involved in alternative carbon metabolism, including those involved in amino acid catabolism. *In vitro* experiments have demonstrated that *C. albicans* cells using amino acids as a carbon source very rapidly alkalinize the environment from acidic to neutral pH by excreting ammonia into the media. Our working model is that during phagocytosis cells catabolize amino acids, with the carbon skeleton used to generate cellular energy and the amino groups excreted as ammonia, resulting in a rise in the extracellular pH. This alkalinization process could counteract the acidification of the phagolysosome and promote the transition to the hyphal form that allows for *C. albicans* escape from the macrophage. Comparison of the transcriptional profile of cells during alkalinization to the profile of phagocytosed cells revealed an overlapping set of up-regulated genes, including several members of the poorly understood *ATO* family. This conserved gene family is dramatically expanded in *C. albicans* as compared to *Saccharomyces cerevisiae* and has been implicated as a transporter of both ammonia and acetate in several fungal species. Like other expanded families of cell surface proteins, we hypothesize that the Ato proteins modulate the interaction of *C. albicans* with macrophages and that this significantly contributes to pathogenicity. Deletion of one of the 10 homologs, *ATO5,* results in a delay in environmental alkalinization, a defect in hyphal formation in autoinducing conditions, and a reduction in the amount of ammonia released from the cell. Further, *ato5*Δ strains form fewer hyphae after phagocytosis and have a reduced ability to escape macrophages. These results suggest that *ATO5* is an important mediator of the host-pathogen interaction in this *ex vivo* model system. Our future studies aim to elucidate the molecular function of Ato5p and its role in virulence *in vivo* in a mouse model of disseminated candidiasis.

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THE COPPER SWITCH: RECIPROCAL REGULATION OF TWO METAL CONTAINING SUPEROXIDE DISMUTASES IN CANDIDA ALBICANS

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Superoxide dismutase (SOD) enzymes are a widely conserved class of antioxidant enzymes that detoxify the cell of superoxide radicals. Cu/Zn-Sod1 is known to be a virulence factor in Candida albicans. C. albicans has a unique collection of SODs, including two SODs in the cytosol, a Cu/Zn-Sod1 and a Mn-Sod3. While both of these types of SODs are common to eukaryotes, the co-localization of both to the cytosol is unique, and we are interested in understanding the purpose of having an extra Mn-SOD3 in this compartment. It has been reported that the two cytosolic SODs are alternatively expressed. Cu/Zn-Sod1 is present during logarithmic growth, and a switch occurs to use Mn-Sod3 during stationary growth. But the mechanism and trigger for this switch is unknown. We hypothesize that this switch has evolved to allow C. albicans to adapt to varying nutrient conditions in the different host environments. Our results show that this regulation of the cytosolic SODs occurs at the transcriptional level. The switch is dependent upon intracellular levels of copper, which is the catalytic cofactor for Cu/Zn-Sod1, but is not affected by manganese, the cofactor for Mn-Sod3. This may serve to conserve copper in copper-depleted cells for use as cofactors in other essential enzymes. Metals like copper are abundant in log phase cells, but intracellular copper levels drop by about 50% in stationary cells. Cu/Zn-Sod1 is retained in stationary phase with the addition of copper sulfate to the media, and is repressed in log phase with the addition of the copper chelator BCS. Cu/Zn-Sod1 is favored when the total intracellular copper concentration is above 5-15 µM, whereas Mn-Sod3 is favored then copper levels are below this threshold, indicating that

a very small change in intracellular copper concentration is sufficient to trigger a change. The switch from Cu/Zn-Sod1 to Mn-Sod3 is inhibited in the knock out strain for Mac1, a copper-regulated transcription factor, suggesting that Mac1 regulates this unique switch. While Mac1 is typically known to be a transcriptional activator of copper uptake genes in low copper conditions, it is a repressor in the case of Cu/Zn-Sod1, revealing a novel mode of action for this well-known transcription factor. Our work has produced insights into an apparent mechanism for copper sparing in C. albicans, which may be partly responsible for this pathogen's remarkable ability to adapt to drastically different environments inside the host.

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MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF BETAINE LIPID BIOSYNTHESIS IN CANDIDA ALBICANS

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Betaine lipid or 1,2-diacylglyceryl-3-*O*-4'-(*N,N,N*-trimethyl)-homoserine (DGTS) is a natural occurring ether-linked glycerolipid present in many algae, lower plants and fungi. The biosynthesis of this membrane lipid has previously been studied in various organisms like purple bacterium *Rhodobacter sphaeroides* (1) and green algae *Chlamydomonas reinhardtii* (2) and it has been observed that this non-phosphorous glycerolipid (betaine) replaces phosphotidylcholine in phosphorous starvation. Expression of DGTS synthase (BTA1) in *E. coli* and *S. cerevisiae* which normally lacks this lipid, results in DGTS accumulation. Recently we have found a homologue of algal BTA1 in *Candida albicans*, which also posses a coregulated cluster of genes responsible for the conversion of phosphotidylcholine into DGTS under phosphate deprived condition. DGTS biosynthesis is regulated by PHO regulatory system, which involves PLD1, DPP1, BTA1, SAM2 and PHO84. We elucidate that the same phosphate starvation regulatory system has been observed in pathogenic yeast *C. albicans*, which thereby correlates with its morphology (yeast to hyphae) and pathogenicity (3). In order to confirm the presence of regulatory pathway, we will be contructing homozygous knockout mutants followed by kinetic assays. Furthermore, phosphate limitation in the gut of critically ill patients also triggers the switching of *C. albicans* into an invasive filamentous form (4). The role of BTA1 in the virulence of *C. albicans* thereby affecting pathogenicity will also be explored. These findings may provide new insight to understand novel targets for the development of anti-fungal chemotherapeutic agents. 1. **Riekhof, W. R.**, Andre, C., and Benning, C. (2005). Two enzymes, BtaA and BtaB, are sufficient for betaine lipid biosynthesis in bacteria. Arch Biochem Biophys 441, 96-105. 2. **Riekhof, W. R.**, Sears, B. B., and Benning, C. (2005). Annotation of genes involved in glycerolipid biosynthesis in Chlamydomonas reinhardtii: discovery of the betaine lipid synthase BTA1Cr. Eukaryotic Cell 4, 242-252. 3. Noble, S. N., French, S., Kohn, L. A., Chen, V., and Johnson, A. D. (2010). Systematic screens of a Candida albicans homozygous deletion library decouple morphogenetic switching and pathogenicity. Nature Genetics 42, 590-598. 4. Romanowski K, Zaborin A, Valuckaite V, Rolfes RJ, Babrowski T, et al. (2012) Candida albicans Isolates from the Gut of Critically Ill Patients Respond to Phosphate Limitation by Expressing Filaments and a Lethal Phenotype. PLoS ONE 7(1): e30119. doi:10.1371/journal.pone.0030119.

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CDC28/CLB2 PHOSPHORYLATION OF SPA2 REGULATES CELL POLARITY IN CANDIDA ALBICANS

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The polarisome is a protein complex that was first identified in the budding yeast Saccharo-

myces cerevisiae for its role in regulating polar growth. The ploarisome has since been found in other fungi as well. Conserved components of the polarisome in fungi include orthologues to S. cerevisiae Bni1 or Bnr1, Spa2, and Bud6. Bni1 and Bnr1 are members of formins that nucleate actin filament assembly. Bud6 acts as actin nucleation promoter, and Spa2 is thought to be a scaffold holding the complex together via direct physical interaction with specific domains of other polarisome components. Although the role of formins in the assembly of actin cables has been extensively investigated, little is known about how the scaffold protein Spa2 is regulated during polarized growth. In this study, we used the dimorphic fungus Candida albicans to investigate whether Spa2 is differentially regulated during different modes of growth. We previously demonstrated that spa2Δ/Δ mutants are characterized by severely swollen filaments, indicating defects in polarity control. To further study the underlying molecular mechanisms, we first asked if Spa2 is regulated by phosphorylation. By Western-blot analysis, we found that Spa2 is phosphorylated during both yeast and hyphal growth, and, furthermore, its phosphorylation level is markedly higher in hyphae than in yeast cells, indicating a growth-mode dependent regulation. Spa2 contains a cluster of CDK phosphorylation sites from residues 613 to 1311, and deleting this region greatly reduced Spa2 phosphorylation. We found that Spa2 phosphorylation depends on the two mitotic cyclins Clb2 and Clb4 in a cell-cycle dependent manner. Deletion of the CDK cluster or mutating the CDK sites to alanine resulted in mis-colocalization of Bud6, Bni1 and the Rab GTPase-activating protein (GAP) Msb3 and loss of apical hyphal growth. The same phenomenon was observed when CLB2 expression was switched off. Co-IP experiments showed that the deletion of the CDK cluster on Spa2 or mutating the phosphorylation sites had no effect on Spa2's interaction with Bud6 and Msb3. However, in these mutants, actin filaments were enriched around the septin ring instead of localizing towards the hyphal tip as normally seen in wild-type cells. Hence, our data suggest that Cdc28/Clb2 phosphorylation of Spa2 is crucial to cell polarity control in C. albicans. We hypothesize that the CDK cluster in C. albicans Spa2, which is not conserved in S. cerevisiae, might be an adaptation required for the modification of polarisome functions to promote the kind of highly polarized growth that occurs during the hyphal development.

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ACE2 IS REGULATED BY THE CAMP-PKA PATHWAY AND MULTIPLE TRANSCRIPTION FACTORS DURING MORPHOGENESIS IN *CANDIDA ALBICANS*

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The morphogenetic transitions displayed by *C. albicans* are affected by a wide range of signaling pathways and transcriptional regulators. How these distinct pathways interact to orchestrate morphogenesis is an area of current interest. Previous genetic interaction studies in our laboratory found that the cAMP-PKA and Regulation of Ace2 and Morphogenesis (RAM) pathways interact during morphogenesis. Here, we described experiments designed to understand the mechanism of this interaction. The promoter region of *ACE2*, the transcriptional regulator of the RAM pathway, contains putative Efg1 binding sites, and *ACE2* expression is increased in an *efg1*Δ/Δ mutant, indicating that Efg1 may negatively regulate *ACE2* expression. Efg1 is regulated by both the protein kinase A (PKA) and Cdc28; mutants with decreased PKA activity also show elevated *ACE2* expression, while interference with Cdc28 phosphorylation had no effect. Chromatin immunoprecipitation (ChIP) experiments indicate that Efg1 is present at the *ACE2* promoter at the initiation of morphogenesis but absent at the time when >90% of cells are hyphal. The promoter of *ACE2* contains consensus binding sites for additional transcriptional regulators including Upc2, Brg1, Tec1, Ace2 and Ada2; in addition, Snf5 and

Crz1 have been shown to regulate both Ace2 and Efg1, respectively. Of these regulators, deletion of Tec1 and Brg1 were the only two to show significantly reduced *ACE2* expression (2-fold) during morphogenesis. ChiP experiments showed that both Tec1 and Brg1 are absent from the *ACE2* promoter at the initiation of morphogenesis but present in cultures predominated by hypha, consistent with these regulators have a direct and positive effect on *ACE2* expression. We were unable to detect the presence of Ace2 at its own promoter during morphogenesis, although this does not rule the possibility of auto-regulation. Taken together, our data indicate that the PKA pathway directly represses *ACE2* expression through Efg1; following relief of this repression, Brg1 and Tec1, two important regulators of hyphal gene expression, contribute to expression of Ace2 in hyphal cells. Details of these experiments as well as an integrated model for the regulation of the RAM pathway during morphogenesis incorporating observations of other investigators will be presented.

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MOLECULAR CROSS-SPECIES APPROACHES TO ELUCIDATE VIRULENCE-RELATED TRAITS OF CANDIDA ALBICANS

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Yeasts of the genus Candida are the most common source of nosocomial fungal bloodstream infections. During life-threatening invasive candidiasis, Candida albicans can be isolated in more than 50% of all cases, while C. dubliniensis is only found in 3 % of these patients [1]. Although the two fungi differ strongly in their epidemiological patterns, C. dubliniensis is genetically the closest relative of C. albicans. Both species share a number of morphological and physiological traits, but they can be distinguished phenotypically using selective cultivation media. To determine the genetic basis of these species-specific properties, a genomic library was designed [2]. Randomized C. albicans DNA fragments, sized from five to seven kilobases, were transferred to a wild-type C. dubliniensis strain. In a complementary approach, C. dubliniensis DNA fragments were integrated into a C. albicans recipient strain. Selection of transformants which acquired phenotypic characteristics of the respective genetic donor, especially related to filamentation, chlamydospore formation, or to osmotic and oxidative stress resistance, allowed the discovery of genes which determine the strain specific traits under typical infection-associated conditions. Several genes related to these conditions were identified and we have begun to characterize selected genes in detail. To this end, we have developed a novel differentiation method based on C. dubliniensis-specific chlamydospore and pseudohyphae formation under nitrogen deprivation at 30 °C. Under these settings, C. dubliniensis transformants (with integrated C. albicans genomic fragments) were identified, which showed a strongly reduced chlamydospore and filament production, resembling C. albicans. Reversely, hyphae producing C. albicans colonies from the C. dubliniensis genomic library were detected on the same medium. All candidate genes of the respective genome fragmentes were then separately introduced cross-species to determine which genes are responsible for the species-specific differences between C. dubliensis and C. albicans. More phenotypic and gene-based analyses are currently in progress to obtain new insights into these two important virulence-related pathways: the pathogen's response to nitrogen depletion and the development of chlamydospore and pseudohyphae. Investigating their species-specific regulation in C. dubliniensis and C. albicans will help to elucidate the striking difference in virulence between these two species. [1] Odds F. C. et. al, One year prospective survey of Candida bloodstream infections in Scotland, J Med Microbiol, 2007 [2] Staib P, Morschhäuser J. Differential expression of

the NRG1 repressor controls species-specific regulation of chlamydospore development in Candida albicans and Candida dubliniensis. Mol Microbiol, 2005

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TRANSCRIPTOME ANALYSIS OF CANDIDA ALBICANS HYPHA-TO-YEAST CONVERSION INDUCED BY GYMNEMIC ACIDS.

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Candida albicans is an opportunistic and polymorphic human fungal pathogen. It alternates frequently between yeast and hyphal growth forms, which is required for virulence. Recently, we have identified gymnemic acids (GAs) as inhibitor of C. albicans yeast-to-hypha transition under several hypha-inducing conditions without affecting cell viability. Further, GAs blocked C. albicans hyphal growth and promoted the conversion of hyphae into yeast cells under hypha inducing condition (PLoS One 8(9): e74189, 2013). Based on these observations, we investigated GAs effect on C. albicans biofilm growth 'in vitro' and determined its gene expression profile during GAs-induced conversion of hyphae-to-yeast cells, which has clinical relevance. GAs effect on C. albicans biofilm initiation (for 8 h) or on mature biofilms (16-24 h old) was examined in microtiter wells using RPMI medium at 37o C. For genome-wide transcriptome analysis (Illumina mRNA Seq), four hours old C. albicans hyphae grown in RPMI medium was divided into two groups and one of them was incubated in RPMI medium containing GAs under static growth condition at 37o C for 2, 5 and 11 hours corresponding to early, mid and late stages of hyphae-to-yeast conversions, respectively. The second group of C. albicans hyphae grown in parallel without GAs was served as control. Three biological replicates within each group were used for RNA Seq analysis. Using C. albicans SC5314 genome v-A21 reference sequences, transcriptome was quantified by htseq-count and differentially expressed transcripts between control and treated samples with false discovery rate $\leq$ 0.05 and $\geq$ 2-fold change, determined by GLM method in edgeR, were included for analysis. As expected, GAs exposure to C. albicans blocked its biofilm initiation. Exposure of mature biofilms to GAs for 8 or more hours led to its detachment and dissolution compared to control biofilms. Temporal gene expression analysis of GAs-induced C. albicans hyphae-to-yeast converted cells showed various groups of differentially expressed genes. Generally, the gene expression data agree with GAs inhibitory effect on C. albicans biofilms. Some of the down-regulated groups include genes involved in adhesion, hyphal growth, sugar transporters and uncharacterized genes. The up-regulated genes include glycerol permease, genes involved in cell wall maintenance and uncharacterized genes. Highest numbers of genes were differentially expressed during mid and late stages, and many of them were common between these two stages than the early stage. We conclude that GAs exposure to C. albicans hyphae impact its carbohydrate metabolism and as a consequence cells may reprogram its metabolic process to adapt. This metabolic change could lead to the inhibition of polarized hyphal growth and promoting budding from hyphae. Genetic and biochemical experiments are in progress to verify these conclusions.

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DISTINCT MECHANISMS OF WHITE-OPAQUE SWITCHING REGULATED BY THE HOG1 SAPK PATHWAY AND SHO1 OSMOSENSOR IN CANDIDA ALBICANS

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White-opaque switching is an epigenetic morphological change in the human fungal

pathogen Candida albicans, and its phenotypic switch regulates many properties including biofilm formation, virulence and sexual mating. The switching frequency is highly associated with different stresses, such as oxidative stress, temperature, CO2, and the growth rate. The osmotic response MAPK gene, HOG1, have been known for helping the cell cope with the osmotic and oxidative stress, although unlike Saccharomyce cerevisiae, Sho1, the osmosensor, does not play a central role in activation of Hog1 in C. albicans. In this study, we have identified a novel but well-known pathway, the Hog1 MAPK pathway, which is involved in the regulation of white-opaque switch. Indeed, the frequency of white-to-opaque switching was 100% compared to those of the wild-type (<10-3) in C. albicans a/a cells, but not in a/α cells, indicating that the phenotypic change is also inhibited by the a1/α2 complex. We further described the role of four upstream components (Pbs2 MAPKK, Ssk2 MAPKKK, Ssk1 and Sln1) of the Hog1 pathway, and the osmosensor Sho1, the one that does not mediate Hog1 activation in C. albicans, respectively. As the expectations, deletion of PBS2 and SSK1 in C. albicans a/a cells resulted in 100% phenotypic transition frequencies from white to opaque cells. Inactivation of SSK2 gene caused 41 ± 8% colonies forming opaque cells, while homozygous sln1 and sho1 mutants remain white colonies on the SCD medium with no glucose. Interestingly, the white-to-opaque switching frequencies of ssk1 mutants are much lower than the switching frequencies of ssk2, pbs2 and hog1 mutants, suggesting the existence of alternative pathway regulating the white-opaque switch in SAPK pathway. Additionally, under pheromone treatment, ssk1, ssk2, pbs2 and hog1 mutants exhibited shorter mating projections compared to those of the wild-type strain. Western blotting revealed that the Hog1 is positively regulated by the upstream component, Ssk1, but shows high basal levels of Hog1 phosphorylation in sln1 mutants in both MTLa/a and MTLa/α strains, indicating that the Sln1 plays a negative role in Hog1 activation under normal osmolarity. Although hog1, ssk1, and sho1 mutants are sensitive to high osmotic and oxidative stresses, the Sho1 does not affect the phosphorylated Hog1 activity. Surprisingly, the white-to-opaque switching experiment showed that sho1 mutants displayed lower switching frequency (white-to-opaque: 13 ± 8%) than those of the wild type (36 ± 8%) on Lee's N-acetylglucosamine medium, implicating that an unknown mechanism is involved in this phenotypic change through Sho1 pathway. Taken together, our study has provided two alternative signaling pathways (Hog1 MAPK and Sho1 osmosensing pathways) involved in this unique phenotypic switch in C. albicans and will elucidate how the interaction happens within the Wor1 and these pathways.

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SKN7, A TRANSCRIPTION FACTOR REGULATING MORPHOGENESIS AND OXIDATIVE STRESS RESPONSE

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Candida albicans is a diploid fungus which is part of the normal human flora and it is also one of the most prevalent human fungal pathogens. An important characteristic of C. albicans lies in its ability to undergo a reversible morphogenetic switch between a unicellular yeast form and filamentous forms (either pseudohyphae or hyphae). This switch plays an essential role in virulence. A number of environmental cues has been identified that triggers the yeast-to-hypha transition, such as temperature, pH, CO2 levels, etc. Signal transduction pathways have been identified that link these environmental cues to the activation of so-called hypha specific genes. Skn7 is a transcription factor highly conserved among fungi. Skn7 was originally characterized in Saccharomyces cerevisiae where it is involved in the regulation of cell wall biosynthesis, cell cycle and resistance to oxidative stress. In C. albicans, SKN7 knock-out mutants are

sensitive to oxidative stress, and also exhibit a filamentation defect. We have shown that, conversely, over-expression of SKN7 triggers filamentation in the absence of hypha-inducing cues, both in wild-type cells or in knock-out mutants defective for filamentation. To get further insight into Skn7 regulation of morphogenesis, we have constructed C. albicans strains that express or not an (HA)3-tagged derivative of Skn7 and conducted transcript profiling and ChIP-chip analyses on cells grown under hypha-inducing conditions. These data revealed that Skn7 binds to ca. 100 regions on the C.albicans genome. Strikingly, most of these regions also exhibit binding sites for transcription factors involved in morphogenesis regulation, namely Efg1, Ndt80, Sfl1 and Sfl2. Only 5 promoter regions were bound uniquely by Skn7, 3 of them controlling genes involved in oxidative stress response, while the function of the other 2 is unknown. Our results confirm the involvement of Skn7 in two different pathways, namely morphogenesis and oxidative stress response. In order to clarify the dual function of Skn7, we have initiated a structure-function analysis to identify the role played by each domain of this protein.

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IDENTIFICATION AND CHARACTERIZATION OF GENES INVOLVED IN THE INTERACTION OF CANDIDA ALBICANS WITH ENTEROCYTES

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Candida albicans is a benign member of the microflora in healthy individuals that can cause systemic infections in immunocompromised patients, by gaining access to the bloodstream mainly through translocation across the intestinal barrier. Using an in vitro model of interaction with the enterocytic-like cell line Caco-2, it has been shown that C. albicans is capable of adhering to, invading and damaging intestinal cells, probably through a combination of phenotypic properties including adherence, the yeast to hypha transition and the secretion of lytic enzymes (Dalle et al., Cell. Microbiol. 12:248, 2010). However, the sequence of events together with the Candida genes involved in these processes remain to be clarified. Here we present the screening of 674 C. albicans knock-out (KO) mutants (Noble et al., Nat. Genet. 42:590, 2010) and 579 C. albicans over-expression (OE) mutants (Chauvel et al., PLoS One 7:e45912, 2012) for alteration of infectivity towards enterocytes using the Caco-2 in vitro model. 18 KO mutants and 12 OE mutants showed alteration in their cytotoxicity towards Caco-2 cell monolayers. Not surprisingly, we observed a strong correlation between the ability of these mutants to undergo the yeast-to-hypha transition and their cytotoxicity towards Caco-2 cells. Nevertheless, alteration of morphogenesis could not explain defects in the interaction with Caco-2 cells for a number of C. albicans mutants. Current work is aimed at understanding the basis for such alterations and evaluating whether observations made using the Caco2 model are transposable to in vivo models of disseminated candidiasis originating from the GI tract and will be presented here.

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REGULATION OF UME6 BY CDC28/CDK1 LINKS MORPHOGENESIS TO PROLIFERATION

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The ability to switch between proliferation in a yeast form and development into a hyphal (mold) form is a hallmark of *C. albicans*. The switch to hyphal growth depends on external

inducing conditions, but its efficiency is affected by the internal physiological state of the cell: for example, quiescent, stationary phase cells are induced to form hyphae more readily than proliferating cells. Ume6, a transcription factor that is transcriptionally induced under hyphal-promoting conditions, is both necessary and sufficient for hyphal morphogenesis. We find that Ume6 is post-transcriptionally regulated by the cell cycle kinase Cdc28/Cdk1. Our data suggest that different Cdc28 cyclins reduce Ume6 activity via different mechanisms. One mechanism involves degradation of Ume6 via the activity of the SCF^{CDC4} ubiquitin ligase, a complex that typically requires phosphorylation of the substrate. *HGC1*, the Cdc28 cyclin gene involved in hyphal morphogenesis, is a key transcriptional target of Ume6. We find that Hgc1/Cdk1 is the kinase required for Ume6 degradation in *C. albicans*, resulting in a negative feedback loop between Hgc1 and Ume6. A second mechanism involves Cln3, a G1 cyclin that is essential for cell cycle progression and yeast proliferation. Our data indicate that Cln3 activity is able to antagonize the activity of Ume6 in the heterologous *S. cerevisiae* system, and that Cln3 suppresses the induction of hyphal morphogenesis by Ume6 in *C. albicans*. This observation suggests that Cln3 activity may explain the antagonistic relationship between yeast proliferation and hyphal development in *C. albicans*.

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EPISTATIC ANALYSIS OF CANDIDA ALBICANS YEAST CASEIN KINASE MUTANT STRAIN: IMPLICATIONS OF GENE EXPRESSION PROFILE ON MUTANT MORPHOLOGY AND STRESS RESPONSE

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Candida albicans is a dimorphic, opportunistic fungal pathogen that, while part of the normal mucosal surface microbiota in the general population, is the leading cause of superficial and disseminated oropharyngeal candidiasis in immunocompromised individuals. Morphology transition is a key virulence trait of C. albicans and is tightly regulated in response to environmental signals. Yeast casein kinase 2 (Yck2), a member of the casein kinase 1 (CK1) family of serine/threonine kinases, is known to play a key role in governing morphogenesis, host cell damage, and stress response in C. albicans. The yck2 deletion mutant strain has severe phenotypic defects including slower growth in glucose, cell wall defects, formation of pseudohyphae under yeast growth condition, significantly reduced damage to endothelial and epithelial cells, and increased susceptibility to cell wall stressors compared to wild type and YCK2 complement strains. In order to identify genes that contribute to the observed yck2 mutant phenotype and to select potential downstream genes that may be transcriptionally regulated by Yck2p, we used qRT-PCR to compare the transcript levels of 16 key genes in the yck2 mutant relative to WT and YCK2 complemented strains in log phage yeast growth condition. We found that there was increased expression of UME6 (70-fold increase compared to WT), likely due to the pseudohyphal growth of the yck2 mutant. This mutant strain also had increased expression of the glucose transporter/sensing genes including RGT2, HGT7 and HXT10 (2.5, 2.2, and 2.4 fold increased compared to WT), potentially as a compensatory response. In addition, the yck2 mutant had reduced expression of the cell integrity genes, HOG1 and MKC1 (2.5 and 4-fold decrease compared to WT), consistent with observed reduced resistance of the mutant to cell wall stressors. Although the yck2 mutant is hypersensitive to antimicrobial peptides, there was no significant change in the mRNA levels of SSD1, which plays a key role in governing antimicrobial peptide susceptibility. Thus, Yck2p likely influences antimicrobial peptide susceptibility by a different pathway than Ssd1p. This study suggests that Yck2p is involved in hyphal transition, glucose transport, and stress responses by regulating

the transcript level of key genes in each functional category.

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BORIC ACID STRESS SUPPRESSES *C. ALBICANS* HYPHAL GROWTH

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The ability to switch from yeast to hyphal growth has been recognized as a major virulence factor in *C. albicans*. Hyphal growth is induced in response to environmental stimuli through the action of at least four signal transduction pathways (Rak1, PAK, PKA and Rim101) by transcriptional activators (Czf1, Cph1, Tec1, Efg1 and Flo8) that stimulate the expression of hyphal genes. During yeast growth and in response to the quorum-signaling compound farnesol, the expression of hyphal genes is inhibited by specific transcriptional repressors (Tup1, Nrg1, Rfg1 and Rbf1). The broad-spectrum anti-biological agent boric acid (BA) specifically inhibits the formation of *C. albicans* hyphae while leaving yeast growth largely unaffected. *We hypothesize that BA has a more severe inhibitory effect on cells undergoing highly polarized growth than on yeast cells displaying isotropic expansion*. A collection of homozygous *C. albicans* deletion strains was screened for mutants with abnormal BA tolerance. It was found that mutants with a constitutive or hyper-filamentous growth phenotype (*tup1/tup1, nrg1/nrg1, sko1/sko1* and *rbf1/rbf1*) showed BA sensitivity whereas mutants with impaired hyphal growth (*efg1/efg1, rim101/rim101* and *rim13/rim13*) were BA-resistant - suggesting that hyphal growth sensitizes mutants to the effects of BA. A gene-expression array analysis was carried out to characterize the genomic response to BA stress. A gene-ontology analysis of induced and repressed genes (GO process category) revealed that cultures grown in presence of BA show an increase in expression of genes required for glycolytic energy generation and host defense. It could also be shown that BA represses the transcription of genes whose products are found in the hyphal cell wall (GO component category), suggesting that BA induces a generic stress response and directly or indirectly represses the transcription of hyphal cell wall genes. An analysis of the expression of the drug-exporting ATPase Cdr1 revealed that, while BA does lead to an induction in stress gene transcription, the subsequent increase in protein amount occurs with a significant delay reflecting a general inhibition of protein translation. We conclude that in *C. albicans* the effects of BA on metabolism, gene expression and protein translation combine to inhibit the polarized growth of hyphal cells more strongly than the isotropic growth of yeast.

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C. ALBICANS YEAST BUT NOT HYPHAE NEED THE PESCADILLO HOMOLOG TO COORDINATELY PROMOTE PROLIFERATION AND GROWTH IN RESPONSE TO TOR

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The ability to switch between unicellular yeast and filamentous hyphae is required for virulence of the human fungal pathogen *C. albicans*. We isolated the *C. albicans pescadillo* homolog *PES1* as a gene required for the hypha-to-yeast switch. Pescadillo is essential in all organisms studied, and *PES1* is essential in *C. albicans* yeast, but hyphae grow robustly during its depletion. *PES1* is required by *C. albicans* yeast, but not hyphae, to respond to Tor inhibition by rapamycin, as is its homolog in *S. cerevisiae*. Pes1 RNA and protein levels are controlled by TOR, and its localization responds to TOR signaling differentially in yeast and hyphae. Inactivation of a Pes1 temperature sensitive allele drastically delays the G1 to S transition of the cell cycle. This is likely to be a direct effect, because Pes1 co-immunopre-

cipitates with Rfc3, a component of replication factor C, required for loading of the replication machinery onto DNA during S phase. Inactivation of Pes1-ts also leads to rapid loss of phosphorylation of ribosomal protein S6, a marker of translational activity, as does starvation, pharmacologic Tor1 inhibition and genetic perturbation of Tor1 activation. Proliferation and growth must be coordinately regulated in most eukaryotic cells, and our data suggest that Pes1 acts downstream of Tor1 in coordinating these processes in *C. albicans* yeast. Identifying the mechanism by which hyphae escape the requirement for *PES1* is expected to shed light on alternative mechanisms of cell cycle control available to *C. albicans* hyphae and perhaps to specific cell types in the animal kingdom.

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CHARACTERIZATION OF THE FUNCTION AND PREVALENCE OF A NOVEL TUP1 MUTANT ALLELE

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The capacity of the opportunistic fungal pathogen Candida albicans to cause disease is linked to its ability to change between different cellular morphologies, including yeast, hyphae, and pseudohyphae. Strains that are unable to switch between these forms are attenuated for virulence in the murine model of haematogenously disseminated candidiasis. The transcriptional repressor encoded by TUP1 is a key regulator of morphogenic transitions. Strains lacking TUP1 are constitutively pseudohyphal, and are unable to form yeast or true hyphae, even under appropriate environmental stimuli. We recently discovered a novel polymorphism in TUP1. This single base pair deletion causes a frameshift mutation, premature stop codons and a substantially shortened open reading frame. We sought to examine the distribution and effects of this mutation. We used RFLP analysis to probe several clinical isolates and SC5314 from numerous different sources for the presence of this mutation. So far we have only found the mutant allele in one SC5314. However, several of the clinical isolates showed restriction patterns matching neither the wild-type, nor the mutant allele, suggesting possible chromosome rearrangements in those strains compared to the SC5314 used in the genome sequencing project. We are continuing to expand this analysis to include other isolates and other wild-type strains. The truncated mutant open reading frame lacks domains thought to be important for interaction with Tup1p binding partners. We therefore predicted that the mutant allele might be defective for morphological regulation. To test this, we constructed a strain in which both endogenous alleles of TUP1 were deleted and an ectopic, regulatable copy of the mutant allele was introduced. When the mutant allele is the sole source of TUP1 expression, under yeast growth conditions this strain grows as pseudohyphae in liquid media, and forms wrinkled colonies indicative of filamentous growth on solid media. In hypha-inducing media such as Spider medium, the strain fails to form true hyphae, but instead grows as pseudohyphae. Our results therefore suggest that the protein encoded by this novel mutant allele of TUP1 is unable to support wild-type C. albicans morphological transitions.

196A

QUANTITATIVE PROTEOMIC ANALYSIS OF PROTEIN PHOSPHORYLATION AT MULTIPLE TIME POINTS OF THE FILAMENTATION PROCESS

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Candidiasis represents the fourth most frequent nosocomial infection in the US and worldwide. These infections carry unacceptably high morbidity, mortality rates and important economic repercussions. C. albicans can grow as yeast cells, pseudohyphae, hyphae or within

a biofilm. The ability to form hyphae and biofilms have been fundamentally linked to the disease causing potential of this organism and way it grows is intimately related to the how it senses and reacts to its surrounding environment. Over the last decade the actual reactions to environmental cues have received significant attention with less effort focused on how the organism is sensing a particular signal and modulating its response. Here we document the use of an in vitro model of filament induction in conjunction with Stable Isotope Labeling with Amino acids in Cell culture (SILAC) to examine protein phosphorylation at multiple time points during early filamentation. We analyzed differences in a phoshorylated protein fraction isolated by IMAC from a cytosolic protein extract. The stable isotope label enabled the quantification of differences in proteins identified following GeLC MS/MS and data searches. Data analysis was carried out with Mascot Distiller. We analyzed several time points associated with key changes early in the filamentation process, namely 30mins, 1 hour and 3 hours post induction of filamentation. For each time point in this analysis set we have identified over 500 proteins with high quality quantitative data. Early filamentation appears to involve significant changes in phosphorylation of proteins involved with amino acid starvation, the core stress response, iron homeostasis and several uncharacterized kinases and exchange factors. Identified proteins also included known hyphal specific proteins and regulators. All the proteins identified in a previous gel-based analysis of phosphoprotein changes during filamentation were validated, demonstrating the robustness of this approach. Here we present data for a subset of the proteins known to be associated with filamentation. The method offers significant insight into the important process of filament formation and we are in the process of developing this approach to study Candida-microbe interactions.

197B

OVERRIDING THE REPRESSION OF FILAMENTATION BY NRG1 USING CDC55

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Candidiasis is a frequent cause of nosocomial infection both in the US and worldwide. The most common cause of candidiasis is Candida albicans, the pathogenic potential of which is intimately related to certain key processes, including filamentation. In Saccharomyces cerevisiae Tap42 and the associated Phosphatase 2A (PP2A) play a role in several downstream effects of TOR signaling. In C. albicans, proteomic but not microarray analysis has implicated the involvement in filamentation of a component of the PP2A complex, 19.1468 (CaCDC55) which encodes a protein similar to Cdc55p, one of two regulatory subunits of the PP2A complex in S. cerevisiae. CDC55 also appears to be associated with filamentation in S. cerevisiae and a strain lacking CDC55 is defective for filamentous growth. A second putative component of the PP2A complex 19.7504, which is predicted to encode a protein similar to S. cerevisiae Rts3, has been shown to be repressed by Nrg1, a key regulator of filamentation in C. albicans. Here we demonstrate that over-expression of CaCDC55 is sufficient to override the repression of filamentation mediated by NRG1 over-expression and in fact restores filamentation in numerous filament-inducing conditions including growth in liquid media and on solid media at 37C and under embedded conditions at 28C. In comparison, the absence of 19.7504 partially restores the ability to filament during NRG1 over-expression under embedded growth conditions, but not under numerous other filament-inducing conditions; its overexpression appears to have little effect. Whilst the deletion of S. cerevisiae RTS3 has been shown to cause rapamycin and caffeine sensitivity, no such phenotype results from the deletion of C. albicans 19.7504. We

also use quantitative real-time PCR analysis to demonstrate the influence of the products of CDC55 and 19.7504 on the Nrg1 repressed filamentation-associated genes during filament inducing conditions. These results suggest that the PP2A complex is involved in both promoting and repressing filamentation, depending on the environmental conditions. In addition they demonstrate the importance of CDC55 in the control of the filamentation process in C. albicans. Furthermore, they suggest that the product of 19.7504 is functioning in a manner distinct from that of Rts3 in S. cerevisiae.

198C

PREVALENCE AND ANTIFUNGAL SUSCEPTIBILITY PATTERNS OF CANDIDA ISOLATES: A STUDY IN MILAD HOSPITAL INTENSIVE CARE UNIT, TEHRAN, IRAN.

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Introduction and objectives: The occurrence of Candida infections has improved during the past two decades as a result of increase in the number of immunocompromised patients. Candida albicans is the most common species causes invasive fungal infection (IFI). However recently there has been an epidemiological shift to non-albicans candidal infections, which are generally prevailed in immunocompromised patients. Candida species other than C. albicans have different antifungal susceptibility patterns and their frequency in the intensive care unit (ICU) may diverge in different parts of the world. In this study the prevalence of Candida species isolated from sterile body sites of patients admitted to Milad hospital ICU during 6 months and their resistance to the antifungal agents contain caspofungin, posoconazole , voriconazole & amphotericin B were determined by using the agar-based E-test method. **Materials and methods:** Candidal isolates were obtained from 50 patients admitted to Tehran Milad hospital ICU from April to September 2012. Specimens were collected from sterile sites of the body. Identification of the isolates was performed by using morphological assay and polymerase chain reaction (PCR). Resistance to the antifungal agents contains caspofungin, posoconazole, voriconazole & amphotericin B was determined via E-test method following NCCLS recommendations. **Results:** Of the 67 candidal isolates, 32 (47.8%) were C. glabrata, 19 (28.3%) were C. albicans, 5 (7.5%) were C. tropicalis and 2 (3%) were C. krusei. Findings show that all of the C.albicans and C.tropicalis strains were susceptible to caspofungin, posoconazole, voriconazole & amphotericin B. C.glabrata was the least susceptible species, with 9.4% of the isolates resistant to amphotericin B and 6.3% resistant to posoconazole and voriconazole. No resistance to caspofungin was observed amongst C.glabrata isolates. One of the C.krusei isolates was resistant to amphotericin B while no resistace to voriconazole, caspofungin and posoconazole was detected among C.krusei strains. **Conclusion:** Increase in the prevalence of antifungal resistant non- C.albicans species in recent years has become a problematic event amongst clinicians caring for ICU patients. C.glabrata as the most common species isolated from ICU patients in this study (47.8%) indicated higher levels of antifungal resistace in comparison with other species. It's noticeable that a shift to non-albicans Candida species, mainly C. glabrata among ICU patients increases the level of Candida strains resistance to the antifungal agents and can become an important problem for clinicians. This event accentuates the importance of managing preventive treatments to avoid development of resistance to the current antifungal drugs. **Key words:** antifungal

susceptibility, ICU, Candida non-albicans species, E-test

199A

IN VITRO EFFECT OF MEDICINAL PLANT EXTRACTS AGAINST HUMAN FUNGAL PATHOGEN CANDIDA ALBICANS

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Candidiasis is one of the most common forms of mycoses caused by Candida spp. especially Candida albicans. Candida is a dimorphic, opportunistic but otherwise commensal organism that turns pathogenic to cause serious infections that ranges from superficial mycoses to life threatening systemic infections as commonly seen in immunocompromised patients. The prevalence of AIDS, malignancies, rising numbers of elderly individuals, invasive surgical interventions and perhaps paradoxically, the progress and sophistication of medical treatments have all contributed to the rising toll of morbidity and mortality caused by candidiasis. Widespread and prolonged usage of antifungal has rapidly led to the increasing incidence of drug resistance which emerges as a threat to the antifungal therapy and therefore there is an urgent need for novel therapies against this pathogen. Medicinal plants represent a rich source of antimicrobial agents. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine. These plant derivatives may be used as adjunct medicine or in combination with already known drugs to increase the efficacy by their synergistic activity. The present project was hence initiated which employed the use of seven known medicinal plants and their anti-fungal activity was checked against Candida albicans. Three of these plant natural products indeed have shown antifungal activity especially, Piper longum or Pippali, Aloe vera and Ashwagandha- Withania somnifera. Drug susceptibility testing was done using three different methodologies, namely toxicological end point determination by MIC, spot assay and filter disc diffusion assay. Growth Inhibition studies further substantiated the results. GCMS-spectra results of the plant extracts recorded peaks as per the already known composition. Findings in this study are preliminary verification of usefulness of medicinal plants as potential antifungal agents. These might help us to develop novel therapeutic strategies and identify new antifungal drug targets for MDR reversal. The project has been funded by grants under University of Delhi- Innovation Project Scheme 2012-2013 (RC101). Key words: mycoses, commensal, secondary metabolites, antifungal

200B

REPURPOSING AS A MEANS TO INCREASE THE ACTIVITY OF AMPHOTERICIN B AND CASPOFUNGIN AGAINST *CANDIDA ALBICANS* BIOFILMS

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Biofilms of *Candida* species are often formed on medical devices and are resistant to currently available antifungal drugs. The aim of this study was to identify potentiators of amphotericin B (AmB) and caspofungin (CAS), the most commonly used antifungals, against *Candida* biofilms. **Methods:** A library containing off-patent drugs was screened for potentiators that increase the in vitro activity of AmB against *C. albicans* biofilms. Biofilms were grown in 96-well plates and growth was determined by the cell titre blue (CTB) assay. Synergy between identified potentiators and the antifungals was further characterized in vitro using FICI values and in vivo using a worm biofilm infection model. In light of application of the compounds on implants,

the effect of the compounds on the growth potential of MG63 osteoblasts was assessed. **Results:** Pre-incubation of *C. albicans* biofilms with subinhibitory concentrations of the potentiators drospirenone, perhexiline maleate or toremiphene citrate significantly increased the activity of AmB against *C. albicans* and *C. glabrata* biofilms. Moreover, the potentiators act synergistically with CAS against *C. albicans* and *C. glabrata* biofilms (FICI < 0.5), while they do not affect growth potential of osteoblasts. Toremiphene citrate was selected to translate these in vitro findings to various in vivo *C. albicans* biofilm infection models. We demonstrate synergy between toremiphene citrate and CAS in curing the infection in these models. **Conclusions:** Our data demonstrate in vitro and in vivo potentiation of the antibiofilm activity of CAS by toremiphene citrate. Furthermore, our results pave the way for implant-related applications of the identified potentiators.

201C

CALCINEURIN CONTROLS HYPHAL GROWTH, VIRULENCE AND DRUG TOLERANCE OF *CANDIDA TROPICALIS*

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Candida tropicalis, a closely related species of *C. albicans*, is an emerging fungal pathogen associated with high mortality rates of 40 to 70%. Like *C. albicans* and *C. dubliniensis*, *C. tropicalis* is able to form germ tubes and hyphae, but genes involved in hyphal growth machinery and virulence remain unclear in *C. tropicalis*. Recently, echinocandin- and azole-resistant *C. tropicalis* isolates have been frequently isolated from various patients around the world, making treatment difficult. However, the studies of *C. tropicalis* genes involved in drug tolerance are limited. Here, we investigate the roles of calcineurin and its potential target Crz1 for core stress responses and pathogenesis in *C. tropicalis*. We demonstrate that calcineurin and Crz1 are required for hyphal growth and virulence in a murine systemic infection model, while calcineurin but not Crz1 is essential for drug tolerance in *C. tropicalis*.

202A

VACCINATION WITH RECOMBINANT NON-TRANSMEMBRANE DOMAIN OF PROTEIN MANNOSYLTRANSFERASE 4 IMPROVES SURVIVAL DURING MURINE DISSEMINATED CANDIDIASIS

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Candida albicans is the most common cause of invasive fungal infections in humans. Because of the rising incidence of life-threatening candidiasis and high failure rates of treatment, it is highly desirable to identify new vaccine targets for prevention and intervention. Different antigenic targets of C. albicans have been investigated to generate immunological tools to combat Candida infections. In our study, several candidate proteins have been cloned, expressed, purified and verified by Western blotting. Their potential use of vaccination in preventing systemic candidiasis in mice has been assessed. Ten BALB/c mice were immunized by subcutaneous injection of 20 μg of purified recombinant proteins mixed with complete Freund's adjuvant at day 0, boosted with 10 μg of proteins in incomplete Freund's adjuvant at day 21, and then infected via the tail vein with the 1×106 cells C. albicans SC5314 at day 35. Among them, the recombinant protein mannosyltransferase 4 (Pmt4p) resulted in efficient immune protection. The rPmt4p vaccination provided significant improvement in survival with 54.5% survival

rate at day 15 post-infection, decreased fungal burdens in the heart, liver, brain, and kidneys at day 5 post-infection, and increased serum levels of both IgG and IgM against both the rPmt4 protein and C. albicans live cells in the immunized mice at day 35. Histopathological assessment demonstrated that rPmt4p protected the tissue structure, and decreased the infiltration of inflammatory cells. The immune serum enhanced mouse neutrophils-mediated killing activity by directly neutralizing rPmt4p effects in vitro. Levels of IL-4, IL-10, IL-12p70, IL-17A and TNF-α in serum were higher in the immunized mice compared to those in the adjuvant control group. Moreover, the anti-rPmt4p serum enhanced the inhibition of fluconazole against C. albicans SC5314 by 64.5%. These data indicated that the rPmt4p vaccine conferred significant protection against experimental disseminated candidiasis by inducing effective killing of phagocytosed C. albicans in neutrophils, and production of protective antibodies IgG/IgM and Th1/Th2/Th17 cytokines, which support the idea that rPmt4p can stimulate both cell-mediated and humoral immunity. In conclusion, our results suggested that rPmt4p may be considered as a potential vaccine candidate against systemic candidiasis.

203B

IN VITRO ANTIDERMATOPHYTIC ACTIVITY OF THE LEAF ESSENTIAL OIL OF PIPER BETLE (PHILIPPINE VARIETY)

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Dermatophytes have become resistant to antifungal drug and the demand for new alternatives are on the rise. This study focuses on the antidermatophytic activity of essential oil coming from Piper betel, Philippine variety. The Betel oil, PbEO, from the leaves of Ikmo (Piper betel) was extracted by hydrodistillation. Freshly extracted PbEO was yellow in color and lighter than water with a percentage yield of 0.2% w/v. The minimum inhibitory concentration (MIC) of the oil was determined using Agar Dilution method supplemented with Tween 20 for good diffusibility. Identification of skin fungal isolates was performed using the Riddle method. Gross colony characteristics, microscopic morphology, micro- and macroconidia were identified and documented. Fluconazole (FLZ) was used as antifungal control drug of choice. The essential oil was found to have significant antifungal activity against dermatophytes and Candida albicans. Growth of control strain C. albicans ATCC 14053 was inhibited at 0.08% betel oil while filamentous dermatophytes, at a range of <0.01% to 0.16%. Fluconazole inhibited the growth of the fungal isolates at higher MICs. Thus, PbEO could provide a new breakthrough in the advancement of antifungal science in the field of medicine.

204C

ANTIFUNGAL ACTIVITY OF PITCHER LIQUID FROM THE CARNIVOROUS PLANT NEPENTHES RAFFLESIANA TO CANDIDA SPP.

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Introduction: Immunocompromised conditions have resulted in increasing number of fungal infection. *Candida spp*. are the most common fungal pathogen. Antifungal chemotherapy is in constant need of new and effective compounds due to the variable efficacy and adverse effect of the drugs in current use. Chitin-induced liquid pitcher of carnivorous plant *Nepenthes khasiana* have considered of having antifungal activity, because of containing naphthoquinones. *N. khasiana* was commonly found in India. Indonesia, as tropical country, have many species of Nepenthes. Distribution Nepenthes predominantly is in Indonesia. One of them is *Nepenthes rafflesiana*. This study aimed to detect antifungal activity of pitcher liquid from *N. rafflesiana*

to *Candida spp*. **Method:** There were 2 types of collected pitcher liquid, non-induced liquid (NIL) and chitin-induced liquid (CIL). NIL was collected from fresh naturally opened pitcher and CIL was collected from closed pitchers after 5 days of 2 ml chitin solution injection. Chitin solution was prepared from dried prawn exoskeleton. The antifungal activities from both of them to *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis* were detected by disc diffusion method. The used medium was Mueller Hinton agar adding with glucosa 2% and metilen blue dye 0.5mg/L. The 5 mm sterile discs were made from Whatman filter paper No. 42. The sterile disc soaked in aquabidest and placed on the seeded medium was used as a control. The test was performed in triplicate. The inhibition zone diameters were recorded. **Result:** The colour of NIL was white cloudy. On chitin induction, the colour of pitcher liquid changed to orange red. Acidity of NIL was 3.1 and CIL was 2.6. All samples were slimy. Diameter of inhibition zones appeared in testing of NIL and CIL to *C. albicans* (36.44 ± 2.49 mm and 30.00 ± 2.00, respectively) and *C. glabrata* (22.55 ± 3.53 and 28.89 ± 1.17, respectively). There were significant differences ($p < 0.05$) between NIL and CIL to both of fungi. No inhibition zones were found in detection NIL and CIL to *C. krusei* and *C. tropicalis*. **Conclusion:** NIL and CIL of *N. rafflesiana* have antifungal activity to *C. albicans* and *C. glabrata*, but no antifungal activity to *C. krusei* and *C. tropicalis*. The antifungal activity of NIL was better than CIL.

205A

CELECOXIB ANALOGS AS ANTIFUNGAL SMALL MOLECULES: IN VITRO ACTIVITY, IN VIVO EFFICACY AND MECHANISM OF ACTION

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Invasive fungal infections are an important cause of morbidity and mortality for people living with diseases and treatments that compromise immune function. Although advances in other fields of medicine have increased the number of people at risk for invasive fungal infections, the pace of new antifungal drug development has been very slow. The echinocandins, the newest class of antifungal drugs, were discovered over 30 years ago and have been in clinical practice for over ten years. Recently, we found that OSU-03012, a pyrazole-based molecule related to the COX2 inhibitor celecoxib, has good antifungal activity against *C. albicans* and *C. neoformans* (MIC 4 µg/mL). OSU-03012 is a multi-kinase inhibitor with reported activity against PDK1 and PAK kinases; it has also entered phase I clinical trials as a targeted anti-cancer therapy. OSU-03012 has additive activity in combination with fluconazole towards *C. albicans* and is synergistic (FIC and time-kill) toward *C. neoformans*. In a mouse model of disseminated candidiasis, OSU-03012 reduced kidney fungal burden ~1 $\log_{10}$ cfu/g relative to untreated controls. In a mouse model of cryptococcosis, OSU-03012 was inactive alone but synergized with fluconazole to reduce brain burdens by 1 $\log_{10}$ cfu/g relative to fluconazole alone. To improve the activity of this promising scaffold, we have performed a structure-activity study focused on two regions of the molecule. In addition, we have begun studies to identify the fungal target of OSU-03012; initial studies indicate that it is unlikely to inhibit Pkh kinases, the fungal orthologs of PDK1. The details of these studies as well as progress toward identification of the mechanism of action of this new class of antifungal molecules will be presented.

206B

FUNGICIDAL EFFECT OF HEPCIDIN 20 VERSUS CANDIDA GLABRATA IN HUMAN VAGINAL FLUID

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Vaginal candidiasis due to *Candida glabrata* is difficult to eradicate as a consequence of the intrinsically low susceptibility to azoles of this species. The human cationic peptide hepcidin 20 (Hep-20) has been considered a potential candidate for the treatment of vaginal candidiasis in view of i) its in vitro fungicidal activity in sodium phosphate buffer (SPB) against *C. glabrata* clinical isolates with different susceptibility to fluconazole and ii) its potentiated antifungal activity in SPB under acidic condition.

To further investigate the potential application of Hep20 in the topical treatment of *C. glabrata* vaginitis, the peptide activity versus *C. glabrata* was evaluated by a killing assay (i) in a vaginal fluid simulant (VFS) and (ii) in human vaginal fluid (HVF) collected from three healthy donors. The results obtained indicated that the activity of the peptide was maintained in VFS and HVF supplemented with EDTA. Interestingly, the fungicidal activity of Hep-20 was enhanced in HVF compared to that observed in VFS, with a minimal fungicidal concentration of 25 μM for all donors. No cytotoxic effect on human (or human derived) cells was exerted by Hep-20 at concentrations ranging from 6.25 to 100 μM, as shown by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide tetrazolium salt (XTT) reduction assay and propidium iodide staining. An indirect evidence of Hep-20 stability was also obtained from co-incubation experiments of the peptide with HVF at 37°C for 90 minutes and 24 hours. Collectively, these findings indicate that this peptide should be further studied as a potential novel therapeutic agent for the topical treatment of vaginal *C. glabrata* infections.

207C

SIGNIFICANT REDUCTION OF GROWTH, BIOFILM FORMATION AND MUTAGENICITY OF CANDIDA ALBICANS BY D,L-2-HYDROXYISOCAPROIC ACID (HICA)

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Introduction: The ability of C. albicans to form biofilms is a major virulence factor and a challenge for management. In addition, biofilm-associated chronic oral-oesophageal candidosis has been shown to be potentially carcinogenic in vivo. We have previously shown that most Candida spp. are able to produce significant levels of mutagenic acetaldehyde (ACH) as part of their ethanol metabolism. ACH is also an important mediator of candidal biofilm formation. HICA is a α-hydroxy-aminoacid and leucine metabolite, which is produced by Lactobacillus species and also found in human muscle and connective tissues. Previous studies from our group show that it significantly inhibits the growth of a spectrum of bacteria and fungi grown planktonically. Our preliminary results suggest that it is also active against candidal biofilms. Antifungal resistance and mutagenicity of these biofilms are significant clinical problems and an agent with activity against Candida biofilms would be an important tool of infection. **Aims:** To investigate the effect of HICA on C. albicans biofilm formation and ACH production in vitro. **Hypothesis:** C. albicans cell growth, biofilm formation and mutagenic potential are significantly reduced by HICA. **Methods:** C. albicans SC5314 biofilms were formed on Thermanox coverslips in 24-well plates. Biofilms were grown for 4h (early), 24h (mature), 48h (old) before 24h treatment with 5% (w/v) HICA or L-cysteine, L-leucine or 0.05% ethanol at pH 5.2 and 7.4 at 37oC. Ca-

spofungin (10 mg/L) was used as a comparator and RPMI and PBS were used as controls. XTT was used to measure biofilm metabolic activity and PicoGreen as a marker of biomass and the biofilms were visualised by scanning electron microscopy (SEM). ACH levels were measured by gas chromatography after 30 min incubation with either 11 mM ethanol, 100 mM D-glucose or PBS (control). Transcriptional changes in ethanol metabolism were measured using qPCR. **Essential results:** The mean metabolic activity and biomass of the biofilms were significantly reduced by HICA at all developmental stages. The reduction was statistically significant at both pHs ($p<0.001$ for both) although more striking at pH 5.2 (71% and 62%, respectively). Caspofungin was only active against early biofilms at neutral pH. ACH production by mature biofilms was also significantly decreased by HICA ($p<0.05$) in contrast to increased production by caspofungin. Expression of genes responsible of ACH catabolism was upregulated by HICA but downregulated by caspofungin. SEM images show aberrant filaments and collapsing hyphal structures in response to HICA at acidic pH. **Conclusion:** HICA has potential as an antifungal agent with ability to inhibit C. albicans cell growth and biofilm formation. HICA also significantly reduces the mutagenic potential of C. albicans biofilms, which may be of importance when treating bacterial-fungal biofilm infections.

208A

EARLY PREDICTION OF INVASIVE FUNGAL INFECTIONS BY β-D-GLUCAN ASSAY, RISK FACTOR ASSESSMENT AND ANTIFUNGAL SUSCEPTIBILITY PATTERNS IN PATIENTS WITH ACUTE PANCREATITIS

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Background: Invasive fungal infections (IFIs) have been increasingly recognized in patients with acute pancreatitis and are associated with significant morbidity and mortality. Successful management require early and effective diagnosis with appropriate antifungal therapy. **Aim of the study:** To assess the diagnostic accuracy of β-D-glucan assay (BDG) for early detection of IFIs in acute pancreatitis patients with coexisting risk factor analysis. Phenotypic identification of clinical fungal isolates and determination of antifungal susceptibility patterns for better outcome and management. **Methods:** Ninety four clinical fungal isolates (blood, pancreatic and peripancreatic fluid, necrotic pancreatic tissue, urine, ascitic fluid and bile) were obtained and phenotypic identification was done as per conventional mycological procedures. Antifungal susceptibility testing was performed for Fluconazole, Amphotericin B, Caspofungin, Micafungin and Posaconazole by Broth Microdilution method following CLSI guidelines M27-A3. E-Test was put for Anidulafungin and Disk-diffusion method was done for Voriconazole following CLSI M44-A. Eighty four sera samples from proven (28), probable (28) and possible (28) cases of IFIs (EORTC/MSG guidelines) were subjected to BDG assay following manufacturer instructions. **Results:** Out of 90 patients with acute pancreatitis, 53 (58.8%) patients had fungal infection (≥1 site) of which 8 (8.8%) patients had bloodstream infections. These patients with fungal infections had evidence of prolonged hospital stay > 4 weeks ($P = 0.002$), Central Venous Access ($P = 0.002$) and prophylactic usage of Broad Spectrum Antibiotics ($P = 0.001$) in contrast to those without fungal infection. The commonest species isolated among these ninety four isolates from fifty three patients was Candida tropicalis (36.1%). Antifungal susceptibility patterns revealed 85.14%, 92.5%, 93.6% of the Candida spp. isolates were sensitive to Fluconazole, Amphotericin B and Voriconazole respectively. All isolates were sensitive to Caspofungin, Micafungin, Anidulafungin and Posaconazole. The diagnostic performance of serum BDG

assay from categorised cases revealed that, at a cutoff of 60 pg/mL, the sensitivity and specificity of the assay were 73.3% and 84.2%, respectively, with a positive predictive value of 78.5% and a negative predictive value of 80%. **Conclusion:** The incidence of fungal infections was found to be high (>50%) in our acute pancreatitis patients. The most common risk factors associated with it were prophylactic antibiotic therapy, prolonged hospital stay and central venous access. This imparts the need of detection of early markers such as β-D-glucan in our diagnostic settings and appropriate antifungal therapy for the better management of the patients.

209B

DISCOVERY OF NATURAL PRODUCTS THAT ENHANCE CASPOFUNGIN ACTIVITY BY ALTERING THE FUNGAL CELL WALL INTEGRITY PATHWAY

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Background: Echinocandin antifungal drugs such as caspofungin (CPF), although potent and well-tolerated, have a narrow spectrum of activity and drug resistance is a growing problem. New therapies are needed that can overcome these limitations. Our approach seeks to improve CPF activity by altering the fungal cell wall integrity pathway (CWIP), which is involved in the adaptation to cell wall stress exerted by this drug. Since the CWIP is regulated by the transcription factor Rlm1 in the model yeast *Saccharomyces cerevisiae*, signaling through this pathway can be monitored with a *lacZ* reporter driven by Rlm1-responsive promoter elements. In this study, using this promoter-reporter assay in a high throughput format, we have identified compounds that alter the CWIP and improve CPF potency in the fungal pathogens *Candida albicans* and/or *Cryptococcus neoformans*. **Methods:** For the high throughput screen, yeast cells carrying the promoter-reporter plasmid were grown in microplates to early log phase, and exposed to either CPF alone or CPF + test compounds. After a 4 h incubation to activate the CWIP, beta galactosidase (beta-gal) activity was measured using the Yeast Beta-Gal Assay Kit. Compounds that altered (either inhibited or induced) CPF-mediated beta-gal activity, by at least 2-fold, were considered hits. Hit compounds were evaluated by checkerboard assays to examine CPF activity enhancement in fungal pathogens. To further investigate one of the CPF-enhancing compounds, transcript profiling was conducted by exposing *S. cerevisiae* cells to compound alone, or CPF alone, or compound + CPF. **Results:** A screen of 880 compounds from our in-house natural products collection identified 43 CWIP inhibitors and 16 CWIP inducers. Among the CWIP inhibitors, 3 classes of compounds were identified (e.g., sesquiterpene quinone, aminoquinoline, and triterpenoid glycoside) that exhibited synergistic or additive activity with CPF in *C. albicans*. The sesquiterpene quinone compound, puupehenone (PUUP) also demonstrated additive and fungicidal activity in a CPF-resistant clinical isolate of *C. albicans*. In the CPF-insensitive pathogen, *C. neoformans*, PUUP exhibited synergistic and fungidical activity with CPF. Among the CWIP inducers, a quinoline alkaloid was identified that was additive and fungicidal with CPF in *C. neoformans*. Transcript profiling studies on PUUP+CPF revealed that CWIP genes that were strongly induced by CPF alone were not induced by CPF+PUUP. Further studies revealed that PUUP targets Hsp90, and that it inhibits the CWIP by regulating Mpk1, a critical CWIP kinase which is an Hsp90 client protein. **Conclusions:** Our work reveals that compounds that alter the CWIP will have potential use in combination therapy with cell wall inhibitors such as CPF, thus providing a means to eliminate CPF-resistant *C. albicans* strains as well as CPF-insensitive *Cryptococcus* sp.

210C

EVALUATION THE ANTIFUNGAL ACTIVITY OF ZINC OXIDE NANOPARTICLES

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In this study, the antifungal activity of Zinc oxide (ZnO) nanoparticles (NPs) against 165 clinical isolates of yeast was evaluated. The test was performed by the broth microdilution method. The media used was Sabouraud dextrose broth (SDB) medium and the concentrations of ZnO NPs were ranging from 1000 to 1.95 mg/L. Results were shown that the ZnO NPs efficiently inhibit growth of yeast isolates. Variation in the inhibitory effect was noticed between different species of Candida. The MIC values for C. albicans, C. tropicalis and C. parapsilosis were ranging from 31.25 to 250 mg/L. However, for C. glabrata and C. krusie, the MIC values were higher and ranging from 125 to 1000 mg/L. In conclusion, ZnO NPs has a good antifungal activity against different type of yeast.

211A

METHANOLIC EXTRACT OF ELAEOCARPUS SPHEARICUS LEAVES HAS POTENT ANTI-CANDIDA PROPERTIES

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From last two decades the instances of pathogenicity and virulence of Candida glabrata has been increased and is now the second most cause of bloodstream infection in humans after Candida albicans. (Fidel et al., 1999; Malani et al. 2001; Hajjeh et al., 2004; Pfaller et al., 2007). Both Candida albicans and C. glabrata together constitute approximately 65-75% of Systemic candidiasis while C. tropicalis and C. parapsilosis comes after them in rank. (Perlroth etal., 2007). Virulence factors for Candida pathogenicity are mainly cell surface hydrophobicity (CSH), adherence to host surfaces, biofilm formation and secretion of hydrolytic enzymes (e.g. proteases, phospholipases and haemolysins) etc. (Silva et al., 2012). Polyenes (Amphotericin B), azole derivatives (Fluconazole, Itraconazole, Voriconazole, Posconazole and Ravuconazole), 5-flucytosine and echinocandins (Capsofungin) are common antifungal agents against Candida species which target fungal cell membrane, ergosterole synthesis, inhibition of DNA and protein synthesis and inhibition of β 1,3-D-glucan synthesis respectively (Fiedel et al., 1999) . C. glabrata has shown resistance to several azole derivatives and to polyenes upto some extent (Silva et al., 2012). Increasing reports on Candida resistance to existing antibiotics & invasive Candidosis have led our attention to develop alternative plant based effective therapeutics. Elaeocarpus sphearicus Roxb., commonly known as Rudraksha is well known for its medicinal and spiritual properties since long back in traditional medicinal systems (Joshi et. al., 2012). Plant parts have revealed the presence of many pharmacoactive constituents like tannins, flavonoids, alkaloids, carbohydrates and acids. In this study, we have found that RDLM (methanolic extract of leaves of E. Sphearicus) have the properties of inhibiting the growth of C. albicans and C. glabrata in broth. 5mg/ml and 1.25 mg/ml of RDLM were found to be lethal dose and LD50 respectively for both the species following NCCLS guidelines. RDLM has shown inhibitory effect on hyphal transition of Candida albicans. We found that RDLM has significantly reduced adhesion and biofilm formation of C. albicans and C. glabrata onto polystyrene surface of microtitre plate (Raut et al., 2013). Our findings declare RDLM as a potent anti-Candida agent which could be developed as first herbal drug against candidiasis after mandatory toxicological investigations. .

212B

DEVELOPMENT OF AN IMMUNOPROTECTIVE STRATEGY AGAINST C. ALBICANS SYSTEMIC INFECTIONS USING CATIONIC LIPOSOMES AS ANTIGENIC DELIVERY SYSTEMS.

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Morbidity and mortality from invasive fungal infections remain unacceptably high despite the availability of new antifungal agents. Cell wall surface proteins (CWSP) of C. albicans are major elicitors of host immune responses during candidiasis, being good candidates for vaccine development. Additionally, cationic liposomes are versatile and robust delivery systems for induction of antibody and T lymphocyte responses towards associated subunit antigens. This study describes the development of an antigen delivery system (ADS) to be used for vaccination against Candida infections based on cationic liposomes. The developed ADS is composed by DTT extractable CWSP from C. albicans and the dioctadecyldimethylammonium bromide and monoolein based liposomes. Liposomes were prepared by the lipid-film hydration method followed by addition of the CWSP. The effect of CWSP surface adsorption to the liposomes on colloidal stability, mean size and surface charge were investigated by dynamic light scattering, using different protein to lipid ratios. The ADS cell uptake and activation was investigated on the cell line J774 using confocal fluorescence microscopy and SEM imaging. Finally, the ADS effectiveness in potentiating an anti-CWSP systemic immune response was evaluated in the BALB/c mice immunized subcutaneously, 3 times, with CWSP, liposomes, ADS1 or ADS2. The results showed that above a defined protein-to-lipid ratio colloidal stable formulations are formed with an average particle size of 280nm, PDI lower than 0.2 and a surface charge around -20mV. From those, 2 formulations were selected for further studies, ADS1 and ADS2, one of them incorporating more than 90% of CWSP. In J774 cell line ADSs showed non-cytotoxicity, at concentrations below 88.7μg/ml of total lipid, and both ADSs were internalized in a concentration dependent manner. Macrophages exposed to ADS1 presented characteristic activation markers, such as, secretion of higher amounts of TNF-α and increased elongated morphology, compared with ADS2 treated cells or with untreated cells. The ADS1 immunized mice showed anti-CWSP IgG titres significantly higher compared to the mice immunized with ADS2 or with CWSP alone. Splenic cells, 3 weeks after the last immunization and stimulated ex vivo for 5 days with CWSP, showed that splenocytes from ASD1-treated mice produced significantly more IFN-γ and IL-17A, than splenocytes from mice treated with ASD2 or CWSP alone. IL-4 and IL-10 were detected only at very low levels. In summary, ADS1 seems to be a suitable delivery system and immunization with this lipid formulation can induce the production of pro-inflammatory Th1- and Th17-associated cytokines, suggesting that immunization with this formulation, previous to C. albicans infection, may result in a Th1 and/or Th17 immune response, which could be protective against this organism.

213C

PROPOSED MECHANISM OF A NOVEL SMALL MOLECULE ANTIFUNGAL FOR *CANDIDA* INFECTIONS: TRANSCRIPTOMICS AND PROTEOMICS

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Candida infections are major problem in immunocompromised populations. The limited number of antifungals and emergence of drug-resistant strains have posed a huge clinical challenge and highlighted the dire need for novel antifungal agents with new mechanism of action. Recently, a high-throughput screening of a library with 50,240 small molecules had led to our discovery of an antifungal small molecule (named "SM21"). Thereafter, SM21 was demonstrated to be a promising lead compound with potent in vitro and in vivo efficacies (US provisional patent No: 61733094). Aim of this study was to investigate the mechanism of action of SM21 by transcriptomic and proteomic approaches. The SM21-induced global differential transcriptional profile was analyzed by microarray with Filgen® Array for *C. albicans*. The SM21-induced global differential proteomic profile was analyzed by label-free quantitative mass-spectrometry. The significantly overrepresented gene ontology (GO) terms of the differentially expressed genes and proteins were assessed by GO enrichment analysis. Among the SM21-induced differential transcriptome, the GO term "cell wall organization" was significantly overrepresented. The involved genes were shown to affect the biosynthesis of β-1,3-glucan, β-1,6-glucan and mannoprotein, the major components of *Candida* cell wall. Moreover, the *ERG* gene family, which is involved in the synthesis of ergosterol, the important component of the fungal plasma membrane, was significantly differentially regulated by SM21. The GO terms "cellular component organization and biogenesis" and "carbohydrate metabolic process" were significantly overrepresented in the SM21-induced proteome. Examples of the proteins involved were Gfa1p, Psa2p, Gal10p and Pmi1p. Regulation of these markers suggested that SM21 affected the *Candida* cell wall integrity. Taken together, it was proposed that, our new discovery SM21, affects the *Candida* cell membrane as well as the cell wall. This novel antifungal small molecule would be a valuable addition to the current limiting arsenal of antifungal agents.

214A

EVALUATION OF MUCOADHESIVE GELS WITH PROPOLIS IN TREATMENT OF VULVOVAGINAL CANDIDIASIS INFECTION IN MURINE MODEL

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Candida spp. is commensally present in approximately 50% of the population and around 80% of women have already suffered from Candida infections, followed with recurring infections in approximately 5% of the cases. In immunocompromised individuals, *C. albicans* can produce mild superficial infections of the mouth and vagina. Clinical treatment of *C. albicans* infections is routinely performed

with polyenes and azole derivatives. However, these drugs are responsible for undesirable side effects and high toxicity. In addition, *C. albicans* azole- and echinocandin-resistance has been described. Propolis is a bee product traditionally used because of its antimicrobial, anti-inflammatory, wound healing and other properties. Previously, our group demonstrated that propolis showed fungicidal action against all three *C. albicans* morphogenetic types and propolis-induced cell death was mediated via metacaspase and Ras signaling. Then, the present work aims to evaluate different propolis gel presentations in the treatment of vulvovaginal candidiasis in murine model. The methodology used involved "in vitro" antifungal evaluation, chemical analysis by HPLC, mucoadhesive properties of propolis based gels and "in vivo" efficacy study in Balb/c mices. Murine model was performed as described by Yano & Fidel (2011) with some modifications. Treatments of animals involved two applications of the product per day during 7 and 10 days. The evaluation of results happened with 7 and 10 days, and finally, 10 days after the conclusion of the treatment. Results obtained demonstrated that the most mucoadhesive behavior happened with carbopol polymeric matrix, followed by both propolis based poloxamer and chitosan polymers. Results of "in vivo" efficacy demonstrated that mucoadhesive propolis based gels (1, 2 or 3%) have antifungal action, similar to commercial available clotrimazole cream (Neo Química). Our results strongly indicate that propolis represents a promising alternative therapeutic with great potential to control vulvovaginal candidiasis. Taken into consideration the fact that propolis is a complex substance with several possible antifungal active compounds, it may be more difficult for resistance to evolve in *C. albicans*, as it would require several concomitant mutations. Then, the results suggest the possibility of use propolis gel in clinical trials.

Yano, J., Fidel Jr., P.L., 2011. Protocols for vaginal inoculation and sample collection in the experimental mouse model of Candida vaginitis. J. Vis. Exp. 58, e3382.

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215B

DYNAMIC HOST-PATHOGEN INTERACTIONS RESULT IN FUNGAL EPITOPE UNMASKING IN VITRO AND IN VIVO.

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The ability of a host to recognize pathogens is the first step in their clearance. The cell wall of *Candida albicans* has many components that the host can recognize, including the β-glucan polysaccharide recognized by the host receptor Dectin-1. While β-glucan is typically masked, it becomes unmasked during disseminated infection. Exposure levels of this epitope during infection may be important, as Dectin-1 mediates protection against at least some strains of *C. albicans* and *in vivo* alterations in the organization and composition of the *Candida* cell wall influences the host response. To understand β-glucan unmasking during infection, we looked to identify the mechanisms whereby host-pathogen interactions alter fungal epitope exposure. We reasoned that β-glucan unmasking could result from direct stripping of the cell wall, by an active mechanism independent of immune attack, or by an active fungal response to immune attack. To examine this, we tested if immune cells can cause β-glucan exposure *in vitro*. We biotinylated cell wall proteins and labeled with fluorescent streptavidin, then incubated with neutrophils. Timelapses show that streptavidin fluorescence is lost rapidly at sites of neutrophil attack, suggesting that there is immune-mediated damage to the cell wall. Staining with soluble Dectin-1-Fc and Calcofluor White revealed that areas where streptavidin was lost had β-glucan exposure and increased chitin deposition. This suggests that neutrophils can cause β-glucan exposure, and to determine if neutrophils are required for unmasking *in vivo*, we examined β-glucan exposure on *C. albicans* in mice with neutropenia. Depletion of neutrophils largely prevented

β-glucan exposure *in vivo*. These lines of evidence suggest that neutrophilic immune attack is necessary for β-glucan unmasking. We then tested if immune-mediated unmasking requires an active fungal response. We found that UV-inactivated *Candida* did not respond to attack with increased chitin deposition or β-glucan exposure, suggesting that these are active processes. Taken together, the data suggests that β-glucan unmasking and chitin deposition result from an active fungal response to immune attack. Current work is focused on the mechanisms that direct this interaction. On the host side, we are testing the importance of neutrophil proteases and reactive oxygen species. We are also exploring the mechanisms required on the fungal side for unmasking in response to attack. Overall, this work helps elucidate host-pathogen interactions in disseminated candidiasis, including insight into immune mechanisms of attack and how *C. albicans* responds. Given the importance of the cell wall as a drug target, understanding how this fungus maintains integrity during immune attack may identify novel therapeutic targets to aid the treatment of candidiasis.

216C

RAB14 RECRUITMENT TO PHAGOSOMES REDUCES MACROPHAGE KILLING BY LIVE *C. ALBICANS*

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Candida albicans virulence is in part due to its ability to switch from yeast to hyphal form and physically rupture and escape from macrophages after phagocytosis {1}. This escape is likely to occur before phagosomes have fully matured into a microbicidal organelle. This makes *C. albicans* a good model organism to study the role of the small GTPase Rab14 in phagosome maturation. Rab14 has been found to recruit to bacterial phagosomes{2}, but its role in fungal phagosome maturation is not fully understood. Here, we demonstrate an important role for Rab14 in protecting macrophages against killing by live *C. albicans*. Macrophages were transfected to express GFP-Rab14, GFP-dominant negative constructs ($Rab14^{S25N}$ and $Rab14^{N124I}$) or siRNA to mediate Rab14 knockdown and then infected with live or UV killed *C. albicans* and studied using confocal and sophisticated live cell imaging and image analysis tools {3}. We showed that phagosomes containing live C. *albicans* became Rab14 positive following phagocytosis. Interestingly, we observed differential recruitment of Rab14 to phagosomes depending on *C. albicans* morphology. Phagosomes containing yeast *C. albicans* retained Rab14 GFP for 5 minutes while hyphal phagosomes retained Rab14 for approximately 12 minutes. Depletion of endogenous Rab14 in J774.1 macrophages did not affect macrophage migration towards live fungi, the rate of engulfment or phagosome acidification of UV killed and live *C. albicans* phagosomes when compared to controls. We also found that a knockdown of Rab14 expression did not influence either the time of Rab5-GFP localisation to phagosomes following uptake or the duration of Rab5-GFP on phagosomes containing *C. albicans*. Importantly, we found that silencing Rab14 disrupts LAMP1 colocalisation, cathepsin activation and Rab7 recruitment to live *C. albicans* phagosomes. Rab14 protein knockdown was associated with a significant increase in macrophage killing by *C. albicans* when compared to controls. Together, these data indicate that in macrophages, Rab14 is mainly localized to early endosomes upstream of Rab7 and plays an important role in the initiation of late stages of phagosome maturation. Our data points to a model where Rab14 may be important in protecting macrophages against killing by hyphal *C. albicans*. 1. McKenzie CGJ, Koser U, Lewis LE, Bain JM, Mora-Montes HM, Barker RN, et al. Contribution of Candida albicans cell wall components to recognition by and escape from murine macrophages. Infect Immun 2010;78(4):1650-1658. 2. Kyei, G.B., Vergne, I., Chua, J., Roberts, E., Harris, J., Junutula, J.R. and Deretic, V. (2006). Rab14 is

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217A

PRESENCE OF THE MULTIFUNCTIONAL PROTEIN ALS3 ON CANDIDA ALBICANS HYPHAE DEPENDS ON THE TUP1 REPRESSOR

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The *Candida albicans ALS3* gene encodes a protein with multiple functions in adhesion and invasion as well as iron acquisition *in vitro* and *in vivo*. *ALS3* belongs to a small set of genes whose expression are increased during the yeast to hyphae transition regardless of the environmental stimulus. These core filamentation response genes are characterized by large promoter regions and are heavily regulated by several transcriptional activators and repressors. In this work, we have analyzed the expression dynamics of *ALS3* in the absence of the repressors Tup1 and / or Nrg1. In response to a hyphal growth trigger, the amount of *ALS3* mRNAs increased by at least 30 fold in wild type as measured by qRT PCR. In contrast to this, it remained stable at an intermediate level in *tup1*Δ, well above of comparable wild type yeast cells, but significantly lower than in wild type hyphae. These expression dynamics were not found in *nrg1*Δ where *ALS3* expression was higher than in wild type yeast cells, but reached the height of wild type hyphae under filament- inducing conditions. The absence of this filament- specific transcriptional shift correlated with very low Als3 protein signal on the surface of *tup1*Δ filaments, regardless of the environmental condition. The mutant was consequently neither able to bind ferritin nor to grow in media with that protein complex as the exclusive iron source. In contast, *nrg1*Δ was able to accomplish growth under this condition, indicating a full functionable Als3 level. A *tup1*Δ / *nrg1*Δ double mutant showed *ALS3* expression dynamics which were similar to that of *tup1*Δ alone and consequently, the double mutant failed to use ferritin as sole iron source. In line with these findings, *nrg1*Δ, but neither *tup1*Δ nor *tup1*Δ / *nrg1*Δ, was still able to invade and damage human host cells in an *in vitro* approach. This was supported by a remaining virulence of *nrg1*Δ in an intraperitoneal mouse infection model. These results indicate that the presence of the Tup1 repressor is required to reach high *ALS3* expression levels as found in wild type hyphae. Additionally, we could show that there seems to be a threshold of mRNA amounts which must be surpassed to generate enough Als3 protein for its full function in iron acquisition. Our data revealed also functional differences between the repressors Tup1 and Nrg1 during the regulation of at least one core filamentation response gene.

218B

INVESTIGATING THE ROLE OF HSF1 IN CANDIDA ALBICANS DRUG RESISTANCE AND MORPHOGENESIS

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Invasive fungal infections caused by Candida albicans are among the leading causes of death in immunocompromised individuals. Treat-

ment is challenging due to the emergence of resistance to the limited number of antifungal drugs in clinical use. A key regulator of C. albicans drug resistance and morphogenesis is Hsp90, an essential molecular chaperone that stabilizes diverse client proteins. Inhibition of Hsp90 also regulates the C. albicans morphological transition from yeast to filamentous growth, which is a key virulence trait. Hsp90 inhibition induces filamentation and abrogates drug resistance, rendering resistant pathogens susceptible to treatment in multiple infection models. It has recently been found that the transcription factor Hsf1, which regulates the expression of HSP90 and ~3% of the yeast genome, is essential for C. albicans virulence. Here, we focus on elucidating the role of Hsf1 in two key virulence traits: drug resistance and morphogenesis. We have found that depletion of Hsf1 has similar effects on drug resistance and morphogenesis as Hsp90 inhibition. Genetic depletion of Hsf1 reduces resistance to echinocandins, which target the cell wall, and induces filamentation, even when HSP90 expression is driven by a constitutive promoter, independent of Hsf1. These phenotypes are also observed upon induction of HSF1 expression suggesting that dramatic changes in HSF1 expression are sufficient to induce filamentation and increase echinocandin susceptibility, independently of HSP90 expression. To determine if these effects on drug resistance and morphogenesis are a result of compromised Hsp90 function, we monitored Hsp90's capacity to enable activation of the client protein Hog1 by monitoring the levels of activated, phosphorylated Hog1. We determined that Hsp90 function in enabling activation of Hog1 is compromised upon Hsf1 depletion. We hypothesize that alterations in the expression of Hsf1 affects drug resistance and morphogenesis by modulating the expression of a co-chaperone or other critical regulator of Hsp90, leading to compromised chaperone function. We are currently taking an unbiased approach to determine Hsf1-dependent genes by performing microarray analysis under Hsf1 induction and depletion conditions. To identify Hsf1-dependent genes through which it controls morphogenesis and drug resistance, we will engineer strains with constitutive expression of candidate effectors to determine those that block the impact of Hsf1 alterations on drug resistance and morphogenesis. This work illuminates a novel role for Hsf1 in regulating drug resistance and morphogenesis of C. albicans. Elucidating the circuitry through which Hsf1 regulates drug resistance and morphogenesis will suggest new targets for treating fungal infections.

219C

CONTROL OF METABOLIC ACTIVITY BY CDK8 INFLUENCES PYOCYANIN SUSCEPTIBILITY IN C. ALBICANS

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A key virulence factor of *Candida albicans* is the ability to form biofilms that are composed of adhesin-expressing yeast and hyphal cells which are together encased in an extracellular matrix. Biofilms, which are structurally and metabolically similar to wrinkled colonies, can readily form on indwelling biomedical devices and cause increased resistance to antifungals. Pyocyanin (PYO), a redox-active molecule produced by *Pseudomonas aeruginosa*, inhibits filamentation and interferes with respiratory activity in *C. albicans*. Here, we used wrinkled colonies to further characterize the effects of PYO on *C. albicans* morphology and metabolism. We screened a mutant collection that revealed the *ssn3* and *ssn8* mutants had increased resistance to the inhibitory effects of PYO (PYO^R). Ssn3 and Ssn8 are components of Mediator, a highly conserved eukaryotic complex that can serve as both a transcriptional co-activator and co-repressor. The identification of Mediator mutants in our screen is consistent with the key role of

transcription in modulating the transition to biofilm or wrinkled colony formation. These proteins are specifically components of the heterotetrameric CDK8 module of Mediator which is primarily implicated in transcriptional repression, and subsequent studies revealed all CDK8 mutants—*ssn3*, *ssn8*, *srb8* and *srb9*—had identical PYO^R phenotypes. These mutants were also found to be hyperalkalinizers, which is indicative of increased amino acid oxidation. Consequently, we hypothesized that mutation of the CDK8 complex resulted in basal level differences in metabolism, and that these differences were even more striking in the presence of PYO. To test this hypothesis, we completed a metabolomics study using the WT, *ssn3*Δ/Δ and *ssn3*Δ/Δ-*SSN3* strains grown under wrinkling-inducing conditions in the presence and absence of PYO. Mutation of *Ssn3* resulted in perturbation of multiple metabolic pathways, and the effect of PYO on these pathways varied tremendously between the WT and *ssn3*Δ/Δ strains. More direct studies revealed that a compromised CDK8 complex caused increased glycolysis and oxidative metabolism, but not fermentation. Furthermore, all CDK8 mutants had higher levels of intracellular ATP compared to the WT and, in contrast to the WT, these levels were not decreased in the presence of PYO. In summary, our results support the hypothesis that the CDK8 component of Mediator regulates aspects of metabolism in ways that alter the response of *C. albicans* to PYO.

220A

A NOVEL FAMILY OF ANTIOXIDANT ENZYMES THAT PROTECTS THE *CANDIDA ALBICANS* PLASMA MEMBRANE FROM LIPID PEROXIDATION

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The plasma membrane (PM) of *Candida albicans* is rich in polyunsaturated fatty acids (PUFAs), which increase the fluidity of the PM and are thought to help this fungal pathogen to adapt to different environments in the host. However, PUFAs also make the PM more susceptible to oxidative stress, as they more readily undergo lipid peroxidation, which can in turn lead to other kinds of oxidative damage to proteins and nucleic acids. Although protecting against lipid peroxidation is extremely important, very little work has been done to define the mechanisms, because most previous studies have focused on antioxidant enzymes that are either secreted or cytosolic rather than in the PM. Based on recent studies in mammalian cells and *S. cerevisiae*, it is likely that ubiquinone, also known as Coenzyme Q, acts as the major antioxidant in the PM. Ubiquinone is a long chain quinone molecule that is best known for its role in the mitochondrial electron transport chain, but it is also abundant in PM of eukaryotic cells. Previous studies have shown that cells must use special mechanisms to safely reduce oxidized ubiquinone to ubiquinol so that it can be reused. One electron reduction of ubiquinone produces a semi-quinone intermediate, which is highly deleterious as it generates reactive oxygen species. Cells try to avoid this by using enzymes that carry out a two-electron reduction of ubiquinone. Our search for candidate proteins identified four flavodoxin-like proteins (FLPs) Pst1, Pst2, Pst3 and Ycp4 that displayed strong sequence similarity to NAD(P)H quinone oxidoreductases in *E. coli* and we found that they localized to the PM. Our data show that mutation of these genes causes increased lipid peroxidation and decreased viability after cells are exposed to oxidized PUFAs. Co-incubation with antioxidant Vitamin E reverses the deleterious effect of PUFAs. Thus, our studies identify a novel mechanism by which fungal cells protect the PM from oxidative stress. Virulence assays are currently being conducted in mice and will be presented at the meeting.

221B

PIPELINE FOR THE ANALYSIS OF COPY NUMBER AND LOSS OF HETEROZYGOSITY CHANGES IN C. ALBICANS GENOME ARRAY AND SEQUENCE DATASETS.

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Candida albicans is an opportunistically pathogenic yeast known for a robust tolerance of even dramatic changes to its genome, such as large-scale copy number changes and the reduction of genetic information caused by loss of heterozygosity events. These changes can strongly impact the ability of C. albicans to survive in the various and changing environments of the human host, including the formation of problematic invasiveness and drug resistance changes in clinical settings. In an experimental context these changes can form concurrently to intended alterations, resulting in secondary phenotypes that confound the intended analyses. Understanding what changes have happened is an important first step in discerning the impact of large-scale genome structural changes or in identifying causes of major unexpected variation in experimental results. To this end, we have developed a generalized genome data analysis pipeline, incorporating freely available and novel in-house tools, to analyze SNP/CGH microarray, whole genome sequence, and double-digest RADseq datasets with a common interface and visualization scheme. The pipeline uses whole-genome ploidy estimates, from flow cytometry or other sources, and local copy number ratios, calculated from the high-density array or sequence datasets, to generate copy number estimates across the genome. The pipeline incorporates known hapmap features for the C. albicans reference (SC5314) extended with whole genome sequence data and can also display changing SNP distributions in clinical strains without pre-determined haplotype information. Combining these features, the pipeline rapidly condenses very large datasets into figures showing copy number and SNP allele changes across each chromosome of a genome using simple to interpret colors. Inferences about observed large-scale genomic alterations can then be made to further the analysis of drug resistance, growth alteration, or other phenotypic changes in C. albicans.

222C

ANALYSIS OF THE CANDIDA ALBICANS TRANSCRIPTION FACTOR FCR1P REGULON

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Candida albicans is a dimorphic human fungal pathogen that causes life-threatening infections in immunocompromised individuals. The C. albicans genome contains 82 genes encoding transcription factors (TFs) of the zinc cluster family. Some of these TFs have been shown to regulate cellular processes such as sugar metabolism, filamentation or drug resistance, yet a majority remains to be characterized. We previously identified Fcr1p, a zinc cluster TF whose deletion increases cell tolerance to multiple drugs. It was also shown in a large-scale screen that an fcr1 heterozygous transposon mutant was hyperfilamentous. These findings indicate that Fcr1p functions as a negative regulator but its transcriptional targets are still unknown. We used genomic approaches to characterize the Fcr1p regulon. To identify where Fcr1p binds in the genome, we tagged it with an HA epitope and performed a ChIP-Chip analysis under standard growth conditions (YPD, log phase). We identified 144 genes bound by Fcr1p (binding ratio $\geq$ 1.5-fold, $p \leq 0.01$), including several genes involved in ammonium, amino acid and oligopeptide transport (MEP1, CAN1, CAN2, CAN3, GAP2, GAP6, OPT1, OPT4, OPT9), nitrogen metabolism (GLT1, GLN1, GDH3, DUR1) and transcriptional regulation of nitrogen utilization (GAT1, STP3). Strikingly, Fcr1p was found to bind predominantly within the open reading frame of its targets, suggest-

ing that it may bind DNA indirectly, through association with the transcriptional or chromatin machinery. To identify the genes whose expression is regulated by Fcr1p, we constructed an fcr1Δ/Δ mutant in the SC5314 background using the SAT1 flipper strategy and performed expression profiling under the same growth conditions. We found 57 downregulated and 30 upregulated genes in the mutant compared to the wild-type (ratio ≥ 1.5-fold, $p \leq 0.01$), indicating that Fcr1p can function as an activator or a repressor of gene expression. Among the most significantly downregulated processes are genes pertaining to nitrogen utilization (CAN1, AAP1) while upregulated processes include genes involved in filamentous growth and biological adhesion (EFH1, HWP1, ALS3, SAP5). Finally, only a minority (6 %) of the modulated genes were bound by Fcr1p, mainly those involved in amino acid transport, suggesting that a majority of the modulated genes are indirect targets of Fcr1p. Taken together, our results indicate that Fcr1p regulates its target genes in a complex manner, acting as a positive regulator of nitrogen metabolism and as a negative regulator of filamentation and cell adhesion. This later proposition was validated by the demonstration that FCR1 overexpression abrogates filamentation. In addition, Fcr1p seems to mediate its function mainly indirectly, possibly through intermediate effectors, a hypothesis that would position this TF upstream of other important regulators.

223A

PPG1, A PP2A PROTEIN PHOSPHATASE, CONTROLS *CANDIDA ALBICANS* MORPHOLOGY AND VIRULENCE

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Candida albicans, the most prevalent human fungal pathogen, is a major cause of hospital-acquired bloodstream infections in the U.S. *C. albicans* infections, which occur frequently in immunocompromised individuals, have become more difficult to treat due to a limited number of antifungal drugs and increased frequency of drug-resistant isolates. *C. albicans* virulence requires the ability to undergo a reversible transition from single ovoid budding yeast cells to elongated cells attached end-to-end known as filaments. This transition allows for efficient tissue invasion, immune evasion and dissemination. While many filament-specific target genes have been identified, considerably less is known about *C. albicans* filamentous growth signaling pathways and regulatory circuits. We have demonstrated that a serine/threonine protein phosphatase type 2A (PP2A), Ppg1, is important for *C. albicans* morphogenesis under a variety of both solid and liquid filament-inducing conditions. Ppg1 also controls down-regulation of *NRG1*, which encodes a key transcriptional repressor of filament-specific target genes, in response to growth in serum at 37°C. We have recently generated a Ppg1 catalytic mutant using site-directed mutagenesis. This mutant shows a filamentation defect equivalent to that of the *ppg1*Δ/Δ strain, demonstrating that Ppg1 phosphatase activity is specifically important for *C. albicans* morphogenesis. The *ppg1*Δ/Δ mutant was also found to be highly attenuated for virulence in a mouse model of systemic candidiasis. Finally, we have demonstrated that a strain expressing high constitutive levels of *PPG1* is defective for filamentation under a variety solid inducing conditions. We are currently investigating the relationship between Ppg1 and several known *C. albicans* filamentous growth signaling pathways. Overall, our data suggest that a PP2A protein phosphatase plays an important role in controlling both morphology and virulence of *C. albicans*. These studies are significant because gaining a better understanding of regulatory circuits that control *C. albicans* filamentous growth and virulence will provide information that may eventually lead to the development of novel and more effective antifungal strategies to treat candidiasis.

224B

UNDERSTANDING THE EVOLUTION OF POST-TRANSCRIPTIONAL GENE NETWORKS IN FUNGI

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Evolutionary rewiring presents a fascinating insight into how related gene networks can develop distinct functions in closely related species. Most studies so far have focused on transcription, and comparatively little is known about the evolution of post-transcriptional networks, and what this means for biological outcomes within the organism. The model yeast Saccharomyces cerevisiae and the fungal pathogen Candida albicans are evolutionarily related, but live in different environments and have had to adapt to specific conditions in their respective niches. It has been proposed that changes to gene expression control have contributed to phenotypic diversification. We are focusing on posttranscriptional regulation of mitochondrial biogenesis by the RNA binding protein Puf3, which binds to a large number of mRNAs encoding mitochondrial proteins, and targets them to the surface of mitochondria for localised translation. Our data suggests that rewiring has occurred at multiple stages of the evolution of gene expression related to the Puf3 network between S. cerevisiae and C. albicans, particularly in regards to the regulation by carbon source. The ability of C. albicans to respond properly to changing nutrient conditions is key for successful host infection, and our studies aim to address the role of Puf3-dependent posttranscriptional gene network in this process.

225C

NOVEL ROLE OF A FAMILY OF MAJOR FACILITATOR TRANSPORTERS IN BIOFILM DEVELOPMENT AND VIRULENCE OF CANDIDA ALBICANS

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We had earlier demonstrated that Candida albicans cells were susceptible to malachite green (MG) and its effect was independent of known antifungal targets. Transcriptional profiling in response to MG treatment revealed that out of total responsive genes, QDR1 (Quinidine Drug Resistance) was among the most induced gene. QDR family of genes encode transporters belonging to Major Facilitator Superfamily (MFS) of proteins. Out of 95 members of MFS super family, Candida genome has three QDR genes annotated in CGD (http://www.candidagenome.org) as QDR1 (orf19.508), QDR2 (orf19.6992) and QDR3 (orf19.136). Present study explores the role of QDR genes. We show that QDR transporters do not play any role in drug resistance. In addition, deletion mutants of QDR1, QDR2 and QDR3 show no impact on susceptibilities towards azoles, polyenes, echinocandins, polyamines, quinolines as well as cell wall inhibitors and many other stresses. However, the deletion of QDR genes individually or collectively led to defects in biofilm architecture and thickness. Interestingly, qdr1, 2 and 3 deletion mutants displayed attenuated virulence but maximum effect was observed in case of qdr2Δ, qdr3Δ and qdr1/2/3Δ strains. Notably, the attenuated virulence and biofilm defects could be reversed

in QDR revertants. The transcription profiling of QDR deletion mutants and its validation confirmed differential expression of many biofilm and virulence related genes in deletion mutants which were down regulated minimum by 2 folds in comparison to WT Candida. Furthermore, lipidomic analysis of QDR deletion mutants suggest remodelling of membrane lipids which may affect cell signalling leading to defect in biofilm and attenuation in virulence. Taken together, our results suggest that QDR genes of Candida differ from its known roles in Saccharomyces cerevisiae and these genes have interesting implications in biofilm formation, virulence and in maintaining membrane lipid homeostasis in Candida cells.

226A

THE ENDOSOMAL GTPASE VPS21P CONFERS AZOLE SUSCEPTIBILITY IN CANDIDA ALBICANS

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Development of resistance to the most commonly used antifungals, the azoles, is an increasing clinical problem. In *Candida albicans* known mechanisms of azole resistance include increased expression of the target enzyme Erg11p and of drug efflux pumps such as Cdr1p and Mdr1p. However, the resistance of many clinical isolates is not accounted by established mechanisms. The azole antifungals block ergosterol biosynthesis through inhibition of lanosterol 14α-demethylase (Erg11p), and result in the accumulation of 'toxic' intermediate sterol species, which are thought to compromise the function of the plasma membrane. However, the consequences of azole treatment and resulting ergosterol depletion upon the function and integrity of *intracellular* membranes is not well characterized. Using a high-throughput screening assay, we recently identified two azoles as causing vacuolar defects in *C. albicans*. The purpose of this study was to investigate the impact of azole treatment upon the integrity of the *C. albicans* vacuole, and determine if vacuolar function can influence azole susceptibility. Specifically, we hypothesized that mutants with vacuolar defects may have altered azole susceptibility. We therefore tested the azole susceptibility of several mutants with defects in distinct vacuolar trafficking pathways, using the CLSI broth microdilution method. Strikingly, mutants lacking the endosomal Rab GTPase Vps21p had significantly reduced susceptibility to several azole antifungals. The azole resistance of the *vps21Δ/Δ* mutant was not dependent upon the Cdr1p or Mdr1p efflux pumps, since *vps21Δ/Δcdr1Δ/Δ* and *vps21Δ/Δmdr1Δ/Δ* double mutants remained resistant. However, inhibition of calcineurin signaling using cyclosporin A, abolished the azole resistance of the *vps21Δ/Δ* mutant. Finally, ergosterol depletion caused by azole treatment has been linked to inhibition of the vacuolar proton pump (V-ATPase), and thus reduced vacuolar acidification. Unexpectedly, we found that quinacrine accumulation increased following fluconazole treatment, suggesting increased vacuolar acidification. Furthermore, in contrast to the equivalent *Saccharomyces* mutant, our *C. albicans vps21Δ/Δ* mutant had increased vacuolar acidification compared to isogenic control strain. We are currently testing if increased V-ATPase activity contributes to the azole resistance of the *vps21Δ/Δ* mutant. Thus perturbation of endosomal trafficking through the Rab GTPase Vps21p may represent a novel, calcineurin dependent mechanism of azole resistance.

227B

SIGNALING BY A BIOFILM SURFACE ADHERENCE PROTEIN

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The cell wall is the fundamental sensing and responding interface between a pathogenic cell and its microenvironment. It is particularly critical for C. albicans, playing roles in protection, morphogenesis and adherence. Cell wall proteins (CWPs), specifically the adhesins, are important for biofilm formation promoting adherence to a substrate during initial biofilm establishment. Als1 is one such critical adhesin. We show Als1 is a key signalling component to a genetic program regulating biofilm formation circuitry. Als1 signals to the transcription factor Brg1 initiating a signature 'biofilm program' and is essential for biofilm formation specifically in the denture stomatitis model. Transient expression of Als1 or Brg1 is sufficient to switch on the biofilm program and form a biofilm. Importantly, we demonstrate that CWPs may signal to diverse regulatory pathways promoting biofilm formation in differing niche environments. This may extend to other eukaryotes. Just as the cell wall is a dynamic structure, CWP gene expression may be dynamic and imperative in signalling in key C. albicans pathways and processes.

228C

SEXUAL BIOFILMS OF CANDIDA TROPICALIS, AND A ROLE FOR A SEXUAL AGGLUTININ

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Candida tropicalis is an opportunistic human pathogen that was recently shown to undergo the white-opaque phenotypic switch, similar to that in Candida albicans. In both species, opaque cells respond to mating pheromones by forming mating projections and completing conjugation, while in C. albicans, white cells respond to pheromones secreted by opaque cells by forming 'sexual' biofilms. Here, we demonstrate that, in contrast to C. albicans, it is C. tropicalis opaque cells that form biofilms when responding to sexual pheromones, while white cells do not. These sexual biofilms are dependent on pheromone receptors Ste2 and Ste3, yet pheromone signaling alone does not result in robust biofilm formation, suggesting that cell-cell contact between mating partners plays an important role in this process. The structure of sexual biofilms produced by C. tropicalis was studied using confocal and scanning electron microscopy. This analysis revealed that biofilms were composed of several layers, with the outermost layer consisting of filamentous cells and extracellular matrix, while yeast-form cells dominated the basal layer. Transcriptional profiling showed that many genes necessary for mating were upregulated in sexual biofilms, including genes involved in conjugation. In particular, FGR23, which encodes an ortholog of the S. cerevisiae sexual agglutinin Aga1, was upregulated in sexual biofilms, and deletion of this gene significantly reduced sexual biofilm formation and also reduced the frequency of sexual mating. Taken together, this work indicates C. tropicalis opaque cells are competent for sexual biofilm formation, and that biofilms exhibit a complex structure consisting of stratified layers of cells and extracellular matrix. In addition, a conserved sexual agglutinin is revealed to be important for mediating both mating and biofilm responses in this species.

229A

CANDIDA ALBICANS-STAPHYLOCOCCUS AUREUS INTRA-ABDOMINAL INFECTION-MEDIATED LETHAL SEPSIS: ATYPICAL REQUIREMENTS OF MORPHOGENESIS AND VIRULENCE

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Background: Intra-abdominal polymicrobial infections cause significant morbidity and mortality. An experimental mouse model of *C. albicans/S. aureus* intra-abdominal infection resulted in ~60% mortality within 48 h post-inoculation concomitant with amplified local inflammatory responses, while monomicrobial infections were avirulent. The purpose of this study was to characterize early local and systemic responses during co-infection, determine the role of *C. albicans* morphogenesis in lethality, a trait involved in virulence and interaction with *S. aureus,* and define which fungal/bacterial interactions induce motility. **Methods**: Outbred mice were inoculated i.p. with *S. aureus* ($8x10^7$) and/or *C. albicans* ($7x10^6$-$3.5x10^7$) and followed for survival over 5 days. Peritoneal lavage fluid, spleen, brain, and serum was collected at defined time points post-inoculation to quantify microbial burden, cytokine levels, and cellular infiltrate (peritoneal). A similar survival design included yeast- and hyphae-locked *C. albicans* mutants, and other yeast species that cannot form hyphae (*C. glabrata* and *Saccharomyces cereviseae)*. **Results**: Local and systemic pro-inflammatory cytokines were significantly elevated during co-infection at early time points (4-12h). Conversely, microbial burden in the organs and peritoneal lavage was similar between mono- and co-infected animals through 24 h, as was peritoneal neutrophil infiltration. After optimizing the model for 100% mortality in 48 h with 5x higher *C. albicans* and the same number of *S. aureus*, co-infection using the *C. albicans* morphogenesis mutants showed similar mortality, dissemination, and local and systemic inflammation as the isogenic control. Conversely, co-infections with *C. glabrata* or *S. cerevisiae* could not replicate the synergistic lethality. Finally, co-infection with the yeast-locked *C. albicans* inoculated i.v. and *S. aureus* inoculated i.p. also failed to induce mortality. **Conclusions**: These results suggest a unique intra-abdominal interaction between the host and *C. albicans/S. aureus* that results in strong inflammatory responses, dissemination and lethal sepsis, which is unique to *C. albicans and* not dependent on morphogenesis.

230B

CGPDR1-DEPENDENT ADHERENCE OF *CANDIDA GLABRATA* TO HOST CELLS: IMPACT OF DIFFERENT YEAST STRAIN BACKGROUNDS AND *CGPDR1* GAIN-OF-FUNCTION MUTATIONS

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There are increasing numbers of multidrug-resistant *Candida glabrata* isolates recovered from patients presenting with disseminated infections non-responsive to therapy [1]. *C. glabrata* frequently develops secondary resistance to azole drugs, the most commonly used drugs to manage candidiasis, usually due to increased drug efflux. The mechanism typically consists on *CgPDR1*-mediated upregulation of ATP-binding cassette (ABC) multidrug transporter genes. Our laboratory reported a collection of matched pairs of *C. glabrata* clinical isolates including over 50 gain-of-function (GOF) mutations leading to *CgPDR1* hyperactivity and azole drug resistance. Surprisingly, the tested GOF mutants in an isogenic strain background (clinical isolate DSY562) were found to display increased fitness *in vivo* and virulence in comparison to the azole-susceptible wild type strains [2]. Follow-up work using *ex vivo* co-cultures of GFP and RFP-expressing yeast cells revealed a decreased adherence and phagocytosis of

three tested GOF mutants (including mutations L280F, R376W and T588A) by murine bone marrow-derived macrophages (BMDMs) in competition with the wild type [3]. In turn, adherence assays to human and murine epithelial cell lines revealed an opposite trend, with GOF mutants displaying increased adherence [3]. In this work we report further studies to extend our previous findings. Reduced adherence of the GOF mutants to macrophages was closely reproduced using murine monocytic macrophage cell line RAW264.7 and human acute monocytic leukemia THP-1 cell line-derived macrophages. Increased adherence to epithelial was also observed with Chinese hamster CHO-Lec2 cells, human Caco-2 and HeLa cells and mouse CMT-93 cells. However, when testing additional CgPDR1 GOF mutations (Y584C, P822L, D1082G and E1083Q) we found that they do not all conserve the previously reported differences in adherence to mammalian cells. Furthermore, expressing the CgPDR1 GOFs in other *C. glabrata* strain backgrounds, including CBS138, BG2 and two additional clinical isolates from our collection, also yielded variable results. Our previous work suggested a dual contribution of *CgPDR1* hyperactivity to the increased virulence of *C. glabrata*: an optimized adherence to and colonization of epithelial tissues, as well as the evasion from the host's innate immune response. The data presented here show that these effects are not inherent to *CgPDR1* hyperactivity, but rather dependent on specific CgPDR1 GOFs and strain backgrounds. Further studies are underway in our laboratory to understand the molecular and genetic mechanisms behind the *CgPDR1*-mediated regulation of *C. glabrata*-host interactions. References: [1] Pfaller MA, (2012) Am J Med, 125, S3-13 [2] Ferrari S *et al.*, (2009) PLOS Pathog 5, e1000268 [3] Vale-Silva LA *et al.*, (2013) Infect Immun, 81, 1709-1720

231C

CANDIDA ALBICANS EED1 IS INVOLVED IN THE RESPONSE TO QUORUM SENSING

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A complex network of regulatory factors controls the morphology of *Candida albicans* and various factors are involved in yeast-to-hypha transition. The Eed1 protein is a regulatory factor of filamentation in *C. albicans* and especially important for the maintenance of hyphal development and growth. Mutants lacking the *EED1* gene retain the ability to initialize hyphal growth by the formation of germ tubes, but do not form elongated true hyphae but pseudohyphae before switching back into an elongated yeast cell-like growth form. The exact molecular role of Eed1 in hyphal elongation is still unclear. We found that filamentation of an *eed1*Δ mutant strongly depends on cell density which suggested a link between *EED1* and quorum sensing. This has been confirmed by filamentation assays, where we observed that *eed1*Δ mutants were significantly more sensitive to the inhibitory effect of farnesol in comparison to the wild type. Hypothesizing that the link between *EED1* and quorum sensing is mediated by farnesol, *eed1*Δ mutants lacking different regions of the *EED1* gene were challenged with farnesol in vitro under various filament-inducing conditions. The results show that the N-terminal region of *EED1* is mainly responsible for farnesol hypersensitivity. To test if the association between *EED1* and the farnesol pathway occurs via cAMP signaling, we tested the effect of dB-cAMP on preventing germ tube inhibition

by farnesol but did not observe a significant effect of dB-cAMP on the sensitivity of the *eed1*Δ mutant. Additional assays such as determination of intracellular cAMP levels are thus needed to provide further insights into this hypothesis. Similar to the results obtained for farnesol hypersensitivity, analysis of *eed1*Δ mutants lacking different regions of the *EED1* gene showed that the N-terminal region of Eed1 is also essential for the hyphal maintenance for cells grown in liquid medium and surface contact. In contrast, the whole protein seems to be necessary for filamentation during agar embedded growth. This suggests that during *EED1*-mediated hyphal elongation, Eed1 may interact with different partners depending on the stimulating condition. Taken together our data indicate that *EED1* is involved in *Candida albicans* response to farnesol, although the mechanisms still have to be revealed. Therefore a His-tagged Eed1 version has been constructed to analyze protein localization. This construct, as well as recombinant Eed1 C- and N-terminal peptides, are currently investigated for interaction with DNA and proteins in gel-shift and pull-down assays, respectively. These experiments will provide valuable information about the possible role of *EED1* in *C. albicans* tolerance to farnesol and contribute to the elucidation of its molecular function in morphogenesis.

232A

DISCOVERY AND CHARACTERIZATION OF POTENTIAL ANTIFUNGAL COMPOUNDS AGAINST CANDIDA ALBICANS

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Candida albicans, a commensal organism living in the gut of many humans, can cause infections in immunocompromised individuals. These infections include vaginal, oral, and systemic infections, the latter being life-threatening. The three main types of antifungals commonly used to treat systemic C. albicans infections are azoles, polyenes, and echinocandins. In past years, drug toxicity, as well as a rise in drug resistant strains of C. albicans, have lessened the efficacy of these drugs and created a need for new antifungal drugs. The fungal phosphatidylserine (PS) synthase (Cho1p) is a good drug target in C. albicans because Cho1p is 1) required for virulence in C. albicans, 2) conserved among fungi, and 3) absent within the mammalian phospholipid biosynthesis pathways. Identifying chemical compounds that inhibit Cho1p is the first step in finding a new, more effective antifungal drug. In order to identify potential Cho1p inhibitors, we developed a novel, high-throughput drug screen based on the compound Papuamide A. Pap-A, a cyclic depsipeptide, binds specifically to PS on the membrane of wildtype (WT) C. albicans where it causes lysis and death of the cells. Since the avirulent cho1ΔΔ mutant does not produce PS, Pap-A cannot bind to the membrane, and these cells survive. Thus, the cho1ΔΔ displays the phenotype for a compound that inhibits the Cho1p enzyme: survival in the presence of Pap-A. We screened over 5,500 compounds with Pap-A and identified the compound SB-224289 as a potential inhibitor of fungal PS synthesis. Further assays confirmed that SB-224289 confers Pap-A resistance to C. albicans at a 50μM concentration. Current studies to elucidate the mechanism of action of SB-224289 are underway, including TLC, LC-MS/MS, PS synthase assays, and fluorescence microscopy.

233B

CHARACTERIZATION AND UTILIZATION OF THE CANDIDA ALBICANS HAPLOID TOOL STRAINS

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The recent discovery of haploids in Candida albicans and construction of a set of stable

haploid tool strains with multiple auxotrophic markers promise to greatly speed up the molecular and genetic study of C. albicans biology and pathogenicity. Although such haploids have essentially the same characteristics as the diploids, their genomes are somewhat unstable and spontaneously duplicate (auto-diploidization) at different frequencies. To facilitate the utilization of these haploid tool strains, we further analyze their genome stability during routine genetic and molecular manipulations. All the haploid tool strains are highly stable upon refreshing from -80 C glycerol stock. During the transformation for targeted gene deletion, the strain (GZY803) containing a single auxotrophy (ura3) consistently shows a correct targeting rate of ~90-100% and an auto-diploidization rate of ~10-20%. In contrast, the rates of auto-diploidization and mis-targeting are a little higher, although varied, in other haploid tool strains (GZY815, GZY822, and GZY823) with multiple auxotrophic markers (ura3, arg4, his1, and lys2). Nevertheless, successful deletion of a target gene can be readily achieved in each of the haploid tool strains. Moreover, typical cell biology and biochemical works, such as visualization of GFP/mCherry-tagged proteins and detection of HA/Myc-tagged protein, have been demonstrated to be applicable in these haploid tool strains.
We have used GZY803 to construct a mini-library containing deletion mutants of ~40 genes encoding GTPase and their regulators. The mutants were screened for various biological defects such as temperature sensitivity, defective hyphal formation on Spider and Lee's plates, sensitivity to DNA damaging agents (HU) and different cell wall stresses (CFW, SDS, and Congo Red). The screens have revealed new regulators of several cellular processes including morphogenesis, membrane trafficking, and cell wall integrity, demonstrating that the haploid strains are useful tools for genetic and molecular study of gene functions in C. albicans. More detailed characterization of these mutants is now in progress.

234C

SMALL MOLECULE INHIBITORS OF CANDIDA ALBICANS FILAMENTATION THAT IMPAIR BIOFILM FORMATION ON MULTIPLE SURFACES

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By high-throughput phenotypic screening of small molecules, we identified compounds that inhibit adhesion of C. albicans to polystyrene. Our lead candidate compound also inhibits binding of C. albicans to cultured human epithelial cells, the yeast-to-hyphal morphological transition, induction of the hyphal-specific HWP1 promoter, biofilm formation on silicone mesh, pathogenesis in a nematode infection model, and alters fungal morphology in a mouse mucosal infection assay. We termed this compound filastatin, based on its strong inhibition of filamentation, and use chemical genetic experiments to show that it acts downstream of multiple signaling pathways (Fazly et al., PNAS 2013). We will present new data on the ability of filistatin and related compounds to affect Candida biofilm formation on a variety of surfaces.

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