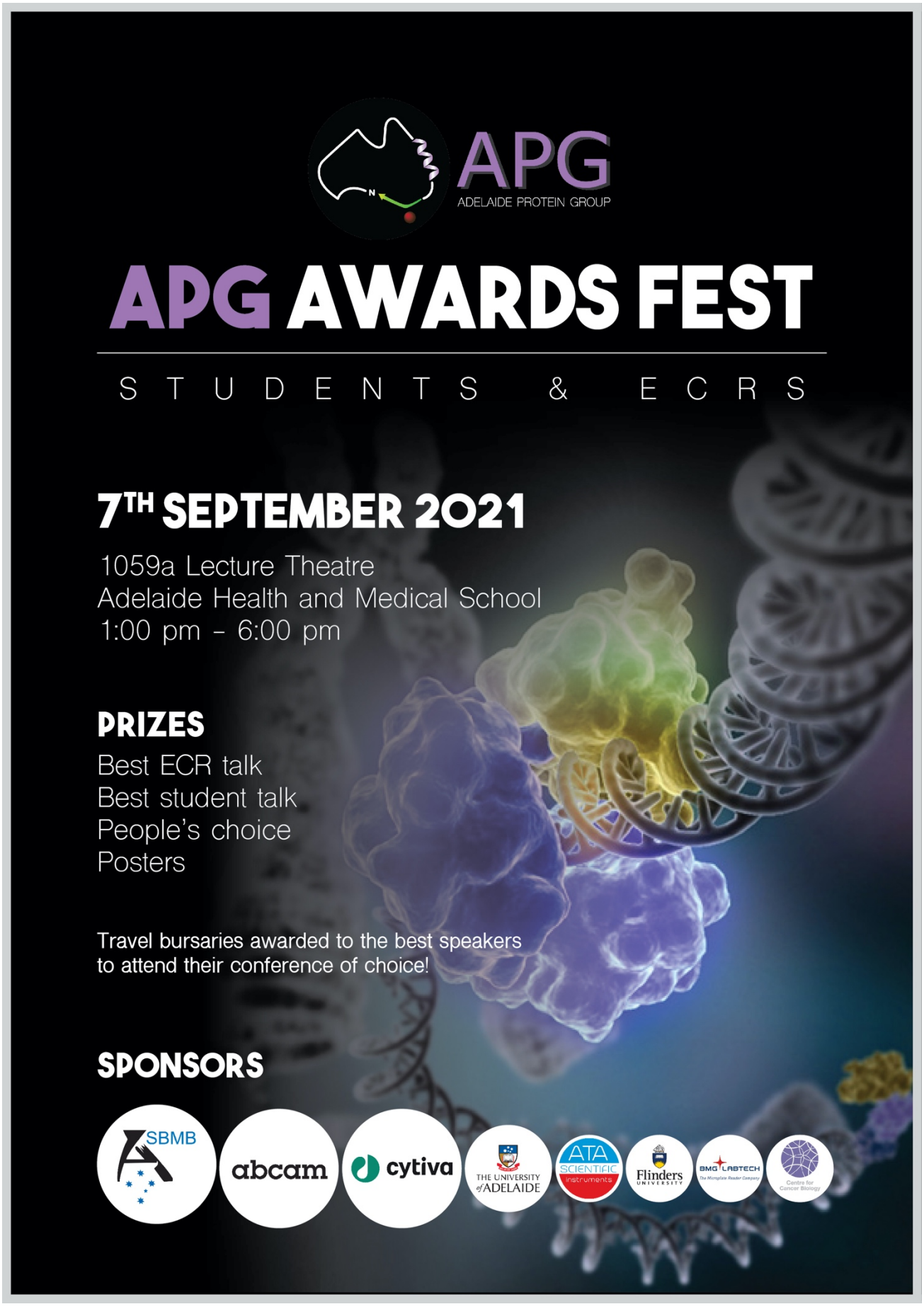
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VIRTUAL ZOOM DETAILS

Time: Sep 7, 2021 1:00 PM Adelaide
https://adelaide.zoom.us/j/84191164459?pwd=ZDFKcWVJdkxQK2xSSG1FUytDVDVhZz09
Passcode: 475207
Meeting ID: 841 9116 4459

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GUEST

Prof. Trevor Lithgow

ECRS

Dr. Sonja Frolich

Dr. Byron Shue

Dr. Andrew Hao

HDRS

Kimberley McLean

Marina Zupan

Isabelle Henshall

Jordan Pederick

Ellen Potoczky

Emily Kirby

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- GUEST SPEAKER -

PROFESSOR TREVOR LITHGOW

Director, Centre to Impact Antimicrobial Resistance
Monash Biomedicine Discovery Institute

ABOUT

For more than ten years (1999–2009) Associate Professor Lithgow held a teaching and research appointment at the University of Melbourne and, in 2009, was awarded an ARC Federation Fellowship to move to Monash University. This provided a means to apply new imaging technology and comparative genomics to understand the evolution of protein transport machines in bacteria. Professor Lithgow also led a mission funded by two cycles of NHMRC Program Grants, with collaborators at other institutes in Australia and the UK, to drive training for the next generation of research leaders in bacterial cell biology, bacteriophages and antimicrobial resistance (AMR) mechanisms. In the period 2014–2019, he was awarded an ARC Laureate Fellowship to bring capacity in cryo-electron microscopy, neutron reflectrometry and super-resolution microscopy to Monash, to address questions in bacterial cell biology using nanoscale imaging. In 2020, Lithgow established the Centre to Impact AMR at Monash and currently serves as Director.

Outside of science, Trevor spends most of his time with family but has also built a garden of indigenous plants on the Mornington Peninsula. Here he divides his time between bird-watching, pulling out weeds and trying to avoid contracting Buruli ulcer.

PROGRAM

12:30-1:00pm (ACST) - Free coffee for registered attendees
Collect your voucher for The Espresso Room when you check in (available from 12:30pm)

OPENING ADDRESS AND KEYNOTE SPEAKER

1:00-2:00pm (ACST)

1:00-1:10pm- Symposium Chair, Daniel McDougal
Welcome to country, opening of symposium and keynote speaker introduction

1:10-2:00pm- Keynote Speaker, Prof. Trevor Lithgow
Working to reverse the evolution of AMR in the bacterial 'superbug' *Klebsiella*.

EARLY CAREER RESEARCHER AWARD PRESENTATIONS

2:00-3:00pm (ACST)

2:00-2:20pm- Dr. Sonja Frölich
Super-resolved view of PfCERLI1, a rhoptry associated protein essential for *Plasmodium falciparum* merozoite invasion of erythrocytes

2:20-2:40pm- Dr. Nan (Andrew) Hao
The pIT5 Plasmid Series, an Improved Toolkit for Repeated Genome Integration in *E. coli*

2:40-3:00pm- Dr. Byron Shue
The characterization of the antiviral protein Viperin in monotremes

POSTER SESSION/AFTERNOON TEA

3:00-3:50pm (ACST)- Poster session
Light refreshments provided

PLATINUM SPONSOR 1

3:50-4:00pm (ACST)- Message from our Platinum Sponsor
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ANNUAL GENERAL MEETING

4:00-4:15pm (ACST)

4:00-4:05- Chair, Dr. Erin Brazel
Chair's annual report

4:05-4:10pm- Treasurer, Kimberley McLean
Treasurer's annual report

4:10-4:15pm- Chair, Dr. Erin Brazel
Other business and election of 2021-2022 Committee

STUDENT AWARD PRESENTATIONS

4:15-5:45pm (ACST)

4:15-4:30pm- Isabelle Henshall
Exploring the function and antibody interactions of the malaria parasite Merozoite Surface Protein 2 during red blood cell entry.

4:30-4:45pm- Emily Kirby
CRISPR Activating your Genome to Identify Novel Viral Host Restriction Factors

4:45-5:00pm- Kimberley McLean
Site specific mutations of GalR affect galactose metabolism in *Streptococcus pneumoniae*

5:00-5:15pm- Jordan Pederick
Characterising the function of a putative ATP-grasp ligase in amino acid metabolism of *Staphylococcus aureus*

5:15-5:30pm- Ellen Potoczky
PR/Set Domain 5: A Critical Transcriptional Regulator of Craniofacial Development

5:30-5:45pm- Marina Župan
The molecular basis for zinc uptake via *Streptococcus pneumoniae* AdcAII

PLATINUM SPONSOR 2

5:45-5:50pm- Message from our Platinum Sponsor
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AWARDS AND CLOSING ADDRESS

5:50-6:00pm (ACST)

5:50-5:55pm- Symposium Chair, Daniel McDougal
Announcement of award recipients

5:55-6:00pm- Symposium Chair, Daniel McDougal
Closing of symposium

EARLY CAREER RESEARCHER AWARDS

EARLY CAREER RESEARCHER FINALIST

Super-resolved view of PfCERLI1, a rhoptry associated protein essential for *Plasmodium falciparum* merozoite invasion of erythrocytes

Sonja Frölich(1*), Benjmain Liffner(1), Garry Heinemann(1), Boyin Liu(2), Stuart Ralph(2), Matthew Dixon(2), Tim-Wolf Gilberger(3) and Danny Wilson(1)

[1] Research Centre for Infectious Diseases, School of Biological Sciences, University of Adelaide, Adelaide, SA 5005, Australia.
[2] Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC 3010, Australia
[3] Bernhard Nocht Institute for Tropical Medicine, 20359 Hamburg, Germany. 5 Centre for Structural Systems Biology, 22607 Hamburg, Germany.

Invasion of human erythrocytes by *Plasmodium falciparum* merozoites involves the coordinated release of ligands from specialised organelles, the micronemes and rhoptries, which secrete parasite proteins onto host red blood cell (RBC) to prime attachment, mechanical entry and establishment of parasitophorous vacuole. Data to date suggests that prior to RBC entry, the two rhoptries fuse to the merozoite plasma membrane before neck contents can be released and the irreversible point of attachment to the RBC forms (the tight-junction). As invasion proceeds, fusion of the two rhoptries commences at the neck and continues to the bulb before the structure partially collapses to facilitate release of rhoptry bulb contents. Despite the importance of the protein network orchestrating the rapid multi-step, process of rhoptry secretion, the proteins involved are largely uncharacterised. Recently, we utilised knockdown studies, biochemical assays, electron and quantitative super-resolution microscopy and identified an essential role for conserved proteins *P. falciparum* Cytosolically Exposed Rhoptry Leaflet Interacting protein 1 and 2 (PfCERLI1 and 2) in rhoptry function and merozoite invasion. While further studies are required to determine exactly how loss of PfCERLI1 and 2 causes these changes in rhoptry function, use of semi-automated quantitative immunofluorescence microscopy highlights how this powerful tool can be used to study RBC invasion.

EARLY CAREER RESEARCHER FINALIST

The pIT5 Plasmid Series, an Improved Toolkit for Repeated Genome Integration in *E. coli*

Nan Hao (1*)(2), Qinqin Chen (1), Ian B. Dodd (1), and Keith E. Shearwin (1)

1. Department of Molecular and Biomedical Science, School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia;
2. CSIRO Synthetic Biology Future Science Platform, Canberra, ACT 2601, Australia;
* Presenting Author

We describe a new set of tools for inserting DNA into the bacterial chromosome. The system uses site-specific recombination reactions carried out by bacteriophage integrases to integrate plasmids at up to eight phage attachment sites in *E. coli* MG1655. The introduction of mutant *loxP* sites in the integrating plasmids allows repeated removal of antibiotic resistance genes and other plasmid sequences without danger of inducing chromosomal rearrangements. The protocol for Cre-mediated antibiotic resistance gene removal is greatly simplified by introducing the Cre plasmid by phage infection. Finally, we have also developed a set of four independently inducible expression modules with tight control and high dynamic range which can be inserted at specific chromosomal locations.

EARLY CAREER RESEARCHER FINALIST

The characterization of the antiviral protein Viperin in monotremes.

Byron Shue (1*), Tahlia J. Perry (2), Emily N. Kirby (1), Linda Shearwin-Whyatt (2), Frank Grützner (2) and Michael R. Beard (1)

1. Research Centre for Infectious Diseases, Department of Molecular and Biomedical Science, The University of Adelaide, Adelaide, SA 5005, Australia
2. The Environment Institute, School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia
* Presenting Author

Viperin is an interferon stimulated gene (ISGs) which is highly upregulated in response to viral infection. It possesses antiviral activity against a wide range of viruses belonging to multiple viral families. Viperin is evolutionary highly conserved from invertebrates such as oysters to chicken, crocodiles and mammals, demonstrating its importance in controlling viral infection. To investigate the evolution of viperin protein structure and associated antiviral activity within mammals, monotremes, comprising the echidna and the platypus were utilised due to their evolutionary divergence from both marsupials and placentals.

In this study, we have for the first time transfected fibroblasts of both echidna and platypus origin with either dsRNA and dsDNA viral mimics or Semliki Forest Virus, which are potent inducers of the interferon signaling cascade and viperin production. qRT-PCR analysis has revealed that these treatments in both species induce a robust innate immune response, resulting in significant upregulation of ISGs including viperin, to generate an antiviral environment within the cell almost identical to that seen in mammalian cell lines. Structural modelling of monotreme viperin genes revealed the presence of an amphipathic helix, which is known to tether viperin to ER membranes and lipid droplets and thus impart antiviral activity against viruses belonging to multiple viral families. In conclusion, this study highlights both the presence and evolution of the antiviral protein viperin and the canonical antiviral innate immune response in monotremes.

STUDENT AWARDS

STUDENT FINALIST

Exploring the function and antibody interactions of the malaria parasite Merozoite Surface Protein 2 during red blood cell entry.

Isabelle Henshall (1)*, James Beeson (2) and Danny Wilson (1)

1. Research Centre for Infectious Diseases, School of Biological Sciences, The University of Adelaide
2. Burnet Institute. Melbourne
*. Presenting Author

The human malaria parasite *Plasmodium falciparum* causes 400,000 deaths every year, the majority of which are in children. Essential for long-term malaria control is the development of a highly efficacious vaccine. A key target for vaccine development is the exposed merozoite form of the malaria parasite which enters red blood cells (RBCs) and is the key first step in disease causing blood stage multiplication. Merozoite Surface Protein 2 (*Pf*MSP2) is an abundant protein on the surface coat which surrounds the merozoite. Antibodies which recognise *Pf*MSP2 are associated with protection and Combination B, a vaccine featuring *Pf*MSP2, in a Phase 1/2b trial reduced parasite density by 62% in a *Pf*MSP2 allele specific manner. However, *Pf*MSP2 function and how antibodies targeting this antigen provide protection are not well understood. Using phylogenetic analysis and CRISPR-Cas9 gene-editing, we show that *Pf*MSP2 is likely an ancient malaria protein but it is not required for RBC entry, debunking long-held beliefs that this protein recently evolved in *P. falciparum* and that it is essential for parasite survival. Inhibitors of early merozoite and RBC interactions, where *Pf*MSP2 was thought to function, did not show additive entry-blocking activity with *Pf*MSP2 knockout, indicating that *Pf*MSP2 is unlikely to have an essential mechanical role in entry. However, *Pf*MSP2 knockout was found to sensitise parasites to growth inhibition from antibodies targeting a different leading merozoite vaccine candidate. Together, these data suggest that *Pf*MSP2 may act as an immune shield protecting key merozoite proteins from the inhibitory effects of protective antibodies.

STUDENT FINALIST

CRISPR Activating your Genome to Identify Novel Viral Host Restriction Factors

Emily N. Kirby (1)*, David C. Bersten (1), Bastien Llamas (2), Byron Shue (1), Ornella Romeo (1), and Michael R. Beard (1)

1. Department of Molecular and Biomedical Sciences, School of Biological Sciences, The University of Adelaide, SA, 5005, Australia
2. Department of Ecology and Evolutionary Biology, School of Biological Sciences, The University of Adelaide, SA, 5005, Australia
*. Presenting Author

The Flaviviruses include the human pathogens Dengue Virus (DENV), Zika Virus (ZIKV) and West Nile Virus (WNV), all which elicit a significant global health burden. Host cellular proteins play a critical role in inhibiting all stages of the viral lifecycle. Identification and characterisation of anti-viral cellular proteins is essential to furthering our understanding of Flavivirus replication kinetics and potential development of effective anti-viral strategies.

Genome wide CRISPR/dCas9 activator (CRISPRa) screens utilise the activity of human and viral transcriptional activators to recruit transcriptional machinery to the proximal promoter of a target gene, subsequently allowing gene upregulation. A CRISPRa screen was employed to identify novel host restriction factors for the WNV variant, Kunjin Virus (endemic to Northern Australia), our screen selected for cells resistant to KUNV induced cytopathic cell death, suggesting activation of anti-viral cellular proteins.

Bioinformatics analysis allowed this novel strategy to identify well characterised anti-viral proteins, such as Interferon Inducible Protein 6 (IFI6) and Interferon Lambda 2 (IFNL2), validating our approach. In addition, we identified putative novel candidates such as Lysine Demethylase 4C (KDM4C) and Sterolin-2 (ABCG8). Confirmation of anti-viral activity, followed by characterisation of a selection of these cellular proteins, will provide a better understanding of the dynamic relationship between Flaviviruses and the cellular intrinsic anti-viral response.

This highlights the capabilities of CRISPRa as a significant genome editing technology, allowing for identification of novel anti-viral proteins and host pathways critical for the inhibition of viral replication, which may aid in development of new anti-viral therapies.

STUDENT FINALIST

Site specific mutations of GalR affect galactose metabolism in *Streptococcus pneumoniae*

Kimberley T. McLean (1)*, Alexandra Tikhomirova (1), Erin B. Brazel (1), Salomé Legendre (1), Gian Haasbroek (1), Vikrant Minhas (1), James C. Paton (1), Claudia Trappetti (1)

1. Research Centre for Infectious Diseases, School of Biological Sciences, University of Adelaide
*. Presenting Author

Streptococcus pneumoniae (the pneumococcus) is a formidable human-adapted pathogen responsible for in excess of 1 million deaths every year. For the pneumococcus to cause disease, it must firstly colonise the human nasopharynx. Colonisation is critical and guarantees that the pneumococcus can spread to other individuals and cause severe disease. A key determinant of colonisation is the ability for the pneumococcus to metabolise the predominant carbon source present in this niche – galactose. Galactose metabolism primarily occurs through the Leloir pathway in *S. pneumoniae*, which is governed by the regulator GalR. GalR is known to possess three putative phosphorylation sites: S317, T319 and T323. A recent study from our group was the first to show that these phosphorylation sites play a critical role in *S. pneumoniae* galactose metabolism and pneumococcal infection. Specifically, mutation of these putative phosphorylation sites to non-phosphorylatable alanine residues (galRAAA) resulted in a decreased ability for *S. pneumoniae* to grow when galactose is the sole carbon source. Gene expression analyses also show that these amino acid substitutions result in shutdown of the Leloir pathway, with expression of key Leloir pathway genes being virtually undetectable. A murine model of infection also showed a decreased ability for galRAAA to persist in the nose, lungs and ears. This study shows the critical importance of not only GalR, but the putative phosphorylation sites in *S. pneumoniae*. Gaining a greater understanding of the mechanisms underpinning colonisation of the nasopharynx creates new avenues for the exploration of novel prevention and treatment strategies for pneumococcal infection.

STUDENT FINALIST

Characterising the function of a putative ATP-grasp ligase in amino acid metabolism of *Staphylococcus aureus*

Jordan L Pederick (1)*, Nan Hao (2, 3), Aimee J Horsfall (4, 5), Andrew D Abell (4, 5), Keith E Shearwin (2), John B Bruning (1)

1. Institute for Photonics and Advanced Sensing, School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia
2. Department of Molecular and Biomedical Science, The University of Adelaide, Adelaide, SA 5005, Australia
3. CSIRO Synthetic Biology Future Science Platform, Canberra, ACT 2601, Australia
4. ARC Centre of Excellence for Nanoscale BioPhotonics, Adelaide, South Australia, Australia
5. Institute for Photonics and Advanced Sensing, Department of Chemistry, The University of Adelaide, Adelaide, South Australia,
*. Presenting Author

-ABSTRACT NOT PUBLISHED-

STUDENT FINALIST

PR/Set Domain 5: A Critical Transcriptional Regulator of Craniofacial Development

Ellen Potoczky (1)*, Sophie Wiszniak (2), Quenten Schwarz (3)

1. Centre for Cancer Biology and University of South Australia
2. Centre for Cancer Biology and SA Pathology
3. Centre for Cancer Biology and University of South Australia
*. Presenting Author

Development of the craniofacial skeleton is dependent on complex cellular processes involving cellular migration, differentiation and morphogenesis. Disruption of these processes can result in craniofacial defects and have a significant impact on quality of life. Utilizing a novel zebrafish line, this work focuses on the role of a transcription factor, PR/Set Domain 5 (PRDM5), in regulating the progression of craniofacial cartilage development. Interest in this protein stems from its involvement in Brittle Cornea Syndrome; a condition with multiple clinical presentations including extreme corneal thinning and spontaneous corneal ruptures, joint hypermobility and hearing loss. The alignment of chondrocytes, the major cellular component of cartilage, is significantly affected in the lower jaw cartilages of *PRDM5* mutant zebrafish, suggesting polarity mechanisms are defective. As chondrocytes are a derivative of the Neural Crest (NC) stem cell population, we have also investigated the major transitional periods during NC development using real time imaging of transgenic models, along with RNA expression analysis. While no significant changes in NC induction and migration were identified, the expression of key patterning pathways that regulate morphogenesis of the facial primordia are altered. In addition, we found a significant reduction in the major collagen subtype *Collagen Type 2 alpha 1 (Col2a1)* in homozygous mutants, suggesting PRDM5 normally functions as a positive regulator of *Col2a1* expression in cartilage precursors. Given the homology between several lower jaw cartilages of the zebrafish with mammalian middle ear structures, this data provides new insight into the mechanisms by which *PRDM5* mutations contribute to the etiology of Brittle Cornea syndrome.

STUDENT FINALIST

The molecular basis for zinc uptake via *Streptococcus pneumoniae* AdcAII

Marina Župan (1)*, Zhenyao Luo (2,3,4), Victoria Pederick (5), Katherine Ganio (1), Evelyne Deplazes (2), Bostjan Kobe (2,3,4), Christopher McDevitt (1).

1. Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia
2. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia
3. Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, Queensland, Australia
4. Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia
5. Research Centre for Infectious Diseases, School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia
*. Presenting Author

Streptococcus pneumoniae is a globally significant human pathogen that scavenges essential zinc [Zn(II)] ions from the host during colonization and infection. This is achieved by the ATP-binding cassette transporter, AdcCB, and two solute-binding proteins (SBPs), AdcA and AdcAII. AdcAII has a greater role during initial infection, but the molecular details of how AdcAII acquires Zn(II) ions remain poorly defined. This can be attributed to the inability of crystallographic approaches to determine a high-resolution structure of ligand-free AdcAII. Here, we overcame this issue by systematically mutating each of the four Zn(II)-coordinating residues and performing structural and biochemical analyses on the variant isoforms. Structural analysis of Zn(II)-bound AdcAII variants revealed how specific regions within the SBP undergo conformational changes via their direct coupling to each of the metal-binding residues. Quantitative *in* vitro metal-binding assays, combined with affinity determination and phenotypic analyses, revealed the relative contribution of each coordinating residue to the Zn(II)-binding mechanism. These analyses also revealed that in contrast to AdcA, AdcAII can interact with other first-row transition metal ions. Intriguingly, the impact of mutant *adcAII* alleles on the growth of *S. pneumoniae* did not generally correlate with SBP affinity, but was instead consistent with the degree of structural perturbation exhibited in mutant AdcAII proteins. Taken together, our data show that SBP conformation, rather than affinity, is the primary determinant of efficacious Zn(II) uptake in *S. pneumoniae*. Collectively, this study reveals a putative metal-binding mechanism for AdcAII, and highlights how ligand affinity and protein conformational changes are coupled within ligand-receptor proteins.