2021 Molecular Biology and Biochemistry Graduate Colloquium

Abstract Booklet

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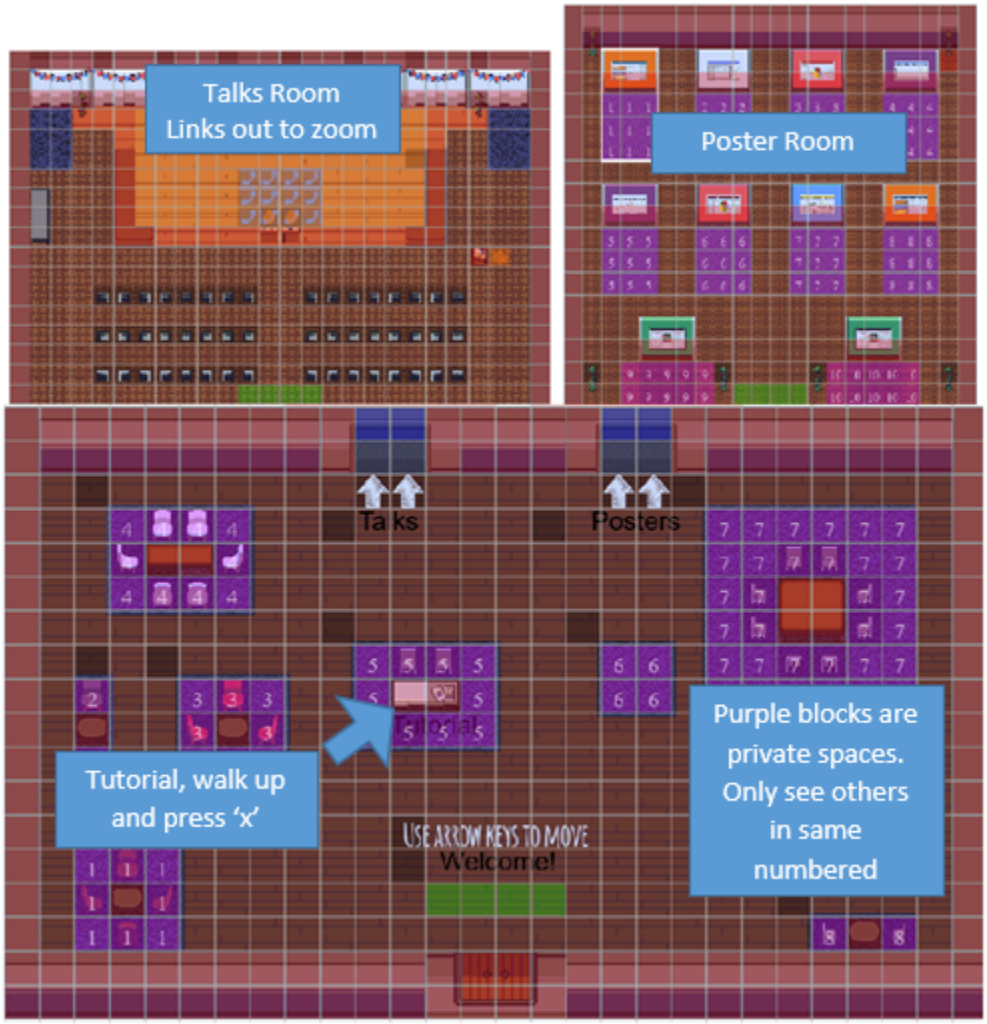
Welcome to the 2021 MBB Colloquium Gather Town!

The MBB colloquium virtual space on Gather.town is similar to an 'onsite' conference, where you can meet your colleagues, friends, and other attendees to strike up conversations just like you would at an in-person event.

Link to the colloquium virtual space:
https://gather.town/app/uPfgw6zL0tyvWrKs/MBBColloquium2021
Password: mbbrocks!

Room Layout & Mini Tutorial



Instructions

- Gather.town is supported on Firefox and Google Chrome on web browsers only (not mobile)

- Click on the link to enter the space. Enter your name and/or change your avatar icon.

- Gather.town will need access to a camera and microphone device.
    - Your microphone and video will automatically start sharing if you move within close proximity of another attendee's avatar.
    - Please turn off your audio and video when you leave the main hallway to not interact with others.
- You can move through the space by using the arrow keys.
- Message people in two ways:
    - Individually by clicking on their name in the participant panel
    - Locally to the people you are video chatting with.
- **Joining the video meetings :**
    - In the keynote, and oral presentation room, press **the phone icon on stage, then press 'x'** and follow the instructions to join the zoom meetings.
    - In the Poster room:
        - As you walk by the posters, you will get a preview of their title and you can view the full poster by pressing 'x' key. Stand in one of the coloured circles near the poster and then press 'x' to interact with the poster and its presenter.
        - You can ask specific questions to the presenter by using a red circular pointer. To activate the pointer, click on and then the part of the poster you wish to point.

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- If you are having bandwidth issues, try turning off your or others' videos by clicking on the video camera icon superimposed on the video (it won't impact the views of others).

Day 1 Schedule (April 22, 2021)

Time	Event		Location	Links
9:30-9:45	Housekeeping and explanation of posters		Zoom	https://sfu.zoom.us/j/69945745266?pwd=WkpMazdsanloWVV1dmg0ck9kc0Fkdz09
9:45-10:30	Prince Kumar Lat Faezeh Borzooee Jenny Liao	10 Minute Talks	Zoom	https://sfu.zoom.us/j/69945745266?pwd=WkpMazdsanloWVV1dmg0ck9kc0Fkdz09
10:30-11:30	Poster Session/ Networking with AbCellera		Gathertown	https://gather.town/i/MFXY2KJu
11:30-11:35	Break			
11:35-11:50	Breanna Raymond Justin Jia Sarah Arthur	Lightning Talks	Zoom	https://sfu.zoom.us/j/69945745266?pwd=WkpMazdsanloWVV1dmg0ck9kc0Fkdz09
11:50-12:20	Mahdi Asgharpour	10 Minute Talks	Zoom	https://sfu.zoom.us/j/69945745266?pwd=WkpMazdsanloWVV1dmg0ck9kc0Fkdz09
12:20-12:30	Closing remarks and reminders for day 2		Zoom	https://sfu.zoom.us/j/69945745266?pwd=WkpMazdsanloWVV1dmg0ck9kc0Fkdz09

Additional Zoom info for Day 1 talks:
Meeting ID: 699 4574 5266
Password: 612854

Poster Session	
April 22nd, 10:30-11:30am	
Poster Presenter	**Poster Number**
Jil Busmann	1
Claire Shih	2
Cally Ho	3
Quiana Ang	4
Alaa Al-Shaer	5
Niveditha Ramkumar	6
Kristen Gray	7
Atefeh Ghorbani	8

Day 2 Schedule (April 23, 2021)

Time	Event Description		Location	Links
9:30-9:40	Housekeeping		Zoom	https://sfu.zoom.us/j/68835991311?pwd=UWhhcWw3RXM2Vi9veHVaejg2WTZUQT09
9:40- 10:00	Elaine (Zhifeng) Wang	Lightning Talks	Zoom	https://sfu.zoom.us/j/68835991311?pwd=UWhhcWw3RXM2Vi9veHVaejg2WTZUQT09
	Stephen Kinsey			
	Casey Engstrom			
	John Zhang			
10:00-11:00	Keynote Speaker	Keynote from Dr. Marco Marra	Zoom	https://sfu.zoom.us/j/68835991311?pwd=UWhhcWw3RXM2Vi9veHVaejg2WTZUQT09
11:00-11:15	Break			
11:15-11:35	Dane Marijan	Lightning Talks	Zoom	https://sfu.zoom.us/j/68835991311?pwd=UWhhcWw3RXM2Vi9veHVaejg2WTZUQT09
	Nicole Thomas			
	Emma Lacroix			
	Franklin Tam			
11:35-12:00	Liliana Vega	10 Minute Talks	Zoom	https://sfu.zoom.us/j/68835991311?pwd=UWhhcWw3RXM2Vi9veHVaejg2WTZUQT09
	Kevin Rey			
12:00-12:30	Closing Remarks		Zoom	https://sfu.zoom.us/j/68835991311?pwd=UWhhcWw3RXM2Vi9veHVaejg2WTZUQT09

Additional Zoom info for Day 2 talks:
Meeting ID: 688 3599 1311
Password: 247819

AbCellera is a Vancouver-based technology company that searches, decodes, and analyzes natural immune systems to find antibodies that its partners can develop into drugs to prevent and treat disease. AbCellera partners with drug developers of all sizes, from large pharmaceutical to small biotechnology companies, empowering them to move quickly, reduce cost, and tackle the toughest problems in drug development. For more information, visit www.abcellera.com.

Networking opportunity during the poster session on Day 1 (April 22) at 10:30-11:30 AM.

10-minute talk abstracts

A Triplex-Quadruplex Hybrid DNA Nanowire Phased and Anchored by G-Quartets

Prince Kumar Lat (MBB, SFU), Clayton Schultz (Chemistry, SFU), Hua-Zhong Yu (MBB & Chemistry, SFU), Dipankar Sen (MBB & Chemistry, SFU)

We report a new DNA nanostructure, an extended 1-dimensional composite built for the first time out of structurally robust yet conveniently disassembled DNA triple helices, interspersed with short stretches of G-quadruplexes. These “TQ Hybrid” 1-dimensional nanostructures/nanowires require potassium ions and modestly acidic pH for their formation and are easily disassembled by changes to either of these requirements. We initially prepared and characterized a “monomeric” TQ Hybrid tile; followed by “sticky” TQs tiles, incorporating unique guanine-only sticky ends, that enable efficient self-assembly via G-quartet formation of nanostructures > 150 nm in length, as seen with atomic force microscopy and transmission electron microscopy. We anticipate that such DNA TQ Hybrid structures will find unique and varied application as communication modules within larger nanostructures, and as sensors, logic gates, as well as in other aspects of DNA nanotechnology.

Examining the role of genome-editing enzymes in anti-tumor immunity

Faezeh Borzooee[1], Krista D Joris[2], and Mani Larijani[1]

[1]Department of Molecular Biology and Biochemistry, Faculty of Science, Simon Fraser University
[2]Immunology and Infectious Diseases Program, Division of Biomedical Sciences, Faculty of Medicine, Memorial University of Newfoundland

Background: Tumor antigens (TA) on cancer cells can trigger immune response TA are peptides that are either not present, or are present to a much lesser extent, on normal cells compared to tumor cells. CD8+ Cytotoxic T-cells (CTL) recognize TAs bound to class I major histocompatibility complex (MHC) molecules on the surface of tumor cells. We and others have shown that viruses subvert the mutational activity of the endogenous DNA-editing APOBEC enzymes in human host cells towards immune escape. Like viruses, cancer cells have genomes that are highly plastic and adaptable through mutation; therefore, we hypothesize that the APOBEC DNA-editing enzymes are exploited by cancer cells for modulating TA immunogenicity.

Aims of the Study

Aim1. To elucidate the impact of A3-mediated mutations on modulating TA immunogenicity for CTL recognition.
Aim 2. To examine whether genomic sequences encoding TAs in the human genome have co-evolved to either attract or avoid APOBEC-driven mutations.

Methods

Aim 1. Using the human reference proteome from EMBL/EBI, we located APOBEC-mutable hotspots in genomic DNA encoding the immunopeptidome. We simulated APOBEC-mediated mutations and translated to peptide TAs. We used NetMHCpan 4 to measure the impact of TA mutations on MHC class I binding affinity. In parallel, we are searching for already-identified APOBEC-mediated mutations in sequenced tumor genomes in cancer genome databases.

Aim 2. To investigate the enrichment of APOBEC-hotspots in genomic sequences encoding TAs restricted to each HLA, we are tabulating APOBEC hotspots in genomic DNA sequencing encoding TAs vs. non-TA-encoding sequences.

Results and Significance

Based on our preliminary data we observe that APOBEC-mediated mutations can diminish or enhance immunogenicity. Understanding the Balance between these two effects is key in informing how APOBEC expression and activity ought to be regarded in the context of cancer immunotherapy.

Cyclin-dependent kinase 8 regulates a Parkinson's Disease Model in Drosophila

Jenny Zhe Liao, Kenneth Kin Lam Wong and Esther M. Verheyen

Cyclin-dependent kinase 8 (Cdk8) is a serine/threonine kinase that acts as part of the Mediator complex to regulate RNA polymerase II-meditated transcription. While its role in transcription has been well established in different model organisms, there is limited information about its potential Mediator-independent functions. We find that when Cdk8 is knocked down ubiquitously in Drosophila, it causes defects in flight and climbing ability, as well as reduced lifespan. Such phenotypes are similar to what is seen in flies mutant for either Pink1 (PTEN-induced putative kinase 1) or Parkin in Drosophila models of Parkinson's Disease. Pink1 is a kinase that functions along with its downstream partner Parkin (an E3 ubiquitin ligase) in quality control of mitochondria. Loss of function in pink1 leads to the accumulation of damaged dysfunctional mitochondria, which in turn impedes processes that require high energy demands such as muscle movement. We find that ectopic expression of Cdk8 can significantly rescue numerous pink1 mutant phenotypes, including locomotor impairment and defects in mitochondrial morphology and integrity. Furthermore, we show that Cdk8 and its obligate partner CycC regulate mitochondrial morphology in the physiological context of a multicellular organism. This is the first description of a role for Cdk8 in modulating Parkinson's Disease phenotypes and mitochondrial morphology under physiological conditions.

Exploring the effects of AID/APOBEC3s genome-mutating enzymes on immune recognition of breast cancer cells

Mahdi Asgharpour (Department of Molecular Biology & Biochemistry, Simon Fraser University)(Immunology and Infectious Diseases Program, Division of BioMedical Sciences, Faculty of Medicine, Memorial University of Newfoundland)
Michael Grant (Immunology and Infectious Diseases Program, Division of BioMedical Sciences, Faculty of Medicine, Memorial University of Newfoundland)
Mani Larijani (Department of Molecular Biology & Biochemistry, Simon Fraser University)(Immunology and Infectious Diseases Program, Division of BioMedical Sciences, Faculty of Medicine, Memorial University of Newfoundland)

Introduction: AID/APOBEC3s are DNA-editing enzymes that mutate immune system genes or virus genomes to boost immunity. However, it is established that AID/APOBEC3s are an endogenous source of DNA damage leading to initiation and evolution of different cancer types, including breast cancer. On the flip side, there are few recent hints in the literatures that AID/APOBEC3s may also play anti-tumor roles. These emerging clues led us to hypothesize that AID/APOBEC3s can play both pro- and anti-tumor roles, depending on the circumstances of their expression.

Methods & Results: Using a Tet-On Advanced gene expression system, we developed stable MCF-7 breast cancer cell lines expressing AID, APOBEC3B, and APOBEC3G with eGFP as the reporter gene. After 24 h of treating cells with different concentrations of Dox to induce different levels of AID/APOBEC3s, the cells were labelled with 51Cr and co-cultured with a human NK cell line. The results showed that at certain expression levels, AID/APOBEC3s could significantly enhance and/or reduce cytotoxicity of NK cells. Using PBMCs, we performed Cr-release assay with primary NK cells and obtained similar results. Expression level of key factors involved in immune recognition of cancer cells, including NK-activating (MICA/B) and NK-inhibitory ligands (PD-1L and HLA-A/B/E), were assessed at both the mRNA and protein levels. The results were in accordance with the observed effects of AID/APOBEC3s on killing ability of NK cells.
Conclusion: We conclude that AID/APOBEC3s could indeed play both pro- and anti-tumor roles, depending on their expression levels. This finding suggests the possibility of future therapeutic avenues through exploiting these enzymes.

Acknowledgment: This research is supported by funding from CIHR and IDRC. Also, Mahdi Asgharpour was a trainee in the Cancer Research Training Program of BHCRI, with funds provided by the TFRI.

Investigating GEI-4, a DSH-2 interacting protein, in Wnt pathway regulation

Liliana Vega, MBB SFU and Nancy Hawkins, MBB SFU.

Asymmetric cell division is one mechanism that generates cellular diversity in multicellular organisms. In C. elegans, a variation of the Wnt pathway, the Wnt/βcatenin asymmetry pathway (WβA), regulates asymmetric cell division. One key component of the Wnt pathway is the cytoplasmatic scaffolding protein Dishevelled. In C. elegans, loss of the Dishevelled homolog, dsh-2, disrupts many asymmetric divisions. Through a yeast two hybrid interactome, DSH-2 has been reported to interact with a protein called GEI-4. GEI-4 is required for embryonic viability but has not been previously shown to have a role in the Wnt signaling pathway. Here, we describe genetic interactions between dsh-2 and gei-4 indicating that their physical interaction is biologically relevant. RNAi knockdown of gei-4 shows partial synthetic lethality with the loss of dsh-2. In addition, genetic interactions are observed between gei-4 and dsh-2 in the asymmetric division of the two somatic gonadal precursor (SGP) cells, Z1 and Z4. In wild-type hermaphrodites, Z1 and Z4 divide along the proximal–distal axis to produce daughter cells with distinct fates. The distal daughters each generate a distal tip cell (DTC), while the proximal daughters generate either an anchor cell (AC) or a ventral uterine (VU) cell. Loss of zygotic dsh-2 function often results in an asymmetric cell defect in which the distal daughter is transformed into a second proximal daughter. gei-4 RNAi knockdown enhances this dsh-2 phenotype. Moreover, gei-4 RNAi knockdown alone leads to AC duplications. Loss o f gei-4 also leads to abnormal number of seam cells, another phenotype seen in mutants in Wnt signaling pathway components. Together, these results suggest that gei-4 is a novel component of the Wnt/βcatenin asymmetry pathway.

Dysbiosis of the gut microbiota, mucin metabolism, and acetate in vascular rejection

K. Rey[1], W. Enns[1], K. Safari[1], E. Guinto[1], T. Van Rossum[1], F.S.L. Brinkman[1], J.C. Choy[1]

[1]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada
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Background: Transplant therapy is effective in treating end-stage organ failure but immune-mediated rejection of transplanted organs is still a major challenge. The regulation of the immune system is influenced the gut microbiota, in part through the production of short chain fatty acids (SCFAs) by bacteria that ferment dietary resistant starches and intestinal mucins. Our study examines the link between the production of the SCFA acetate from mucin degradation by the gut microbiota and vascular rejection.

Methods: The gut microbiota was disrupted in C57BL/6 mice via administration of an antibiotic cocktail (ampicillin, vancomycin, metronidazole, neomycin sulfate) for only the first 3 weeks of life. Some antibiotic treated mice were cohoused with untreated mice starting at 3 weeks old to normalize their microbiota. The composition of the microbiota was determined by 16S and whole genome shotgun sequencing of fecal samples. Vascular rejection was then examined by transplanting allogeneic aortic segments from BALB/c donors into the C57BL/6 recipients.

Results: Analysis of 16S sequencing indicated that antibiotic treatment persistently altered the relative abundance of several bacterial classes. Inference of metabolic genes from 16S sequences suggested that antibiotic treatment decreased several enzymatic processes that metabolize mucin. When the metagenome of the gut microbiota was examined by whole genome shotgun sequencing, there was an elimination of Akkermansia muciniphila, a bacterium that degrades mucin. Reduction of A. muciniphila was associated with a decrease in the mucin degrading enzyme acetyl-N-hexosaminidase. Cohousing mice normalized the levels of A. muciniphila and acetyl-N-hexosaminidase. Antibiotic treatment dramatically increased the neutrophil accumulation in aortic transplants, and cohousing was sufficient to reverse this. Finally, providing magnesium acetate in the drinking water also prevented neutrophil accumulation in allograft arteries from antibiotic-treated mice.

Conclusion: The composition of the gut microbiota influences the development of acute vascular rejection, potentially by regulating neutrophil responses via A. muciniphila metabolism of mucin and resultant production of acetate.

3-minute talk abstracts

Molecular identification of green life-stage in the red-celled snow algal genus Sanguina

Breanna Raymond, Casey Engstrom, Kurt Yakimovich, Lynne Quarmby

Coloured algae are contributing to the melting of snowfields in polar and alpine regions across the globe. Particularly common are microalgae belonging to the genus Sanguina, which express high levels of a red pigment. In contrast to white snowfields, streaks and patches of snow turned red by algal growth reduce the albedo of the snow and increase the amount of snow melt. Sanguina is a newly delimited snow algae genus, so far composed of only two species, S.aurantia and S.nivaloides. They are distinguishable morphologically by colour, S.aurantia cells appears orange and S.nivaloides as red, and molecularly by one compensatory base change (CBC) in their ITS2 rDNA secondary structures (CBC analysis serves as a proxy for speciation in the green algae). Little else is known about the diversity of these organisms. To date, there are no published reports of a successful culture of this genus. Our lab has now cultured a strain of green-coloured cells, which we identify as Sanguina, based on two genetic markers, ITS2 and rbcL. Based on maximum likelihood and Bayesian phylogenetic trees, we hypothesize the green cells to be a life stage of S.aurantia. Alternatively, the green cells may be a divergent strain of S.aurantia or a new species within the genus Sanguina. CBC analysis revealed that the green cells have a single CBC difference to S.nivaloides and zero CBC's to S.aurantia. As a baseline for future phylogenetic work within the Sanguina clade and other algae, we are currently sequencing the complete chloroplast genome of the green culture using Oxford Nanopore technologies. In summary, we have for the first time identified the long-hypothesized green life stage of the red snow alga now known as Sanguina. We have also achieved the first successful culture of the genus.

The Endangered St. Lawrence Estuary Beluga's Skin Microbiome and Its Potential Relationship with Halogenated Flame Retardant Exposure

Jia, B.[1*], Lo, R. [1], Allison, M. J. [2], Round, J. M.[2], Helbing, C. C.[2], Verreault, J[3], and Brinkman F.S.L.[1].

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2. University of Victoria, Victoria, BC, Canada
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Multiple sources of contaminants (e.g. industry runoff) represent toxicological hazards to coastal marine mammals. Persistent chemicals such as polybrominated diphenyl ethers (PBDEs) accumulate at high levels in the blubber of St. Lawrence Estuary belugas, posing a continuous threat to an already endangered population. Current beluga contaminant monitoring relies on skin biopsies, a highly invasive procedure that is challenging for the study of marine mammal populations. Skin microbiome analyses represent an innovative and non-destructive biomarker approach to monitor environmental contaminants and animal health. Presented here is an investigation of the beluga skin microbiome communities to understand the relationship between biopsy concentrations of halogenated flame retardants and the skin microbiome and, to identify potential biomarkers for early detection of altered ecosystem health. Biopsy samples were collected from the dorsal regions of adult St. Lawrence Estuary belugas and analyzed for the concentrations of 35 PBDEs and 13 emerging halogenated flame retardants. The skin microbiome was obtained through 117 samples, including skin swabs, sea water controls and sequencing controls using 16S amplicon-based DNA sequence analysis on the Illumina MiSeq platform. Further metadata analysis, including contaminant-microbiome differential abundance analysis, was initiated using the taxonomic profiles of the skin microbiomes, sample metadata, and contaminant metadata. Skin microbiome analysis revealed that belugas have their own distinct skin microbiome, which differs from the surrounding seawater. There were no significant differences between the skin microbiome of male and female belugas nor at different geographic regions within the St. Lawrence Estuary. However, notably, we identified several bacterial taxa at the phylum and genus level that were strongly correlated with concentrations of contaminants, which warrant further investigation as potential biomarkers. Results to date suggest a potential utility of skin microbiome analysis for non-invasive monitoring of contaminants in belugas that warrants further study as a tool to aid investigation of such species at risk.

Characterization of NFKBIZ 3' UTR Non-Coding Mutations in Diffuse Large B-Cell Lymphoma

Sarah E Arthur[1,2], Nicole Thomas[1], Christopher Rushton[1], Jeffrey Tang[1], Miguel Alcaide[1], Adèle Telenius[2], Shannon Healy[2], Anja Mottok[2], Razvan Cojocaru[1], Peter Unrau[1], David W Scott[2], Christian Steidl[2] and Ryan D Morin[1,3]

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The activated B-cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL) is characterized by activation of NF-κB signaling. Although many genes with recurrent mutations activating this pathway have been discovered in ABC DLBCL, there still remain cases with no known genetic basis for NF-kB pathway activation, suggesting our understanding of ABC DLBCL drivers remains incomplete. Previously, NFKBIZ was shown to be amplified in 10% of ABC DLBCLs and to contribute to activation of NF-κB signaling. We have recently described a novel pattern of non-coding mutations affecting the 3' UTR of NFKBIZ, resulting in an overall mutation rate of 25% (UTR or AMP) in ABC DLBCL. These NFKBIZ UTR mutations are mutually exclusive with MYD88 mutations, a known driver of ABC DLBCL, thus suggesting they may also lead to activation of NF-κB signaling. We hypothesized that NFKBIZ UTR mutations affect the normally rapid degradation of this mRNA by disrupting secondary structures recognized by RNA-binding proteins. The resulting elevated NFKBIZ mRNA levels cause accumulation of protein and act as a novel mechanism to promote cell growth and survival in ABC DLBCL. CRISPR-induced NFKBIZ mutations in DLBCL cell lines confirmed that UTR mutations lead to elevated mRNA and protein levels. Additionally, these mutant lines had a selective growth advantage over WT when grown together, both in vitro and in vivo. RNA-sequencing of mutant and WT lines revealed possible transcriptional targets of NFKBIZ including some genes commonly over-expressed in ABC DLBCL. Targets of NFKBIZ implicated in treatment response were also discovered, including PD-L1 and HCK. To evaluate the effect of NFKBIZ mutations on drug responses, several drugs were tested on WT and mutant lines; NFKBIZ mutant cells had a significantly higher IC50 for ibrutinib, idelalisib and masitinib, but not bortezomib. This highlights the potential utility of NFKBIZ mutations as predictive biomarkers of treatment response.

Tip-associated protein PilY1 as a potential target for antibiotic delivery

Elaine Wang and Dr. Lisa Craig

Multidrug-resistant Acinetobacter baumannii is a rapidly emerging pathogen in the health care setting. Like all Gram-negative bacteria, A. baumannii is intrinsically resistant to large antibiotics due to its outer membrane, which acts as a physical barrier. To overcome this barrier, we in the Craig lab are investigating a natural uptake system, the Type IV pilus (T4P), as an antibiotic delivery system to sensitize Gram-negative pathogens like A. baumannii to large antibiotics (e.g vancomycin). The virulence factor T4P are dynamic, retractile, and multi-functional filaments present on many bacteria surface, often essential for host cell adherence, biofilm formation, cell twitching motility, and acquisition of foreign DNA. My research focuses on a putative T4P tip-located protein, PilY1, which is indispensable for efficient pilus assembly and for T4P functions including DNA up-take, yet is poorly understood. The goal of my project is to characterize PilY1 and determine if it is a good target for pilus-mediated antibiotic delivery for A. baumannii. I have expressed and purified the N-terminal domain of PilY1and obtained crystals for structure determination. I am investigating the localization of PilY1 and its role in natural transformation. These results with A. baumannii T4P may be generalizable for other Gram-negative bacterial pathogens and inform us on the validity of PilY1 as a target molecule for antibiotics delivery.

Using Drosophila to compare the functions of Human HIPKs

Stephen Kinsey, Esther Verheyen

Homeodomain-interacting protein kinases (Hipks) are a family of conserved proteins that are necessary for development in both invertebrate and vertebrate organisms. Vertebrates have four paralogues, Hipks 1-4. Mice lacking Hipk1 or Hipk2 are viable, however loss of both is lethal during early embryonic development, with embryos exhibiting homeotic skeletal transformations and incorrect HOX gene expression. While these results suggest Hipks have a role in regulating HOX genes, a regulatory mechanism has not been characterized, and further comparisons of the roles of Hipks in development has not progressed. A major challenge with characterizing developmental regulators in vertebrates is the extensive redundancy of genes. For this reason, we used Drosophila melanogaster, which has reduced genetic redundancy, to study the functions of the four human HIPKs (hHIPKs). In Drosophila, zygotic loss of the single ortholog dhipk results in lethality with distinct eye and head defects. We found that replacing dhipk with either hHIPK1 or hHIPK2 rescued lethality, while hHIPK3 and hHIPK4 only rescued minor dhipk mutant patterning phenotypes. Following this evidence of conserved functions between human and Drosophila Hipk's, we directed our efforts to identify and compare specific roles of hHIPKs by expressing them in well-defined tissue domains and monitoring changes in phenotypes. We observed unique patterns of homeotic transformations in flies expressing hHIPK1, hHIPK2, or hHIPK3 caused by ectopic induction of Hox proteins, including wing-to-haltere transformation, ectopic sex combs, malformed legs, and partial arista-to-leg transformation. These results were indicative of inhibited Polycomb-group complex (PcG) components, suggesting that hHIPKs play a role in regulating its activity. Preliminary data suggests that HIPKs colocalize with PcG components in the nucleus as puncta on chromatin. Together, this data shows that hHIPKs function in Drosophila, where they appear to have variable ability to inhibit PcG, which may reflect their roles in development.

Remote Sensing Interannual Trends in Snow Algae Blooms

Casey Engstrom (1), Scott Williamson (2), David Hik (3), Lynne Quarmby (1)

1: Dept. Molecular Biology and Biochemistry, Simon Fraser University
2: Dept. Geography, University of Northern British Columbia
3: Dept Biology, Simon Fraser University

Pink snow algae blooms grow in polar and alpine regions worldwide, reportedly covering vast areas of summer snowfields. These blooms reduce albedo, thereby increasing snowmelt and accelerating glacial retreat. Snow algae growth requires liquid water, nutrients, and sunshine so an obvious hypothesis is that higher summer temperatures with more snowmelt would result in larger blooms. Ultimately, we would like to know whether global warming is increasing the intensity, duration, and extent of snow algae blooms. The Snow Darkening Index (SDI) has previously been used as a proxy for snow algae abundance, so we used 20 years of MODIS satellite data to track SDI at six glacier sites in western North America. Consistent with expectations, SDI began to increase shortly after the onset of melting temperatures, when snowmelt releases nutrients and liquid water. Positive SDI values can also indicate mineral dust, so we trained a random forest classifier to distinguish snow algae from mineral dust in Sentinel-2 images; across all available images at our study sites 98% of pixels were classified as algae. We used the yearly sum of SDI (“SDI day sum”, i.e. area under the curve) as a proxy for intensity and duration of snow algae blooms. SDI day sum was highly variable between years and regions, and southern sites had consistently higher SDI values than northern sites. SDI day sum increased at all sites over the past 20 years, and increased as a function of air temperature (degree day sum). These initial results suggest that hotter summers result in more intense and prolonged snow algae blooms. Currently I am using high resolution imagery to map annual bloom extent; we predict that bloom area will also increase with warmer temperatures.

Exploiting T4P as a novel method of antibiotic delivery

John Zhang, Lisa Craig

The rise in antibiotic resistant bacterial pathogens coupled with a dearth of new antibiotics in development poses a significant threat to the global health system. Gram-negative pathogens are inherently resistant to large antibiotics such as vancomycin owing to their outer membrane barrier, emphasizing the need for alternative therapeutic strategies. I am investigating the use of Type IV pili (T4P) as a novel antibiotic uptake system for Gram-negative bacterial pathogens. T4P are long, thin, retractile protein polymers expressed on the surfaces of human pathogens including Vibrio cholerae, enterotoxigenic Escherichia coli (ETEC) and Neisseria gonorrhoeae. These filaments are composed of thousands of major pilins and a few minor pilins, the latter of which form a priming complex that initiates pilus formation and are thought to localize to the pilus tip, with roles in adhesion and DNA uptake. The minor pilin TcpB is located at the tip of the Vibrio cholerae T4P and is the receptor for the filamentous bacteriophage CTXΦ. I hypothesize that TcpB and other minor pilins can be targeted by antibody and DNA aptamer carriers to deliver antibiotics into bacterial pathogens via T4P retraction, bypassing the outer membrane. To this end, I identified, from phage display libraries, antibody fragments (Fabs) specific for V. cholerae TcpB as well as for minor pilins from Neisseria gonorrhoeae, Neisseria meningitidis and ETEC. I cloned, expressed, and purified four TcpB-specific Fabs and demonstrated their ability to bind to recombinant TcpB. The Fab candidates are then assessed for binding to the pilus tip and their ability to block T4P-mediated phage uptake. Strong, specific binders will be conjugated to vancomycin and assessed for T4P-mediated killing of Vibrio cholerae. High affinity Fabs specific for T4P tips could repurpose potent antibiotics such as vancomycin to act on Gram-negative bacteria, providing novel avenues of curbing antibiotic resistance.

Aggregation Discrimination Regulation

Dane Marijan, Tim Audas

All cells must respond to changing conditions if they are to survive. One of the ways mammalian cells react to harsh stimuli is by forming physiological, RNA-seeded amyloid bodies (A-bodies) within nuclei. Amyloids are highly organized structures commonly associated with debilitating conditions such as Alzheimer's disease. Formed through the polymerization of proteins adopting a beta sheet-rich conformation, mature amyloid fibers are extremely resistant to degradation, which has heavily contributed to their reputation as an irreversible protein state. A-bodies, however, disassemble upon stimulus termination, highlighting that amyloid aggregation does not always generate toxic structures.

The proteomes of A-bodies induced by different stimuli vary significantly, as their constituent proteins can be sequestered by one or more stressors. This project examines the mechanism and biological effects of this stress-specific amyloid aggregation by using the 90% identical DDX39A and DDX39B proteins as a model. These RNA helicases are differentially detained in A-bodies during heat shock, with only DDX39A consistently being targeted. Our data shows that selectively mutating even single key residues at specific locations has the ability to dictate their respective heat shock targeting. We hypothesize that these key residues are parts of distinct hydrophobic pockets which have a stabilizing effect at high temperatures, preventing A-body targeting by several possible mechanisms. Either directly blocking a necessary shift into a more aggregation prone state; or indirectly, by masking / exposing a post-translational modification or protein binding site, which in turn would regulate recruitment to A-bodies by affecting aggregation propensity or interaction with the seeding RNA.

Discovering the exact mechanism of this stimulus specific amyloid aggregation and its consequences on cell function will advance fundamental knowledge of how cells interact with their surroundings, and clue into how this pathway can be disregulated, which might have interesting implications in protein misfolding disease settings.

Key genetic and molecular aberrations identified in adult Burkitt lymphoma

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Burkitt lymphoma (BL) is the most common B-cell non-Hodgkin lymphoma (NHL) in children accounting for ~50% of pediatric NHLs, while accounting for only 1-2% of adult NHLs. The genetic hallmark of BL is translocations that place MYC under the regulation of an immunoglobulin (IG) heavy or light chain enhancer. The limited available studies comparing pediatric BL (pBL) to adult BL (aBL) have revealed the latter more frequently harbor non-IGH-MYC translocations and possibly display some distinct driver mutation profiles. Because aBL patients have inferior survival rates, there is a need to better understand the genetic and molecular features of aBL to enable more effective treatments and prognostication within this population.
We performed whole genome sequencing and RNA-seq on 208 BL tumors: 124(92 EBV+) pBL and 84(27 EBV+) aBL cases. We analyzed mutation patterns to identify significantly mutated genes (SMGs) and compared their frequencies between aBL/pBL.
In analyzing simple somatic mutations, we identified 4 SMGs not previously associated with BL: TET2, HNRNPU, BRAF, and EZH2. Three of these are commonly mutated in other cancers and at variable rates in diffuse large B-cell lymphoma. We specifically associate TET2 mutations with aBL and TP53 mutations with significantly inferior progression free survival (PFS) in these same aBL patients. HNRNPU mutations have not previously been attributed to any cancer.
This work highlights key mechanisms underlying BL pathogenesis and key genetic differences based on patient age. We show the first evidence of mutations in TET2, HNRNPU, BRAF, and EZH2 to be associated with BL, with TET2 mutations specifically associated with aBL. Among the SMGs, TP53 mutations were predictive of inferior PFS in aBL, presenting a subset of patients to be considered for novel treatment approaches. These findings further elucidate differences between aBL/pBL and highlight model systems for the further development of novel therapeutics exploiting these differences.

Evolutionary Conservation of Systemic and Reversible Amyloid Aggregation

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In response to specific environmental stimuli (heat shock, extracellular acidosis, transcriptional/proteotoxic stress), human cells have been shown to target proteins to the nucleolus where they disrupt ribogenesis, adopt an amyloid conformation, and aggregate together to form a novel structure termed an amyloid body (A-body). Notably, these structures share many of the biophysical characteristics of the pathological amyloid aggregates that are commonly observed in neurodegenerative diseases such as Alzheimer's disease, and Parkinson's disease. In contrast to pathological amyloid aggregation, A-body formation is a reversible adaptation mechanism that induces a state of cellular dormancy to conserve energy during periods of cell stress. To date, A-body formation has been observed only in cultured human cells. Here, we investigate the formation of A-bodies in different species to determine if this pathway is evolutionarily conserved. By subjecting cells derived from different species to environmental stressors, we have found that A-body formation is a pathway conserved in mammals, chickens, fish and fly species, and the conditions forming A-bodies differ based on the organism's environmental norms. Additionally, stress treatments performed on both embryonic and adult flies revealed the formation of these structures in embryonic tissues, and egg chambers. This represents the first observation of A-body formation outside of a cultured tissue environment, and demonstrates the ability of a variety of tissues to harness this reversible amyloid aggregation strategy to withstand stress.

Novel regulatory mechanisms controlling human inducible nitric oxide synthase (iNOS) expression

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Introduction:
Nitric oxide (NO) is a bioactive gas produced by a family of enzymes known as the nitric oxide synthases (NOS) which has many biological effects. When produced by the inducible form of NOS (iNOS) through proinflammatory cytokine stimulation, NO plays a role as an immune effector molecule. Importantly, dysregulation of iNOS and the resultant production of NO is implicated in the pathogenesis of many immune-mediated diseases where it affects tissue injury and regeneration. Therefore, elucidating novel regulatory mechanisms controlling iNOS expression is critical to understanding its role in these diseases. Here, we analyze novel protein interactors with iNOS that may have potential roles in regulating iNOS protein expression and function at the post-translational level.

Approach and Results:
iNOS fused to a protein tag (Halotag) was transfected into A549 human lung epithelial cells to overexpress iNOS protein. Utilizing the Halotag, iNOS was immunoprecipitated and analyzed using a Tandem Mass Tag mass spectrometry workflow. The experiment found a significant and novel interaction with the BAG family molecular chaperone regulator 2 (BAG2) protein that is known to regulate protein ubiquitination. Specifically, BAG2 inhibits the carboxyl-terminus of Hsc70 interacting protein (CHIP), which is a ubiquitin ligase known to target iNOS towards proteasomal degradation. This suggests that BAG2 may regulate iNOS protein levels by preventing CHIP-mediated iNOS proteasomal degradation. The interaction between BAG2 and iNOS is currently being investigated.

Conclusions:
Potential novel iNOS protein interactors have been identified and are currently being studied. The findings made here will elucidate novel regulatory mechanisms by which iNOS protein levels are controlled.

Poster abstracts

#1 Affinity Purification of Endogenous O-GlcNAc Transferase (OGT) in Acute Myeloid Leukemia (AML)

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O-GlcNAc Transferase (OGT) catalyzes the installation of O-linked N-acetylglucosamine (O-GlcNAc) to serine and threonine residues of nuclear and cytosolic proteins. This post translational modification (PTM) has emerged as a regulator of central cellular processes and is of interest due to its importance in health and disease. Elevated OGT and O-GlcNAc levels are a common signature of many cancers including acute myeloid leukemia (AML) and are associated with tumor progression and metastasis. Moreover, knockdown of OGT preferentially impairs cancer cell proliferation. However, the molecular mechanisms that regulate OGT activity are largely unknown. This is, in part, due to a lack of chemical tools to study the structure and function of endogenous OGT and its association with other cellular factors. In this work, I validate and demonstrate the utility of a chemical probe (Fig. 1) that enables affinity purification of endogenous OGT from cellular lysates including AML cells. This probe permits the mapping of native post-translational modifications to OGT as well as the identification of interacting binding partners through mass spectrometry-based proteomic analyses. The results of this study will improve the fundamental understanding of the factors which may govern regulation of OGT in cells and will help unveil mechanisms that are involved in elevating O-GlcNAc levels in AML. This may open the door to exploiting OGT or other actors in the O-GlcNAc pathway as therapeutic targets for AML and other cancers.

#2 Drosophila adducin has a nuclear role in Dlg-mediated synaptic adhesion during larval neuromuscular junction development

Simon Ji Hau Wang, Soo Hyun Yoo, Hae-yoon Kim, Claire Ren Yun Shih, Byoungjoo Yoo, Andrea Di Lorenzo, Nicholas Harden and Charles Krieger

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the loss of contact between presynaptic motor neurons and postsynaptic muscle cells. While there are certain genes associated with familial ALS, most cases of ALS are sporadic, and the molecular mechanism by which this disease causes neurodegeneration is not well understood. Mammalian adducin is a protein involved with regulating synaptic contacts, and there has been evidence that adducin interacts with ALS associated genes. To study this protein and its implication in the mechanism of ALS, the Drosophila melanogaster orthologue of adducin, Hu-li tai shao (Hts), was studied in third instar larvae. The role of nuclear Hts was investigated through UAS-HtsNLS and UAS-HtsNES transgenes that prevent Hts protein from entering and exiting the nucleus, respectively. Here, we show that Hts influences phosphorylation levels of the scaffolding protein Dlg, which appears to regulate neuromuscular junction (NMJ) growth by facilitating adhesion molecules between the motor neuron and muscle. By co-expressing wild-type Hts with transgenic RNAi against nuclear export proteins, we show that delocalization of Dlg from the post-synaptic membrane is affected when Hts is unable to move out of the nucleus. We also provide evidence that Hts binds and regulates the nuclear localization of camkII and par-1 mRNA, which encode kinases that act downstream of Hts and are known to phosphorylate Dlg. With this project, we ascertained the presence of a molecular pathway between Drosophila Adducin with other proteins that may be relevant in regulating synaptic adhesion at the NMJ.

#3 At the crossroads of autophagy and metabolism: discovery of a novel interaction between ATG4B and IMPDH2

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Autophagy is an intracellular catabolic process that plays both pro- and anti-tumorigenic roles depending on the cancers' stage and context. Previous studies from our group have uncovered a role for ATG4B, a core cysteine protease in autophagy, in modulating responses of HER2+ breast cancers to nutrient deprivation and targeted therapy. To date, the molecular mechanisms underlying ATG4B's role in regulating nutrient stress and treatment responses of HER2+ breast cancers remain undefined. To this end, we performed a pilot immunoprecipitation-mass spectrometry study to identify potential protein-protein interactions that may be contributing to ATG4B's roles in breast cancers that overexpress HER2. Here, we describe our discovery of a novel protein-protein interaction between ATG4B and IMPDH2. Using forward and reciprocal immunoprecipitation-western blots, we successfully validated the interaction between ATG4B and IMPDH2 in multiple HER2+ and HER2- breast cancer cell lines. IMPDH2 is a key rate-limiting enzyme in guanine nucleotide biosynthesis, and little is known about its role in breast cancers. Using a publicly available patient dataset of 52 matched breast tumor and normal tissue samples, we found that IMPDH2 protein levels were significantly elevated in breast tumors compared to normal breast tissues. High IMPDH2 protein expression was also associated with significantly reduced overall breast cancer patient survival, supporting a potential role for IMPDH2 in breast tumorigenesis. Functional studies investigating the significance of this novel ATG4B-IMPDH2 interaction and potential links to HER2 signaling, autophagy and cancer metabolism are currently underway. Together, this study has the potential to uncover novel molecular mechanisms underlying ATG4B's role in breast tumorigenesis through interactions with IMPDH2, and may reveal crosstalk between the autophagy pathway and guanine nucleotide metabolism.

#4 Evaluating the antigenicity of CMV gB-based antigens designed to preserve conserved nAb-sensitive epitopes for the elicitation of cross-reactive antibodies.

Quiana Ang and Ralph Pantophlet

Congenital cytomegalovirus (cCMV) infection is a common intrauterine infection in the world, with an overall CMV birth prevalence estimate at 0.7% as of 2007. This virus can lead to devastating effects, including perinatal mortality, and long-term neurologic damage and deafness in 40-58% of the surviving infants. An effective vaccine, given to infants or to pregnant women, is widely considered the best way to prevent cCMV infection. However, no candidate vaccine has yet shown the desired level of efficacy in human clinical trials; at best only 50% efficacy has been achieved. Most human CMV (HCMV) vaccine strategies are focused on the virus envelope glycoprotein B (gB) antigen with the goal of inducing neutralizing antibodies (nAbs), which are antibodies that would block virus infection to prevent CMV acquisition. Here, the construction of more effective gB-based immunogens is explored. CMV gB is an attractive target for vaccine design because of its central role in CMV infection of all cell types. Of the five distinct antigenic sites (AD 1-5) defined by gB-specific antibodies, site 1 on AD-2, AD-4 and AD-5 are known to be vulnerable to nAbs, thus constituting obvious sub-targets within the overall gB antigen. A series of gB-based proteins were produced and assessed through an ELISA to select for ones that are best recognized by anti-gB nAbs relative to non-nAbs, as a surrogate for selecting which gB-based antigens might best be able to evoke nAbs upon immunization.

#5 Sequence-dependent analysis of collagen mechanics

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Extracellular matrix mechanics influence diverse cellular functions, yet surprisingly little is known about the mechanical properties of their constituent collagens. Because of collagens high molecular weight (>300 kDa), there are limitations in using conventional methods to gain structural insight on the protein. New methods for investigating the sequence-dependence of collagen structure in the context of its full-length are needed. Here, we used atomic force microscopy (AFM) image different collagen types and performed statistical analysis to calculate the persistence length – a mechanical property used as a measure of flexibility. By analyzing flexibility in a sequence-dependent manner, we learned that discontinuities in the triple-helix-defining sequence (Gly-X-Y) in collagen IV led to a generally more flexible polymer with notable flexible “hinges” that correlated to the non-helical regions. We contrast these findings by studying collagen III – a continuously triple-helical collagen – and find that it also displayed variable flexibility along its contour. Notably, possessing a high-flexibility region around its matrix-metalloprotease (MMP) binding site, suggesting a unique mechanical fingerprint of this region that is key for matrix remodeling. By comparing collagens with continuous and discontinuous triple-helix-forming sequences, we find that helix interruptions correlate with local flexibility, providing the first steps towards a much-needed map between sequence, structure, and mechanics in these large proteins.

#6 Siks are multifunctional kinases bridging nutritional response and tissue growth

Esther Verheyen, Niveditha Ramkumar

Obesity has been an established risk factor for increased incidence of many cancers. However, the underlying mechanism linking nutrition to tumor growth is still unclear. Siks (Salt-inducible kinases) are proteins that act as molecular sensors for nutrition, promoting energy production in cells taking up glucose. In a former preliminary screen, Drosophila Siks, Sik2 and Sik3, were found to be potential novel regulators of Hipk (Homeodomain interacting protein kinase), an oncogenic protein. Overexpression of Hipk yields overgrowth and this phenotype is rescued when levels of Siks are reduced, and enhanced when Sik levels are increased. Siks can thus promote Hipk function. When Hipk-overexpressing flies are raised on a high-sucrose diet leg malformations are enhanced. These defects could also be rescued when Siks are mutated and lose their functions. These findings indicate that Siks play a role in mediating nutritional response in Hipk-driven tumourous growth. Siks have previously been shown to regulate a multitude of other pathways like the Hippo, Wingless and Notch cascades. Hipk also interacts with these pathways to exert overgrowth effects. Understanding the mechanism by which Siks interact with Hipk, whether directly or via other pathways, and/or whether Siks and Hipk act in parallel to regulate these pathways at various levels, will enable us to better understand the etiology of a multifaceted ailment like cancer.

#7 Impacts of Gene Annotations on Genomic Island Predictions

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Responding to infectious disease outbreaks is complicated by the ongoing evolution of microbial pathogens, necessitating an equally dynamic public health response. Bacterial species, including pathogens, possess a remarkable ability to evolve rapidly and exchange genomic DNA with unrelated lineages in a process known as horizontal gene transfer. Clusters of these horizontally transferred genes, called genomic islands, are of high interest to computationally predict in pathogen genome sequences since they disproportionately encode genes that enable a pathogen to cause disease or resist treatment by some antimicrobials. While tools have been developed to accurately predict genomic islands in completely sequenced genomes, previous computational methods for predicting GIs were not amenable to scalable analysis and visualization of multi-isolate genome sets, despite large-scale genomic approaches to investigating outbreaks becoming increasingly common. To fill this gap, we are developing IslandCompare, an open-source computational tool with web-based visualization that enables comparison of GIs across genomes. Early analysis with this new platform exposed a previously underappreciated impact of inconsistent gene annotations on downstream genomic island predictions. This has been corrected for in IslandCompare to ensure reliable, consistent predictions and enable the comparison of genomic island content across isolates. IslandCompare will facilitate improved risk assessment of AMR/virulence gene spread, and more robust, rapid responses to disease outbreaks, ultimately reducing both the economic and public health impacts of infectious diseases where genomic islands are involved in pathogenesis.

#8 Evolutionary trajectory of the enzyme activation-induced cytidine deaminase (AID) within the Gadiformes lineage

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In vertebrates, the enzyme activation-induced cytidine deaminase (AID) introduces somatic mutations at the immunoglobulin (Ig) loci to instigate antibody affinity maturation, generating high-affinity antibodies. Unlike other studied vertebrates, the Atlantic cod (Gadus morhua) humoral response lacks affinity-matured antibodies. Since AID is responsible for generating high-affinity antibodies in other vertebrates, we sought to examine the genetics, expression, and function of Atlantic cod AID. Previous studies have uncovered a drastic remodeling of the immune system in Gadiformes species, including Atlantic cod, where many genes involved in B cell activation were lost from their genomes. To shed light on the extent of immune system remodeling in this lineage, we also investigated AID's evolutionary trajectory within Gadiformes species. We found that although the AID gene synteny and transcript expression seems to be conserved in Atlantic cod, the enzyme itself exhibited deficient catalytic activity compared with other studied homologs. Our biochemical analyses of 35 other bony fish AIDs revealed a vast diversity in the enzymatic properties of AID homologs. By predicting and resurrecting the ancestral AIDs within and outside of Gadiformes lineage, we showed that the functional impairment of AID most likely has happened in the Gadidae's common ancestor. Since Gadidae species have successfully populated their natural habitats, their AID enzyme's functional impairment did not hamper their fitness. Our findings of the first examples of vertebrate species with dysfunctional AID challenge the long-standing immunological concept that the loss of AID activity leads to immunodeficiency.