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June 25 to 28, 2023

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Conference Content

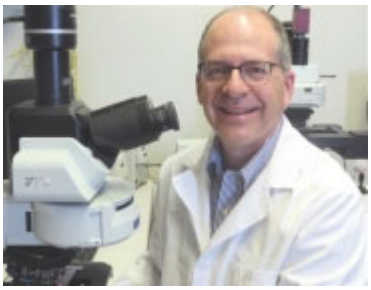
RiboWest2023

RIBOWEST 2023

EVENT SCHEDULE

Sunday June 25th 2023		
5:00 pm – 7:30 pm 6:00 pm – 7:30pm	Registration Please drop by to pick up registration packages. UNBC Campus Tours available. Registration will also be open Monday during breakfast. Welcome Reception Food and drinks available so please join us!	7-172 Bentley Centre
7:30 pm to 8:30 pm	KEYNOTE 1 – Michael Levin	8-164 Teaching Lab Building
Monday June 26th 2023		
7:30 am – 8:30 am	Breakfast (registration)	7-172 Bentley Centre
8:30 am – 9:00 am	Welcome from UNBC	8-164 Teaching Lab Building
9:00 am – 10:00 am	KEYNOTE 2 – Tracy Johnson	8-164 Teaching Lab Building
10:00 am – 10:30 am	Coffee Break	Outside 8-164
10:30 am – 12:00 pm	EDI Fixing Academic Hiring	8-164 Teaching Lab Building
12:00 pm – 1:00 pm	Lunch	7-172 Bentley Centre
1:00 pm – 2:30 pm	Session I	8-164 Teaching Lab Building
2:30 pm – 3:30 pm	Poster Session I (coffee break)	Bentley Centre Hallway
3:30 pm– 5:00 pm	Session II	8-164 Teaching Lab Building
5:15 pm- 5:30 pm	Group Photo	Agora Courtyard Steps
6:00 pm – 7:30 pm	BBQ Dinner	Agora Courtyard (or Dining Hall) weather dependent
7:30 pm – 8:30 pm	Free time	
Tuesday June 27th 2023		
7:30 – 8:30 am	Breakfast	7-172 Bentley Centre
8:30 am – 10:00 am	Session III	8-164 Teaching Lab Building
10:00 am – 10:30 am	Coffee Break	Outside 8-164
10:30 am – 12:00 pm	Session IV	8-164 Teaching Lab Building
12:00 pm – 1:00 pm	Lunch	7-172 Bentley Centre
1:00 pm – 2:30 pm	Session V	8-164 Teaching Lab Building
2:30 pm – 3:30 pm	Poster Session II (coffee break)	Bentley Centre Hallway
3:30 pm – 4:30 pm	Job Fair	Outside 8-164 Teaching Lab Building
4:30 pm – 5:30 pm	KEYNOTE 3 – Karissa Sanbonmatsu	8-164 Teaching Lab Building
6:00 pm – 8:30 pm	Banquet Dinner	Atrium, Charles J McCaffray Hall
Wednesday June 28th 2023		
7:30 – 9:00 am	Breakfast	7-172 Bentley Centre

Keynote Speakers



Michael C. Levin
Professor of Neurology, University of Saskatchewan
Saskatchewan Multiple Sclerosis Clinical Research Chair
Cameco Multiple Sclerosis Neuroscience Research Centre

Michael C. Levin, MD, is the inaugural Saskatchewan Multiple Sclerosis Clinical Research Chair and Professor of Neurology and Anatomy, Physiology & Pharmacology at the University of Saskatchewan. He received his Bachelor of Science degree in Chemistry with special honors at George Washington University, his medical degree at Pennsylvania State University and basic neuroscience training at The Salk Institute with Drs. Max Cowan and Paul Sawchenko. Dr. Levin completed his residency training in Neurology at the New York Hospital/Cornell Medical Center – Memorial Sloan Kettering Cancer Center where Drs. Fred Plum and Jerry Posner mentored him including while he was chief neurology resident. He then completed his Multiple Sclerosis post-doctoral fellowship in the Neuroimmunology Branch at NIH with Drs. Henry McFarland and Steve Jacobson.

He was recruited to the University of Tennessee in Memphis where he moved up the ranks to professor with tenure, was Chief of the Neurology Service at the Memphis Veterans Affairs Medical Center and led the MS clinic and developed a translational research program based on the role that dysfunctional RNA binding proteins play in the pathogenesis of neurodegeneration in MS and relevant MS models. His work has been published in The New England Journal of Medicine, Nature Medicine, Glia, Annals of Neurology, Neurology, the Journal of Comparative Neurology, and the Journal of Neuroscience Research. Dr. Levin has received more than 30 awards for academic excellence and his work has been recognized by the National Multiple Sclerosis Society, American Academy of Neurology, and the Society for Neuroscience. Most recently his work on dysfunctional RNA binding proteins in MS has been recognized by a Canadian 'Science, Technology, Innovation and Collaboration' Award for the discovery of stress granules in brain tissue of an MS patient and a Canadian Tri-agency New Frontiers Research Grant – one of the most competitive in Canada - which is awarded for high risk, high reward interdisciplinary research that has the potential for significant impact.

Dr. Levin is married to his lovely wife of more than 30 years, Dr. Audrey Zucker-Levin, an academic physical therapist. He has two strappingly handsome sons and is an avid sailor and scuba diver.

Peptide-derived small molecules targeting the RNA binding protein hnRNP A1 inhibit neurodegeneration – implications for the pathogenesis and treatment of multiple sclerosis

Michael C. Levin[1-4]

[1]Office of the Saskatchewan Multiple Sclerosis Clinical Research Chair, [2]Cameco MS Neuroscience Research Centre, [3]Neurology Division - Department of Medicine, [4]Department of Anatomy, Physiology, Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan

KEYWORDS: multiple sclerosis, heterogeneous nuclear ribonucleoprotein A1, RNA metabolism, neurodegeneration, small molecule therapies

Neurodegeneration, the death and damage to neurons and axons, underlies permanent disability in multiple sclerosis (MS). Yet, regardless of their marginal effect on neurodegeneration, immunotherapies are still the primary treatment for MS. We discovered that dysfunction of the RNA binding protein heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), including its cytoplasmic mislocalization and aggregation in neurons, drives neurodegeneration in MS and its models. We hypothesized that correcting hnRNP A1 dysfunction will rescue neurodegeneration in relevant MS models. Multiple in vitro and in vivo model systems were used to assess hnRNP A1 dysfunction and its treatment. An hnRNP A1-specific peptide was used to inhibit hnRNP A1 dysfunction. In silico modeling and thermal shift binding assays were used to model peptide - hnRNP A1 interactions, which identified small molecules with the potential to inhibit hnRNP A1 dysfunction. The small molecules were then examined for their ability to inhibit hnRNP A1 dysfunction and neurodegeneration in the model systems. hnRNP A1 nucleocytoplasmic mislocalization and cytoplasmic hnRNP A1 aggregation caused decreased neurite length, a marker of neurodegeneration. The hnRNP A1-specific peptide reduced hnRNP A1 mislocalization and hnRNP A1 aggregation and rescued neurite length in multiple model systems. In-silico modeling identified peptidomimetic small molecules that bound hnRNP A1 and reduced hnRNP A1 mislocalization and aggregation and rescued neurites like the hnRNP A1-specific peptide. The small molecule with the greatest in vitro effects was non-toxic and efficacious in mice with experimental autoimmune encephalomyelitis, the most common preclinical model of MS. In summary, hnRNP A1 dysfunction contributes to the pathogenesis of MS and hnRNP A1 specific small molecules inhibited hnRNP A1 dysfunction and ameliorated neurodegeneration, the root cause of disability in MS, thus fulfilling a major therapeutic gap in the treatment of MS.

Tracy L. Johnson, Ph.D.
Keith and Cecilia Terasaki Presidential Endowed Chair
Professor, Molecular, Cell and Development Biology
Dean, Life Sciences, UCLA College

Dr. Tracy Johnson earned her bachelor's degree from UCSD in Biochemistry and Cell Biology and her Ph.D. in Molecular and Cell Biology form UC Berkeley. She was a Jane Coffin Childs postdoctoral research fellow at the California Institute of Technology (Caltech). Dr. Johnson began her first faculty position at UCSD in and moved to UCLA to join the faculty in 2013. In 2020, Dr. Johnson was appointed Dean of Life Sciences at UCLA. Her research lab laboratory focuses on understanding mechanisms of gene regulation, particularly RNA splicing, chromatin modification and the intersection between these reactions.

In addition to her activities at UCLA, Dr. Johnson play a leadership roe in a number of professional societies. She is the current President of the Genetics Society of America. She has served on the RNA Society Board of Directors, the National Cancer Institute Board of Scientific Advisors, and as the chair of the Molecular Genetics NIH study section. She is currently on the Executive Board for the Society of HHMI Professors and recently served as its chair. Dr. Johnson is a trustee of the Cold Spring Harbor Laboratory.

Dr. Johnson has been the recipient of numerous awards. These include the NSF CAREER Award, the Presidential Early Career Award for Scientists and Engineers (PECASE), and in 2013 was named of the Top 20 Women Professors in California. In 2022 she received the Ruth Kirschstein Diversity in Science Award from the American Society for Biochemistry and Molecular Biology.

To this end, Dr. Johnson has been actively involved in a number of education initiatives to support the development of students, particularly those from underrepresented groups, including the HHMI Pathways to Success program, which fosters academic success for students, in part by early exposure to research. In 2017, Dr. Johnson received the 2017 Academic Senate Award for Career Commitment to Diversity, Equity, and Inclusion and in 2018 received the Life Sciences Award for Excellence in Promoting Diversity, Equity, Inclusion.

Karissa Sanbonmatsu
Los Alamos National Laboratory
Theoretical Biology and Biophysics Group, Theoretical Division

Dr. Karissa Sanbonmatsu is a structural biologist and fellow of Los Alamos National Laboratory, where she leads the Sanbonmatsu Laboratory established in 2001. She received her B.A. in Physics from Columbia University in 1992 and Ph.D. in Astrophysical, Planetary and Atmospheric Sciences from University of Colorado at Boulder in 1997. Her team uses computational and experimental approaches to understand the mechanism of a diverse array of epigenetic and non-coding RNA systems, including chromatin, ribosomes, riboswitches and long non-coding RNAs. She is a fellow of the American Physical Society and an advocate for LGBT scientists. Her TED talk, *The biology of gender, from DNA to the brain,* has over 2.5 million views on TED.com.

Oral Presentations

Abstract # 4

Lnc35682/PAN3-AS1: A De Novo Discovered Conserved Long Noncoding RNA Fueling the Progression of Acute Myeloid Leukemia

Zhen Jin[1,2], Maryam Ghashghaei[1,2], Kyle McPherson[2], Ly P. Vu[1,2]

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[2]Terry Fox Laboratory, British Columbia Cancer Research Centre, Vancouver, BC, Canada
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KEYWORDS: LncRNA, Leukemogenesis, Acute myeloid leukemia (AML), Leukemia stem cell, Hematopoiesis.

Long non-coding RNAs (lncRNAs) have recently emerged as an important class of regulators of tumorigenesis. Although more than 100,000 lncRNAs are identified in humans, most of them have not been functionally characterized. Insights into identities and mechanisms of action for lncRNAs can uncover novel pathways and targets for the treatment of cancers. Here, by performing a de novo discovery of murine lncRNAs using comparable transcriptome profiling, we identified *Lnc-35682*, a lncRNA expressed in mouse hematopoietic stem/progenitor cells and highly upregulated in the leukemia stem cells (LSCs)-enriched population. To explore the role of *Lnc-35682* in leukemia, we depleted *Lnc*-35682 using three independent shRNAs and observed significant inhibition of growth and the ability of leukemia cells to form colonies. Knockdown of *Lnc-35682* also delayed leukemia development in the xenograft leukemia model. To further delineate the biological function of *Lnc-35682 in vivo*, we employed CRISPR/Cas9 to create a murine germline knockout (KO) of *Lnc-35682. Lnc-35682* KO exhibited minimum effects on normal hematopoiesis but efficiently delayed leukemia progression.

Moreover, to determine the relevance of *Lnc*-35682's function in human disease, we performed a Syntenic analysis and identified *PAN3-AS1* as the putative human homolog of *Lnc-35682*. Intriguingly, we found that *PAN3-AS1* is highly upregulated in primary acute myeloid leukemia (AML) cells (compared to healthy donors' cells) and elevated *PAN3-AS1* expression correlates with unfavorable prognosis in AML patients. Similar to *Lnc-35682* loss of function, the depletion of *PAN3-AS1* significantly inhibited the survival of human leukemia cells *in vitro* and *in vivo* xenografted animals. Conversely, *Lnc-35682/PAN3-AS1* overexpression (OV) promoted leukemia cell growth and colony formation. Importantly, *Lnc-35682/PAN3-AS1* OV efficiently rescues human leukemia cell growth caused by *PAN3-AS1* depletion, indicating functional conservation of the mouse and human lncRNA. In situ hybridization for RNA FISH demonstrated that *Lnc-35682* as well as *PAN3-AS1* localizes to the perinuclear region, suggesting a potential role in modulating local gene expression. In fact, our preliminary data suggested a potential link between the sense strand-derived coding gene *Pan3* by the antisense *Lnc-35682/PAN3-AS1*. In conclusion, our study demonstrated that the conserved lncRNA, *Lnc-35682/PAN3-AS1*, is required for leukemogenesis and represents a leukemia-specific vulnerability that can be targeted for AML therapy. The findings will offer valuable insights into the role of lncRNAs in regulating AML, providing novel avenues for future research and the development of targeted cancer therapies.

Abstract # 5

Application of an integrated Illumina RNA-seq and shotgun proteomics workflow for identifying alternative proteoforms in a leukemia cell line with a recurrent point mutation in SF3B1

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KEYWORDS: Proteogenomics, proteoforms, shotgun proteomics, Illumina RNA-seq, alternative splicing, SF3B1, chronic myelogenous leukemia

Splicing factor 3b subunit 1 (SF3B1) is one of the largest components of the spliceosome and is frequently mutated in hematological malignancies and cancers. Particularly, the recurrent point mutation of K700E on SF3B1 is associated with aberrant 3' splice site selection and increased nonsense-mediated decay. Thus, there is a need for discovering aberrant alternatively spliced protein isoforms (i.e., alternative proteoforms) to better understand how SF3B1-K700E affects disease phenotypes; this is challenging due to the presence of highly homologous peptide sequences between different proteoforms of the same gene. We applied a workflow combining Illumina RNA-seq and shotgun proteomics to analyze a stable chronic myelogenous leukemia cell line (K562) with $SF3B1^{+/+}$ or $SF3B1^{+/K700E}$. Sample-matched RNA-seq global transcriptome profiling data (n=3 each) were used for differential alternative splicing (AS) analysis by rMATS[1] and the generation of a database of ~22k alternative proteoform sequences via JCAST[2]. Searching the database against shotgun proteomics global proteome profiling data (n=5 each) identified nearly 200 alternative proteoforms that are absent from the UniProt/Swiss-Prot human canonical proteome of ~20k sequences, and ~100 of them are novel to UniProt/Swiss-Prot's alternative isoforms and the Ensembl human proteome database (~22k and ~86k sequences, respectively). Gene set enrichment analysis (GSEA) showed a few gene ontology (GO) biological processes (cytoplasmic translation, protein folding, double-stranded break repair via break-induced replication) were commonly enriched between protein-level differentially expressed (DE) genes and RNA-level DE genes or differentially spliced genes. The custom database was also searched on a subset of CPTAC breast cancer (BRCA) cohort containing two patient samples with $SF3B1^{+/K700E}$, identifying over 60 non-canonical alternative proteoforms, half of which have a relative expression consistent with that from our in-house dataset. Notably, multiple proteoforms for each of FMR1 (involved in cytoplasmic translation) and PDIA4 (involved in protein folding) were detected in both our in-house dataset and the CPTAC BRCA dataset, highlighting them as potential candidates for future experimental validation. This study demonstrates the power of integrating RNA-seq and shotgun proteomics to identify and prioritize potential alternative proteoforms that result from aberrant splicing.

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Abstract # 6

Investigating the role of ribosome biogenesis in the translation of the interferon response

Brenna N. Hay, R. Greg Stacey, Stephane Flibotte, Leonard J. Foster, Eric Jan

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PresenAng author level: PhD candidate

KEYWORDS: TranslaAon, translaAonal regulaAon, interferon response, ribosome biogenesis, viruses
Submission type: oral presentaAon

Introduction: The interferon response is a signaling pathway unique to vertebrates that links the innate and adaptive immune responses. Interferon signals through a cascade of factors including the JAK-STAT pathway to induce the transcription of hundreds of interferon stimulated genes (ISGs). Although the main signal transduction pathways and ISGs induced by interferon have been elucidated, translational regulation of ISG transcripts has not been fully investigated. Prior work demonstrated that ribosomal protein RPL28 expression is induced and RPL28 is more ribosome-associated under interferon stimulation. Knockdown analysis suggested RPL28 negatively regulates a subset of ISGs. Recent findings suggest that ribosome biogenesis may be regulated by interferon signaling and impacts the translational regulation of ISGs. The aim of this project is to examine the relationship of ribosome biogenesis and ISG translation under interferon stimulation.

Methods: Host proteome of lung epithelial A549 cells is assessed through label-free quantification by liquid chromatography-mass spectrometry and western blotting. RNA quantification is measured by RT-qPCR.

Results: Initial mass spectrometry results support prior evidence demonstrating a pattern of ISG protein upregulation in interferon-stimulated RPL28-depleted cells. Further, we observe a broad ribosomal protein depletion under RPL28 knockdown, suggesting that ribosome biogenesis is impacted which then affects ISG expression. To determine whether ribosome biogenesis and the interferon response are associated, mass spectrometry analysis was performed following the knockdown of a key ribosome biogenesis factor, BOP1, in A549 cells treated with interferon. Initial quantification data shows that eight of the ten previously identified subset of ISGs had higher abundance in the BOP1-depleted condition, further supporting that the interferon response appears to be uniquely regulated. To investigate the interplay between the interferon response and viral infection more directly, BOP1-depleted cells were infected with Sendai Virus, a virus that induces a strong interferon response. Protein samples were collected over a time course for mass spectrometry and RNA samples were collected in parallel for RT-qPCR. RT-qPCR shows a reduction in viral RNA of up to 50% in the knockdown condition across several time points, suggesting a defect in viral infection. This has been further supported by mass spectrometry data demonstrating a reduction of viral protein abundance in the BOP1-reduced samples.

Future work and conclusion: Future work includes a thorough differential expression analysis of the mass spectrometry datasets and RNA-seq to gain global insights on transcription. This work will elucidate the intricate translational regulation of the interferon response and gain insights into impacts on virus translation.

Abstract # 7

Characterization of the North American Sin Nombre *orthohantavirus* (SNV) 3' Medium Non-coding Terminal Regions and their Interactions with Human RNA-Binding Proteins

Mitchell J. Geeraert[a], Michael H. D'Souza[a], Angela I. Frederickson[a], Carmen Agenbacht[a], M. Quadir Siddiqui[a], Liam Kerr[a], Chantelle Plumb[a], Trushar R. Patel[abc]

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Presenting Author Level: MSc student.

KEYWORDS: Hantavirus, Sin Nombre *orthohantavirus*, Staufen, PACT, Non-coding Terminal Region, Small Angle X-ray Scattering, Multi Angle Light Scattering, Microscale Thermophoresis

Hantaviruses are segmented, negative-sense RNA viruses from the *Bunyaviridae* family. These Category C Pathogens threaten agricultural industries, global trade, and human health, due to their dispersibility and high mortality rates. North American populations are at risk from the New World Hantavirus species known as Sin Nombre *orthohantavirus* (SNV) that is spread by the common deer mouse (*Peromyscus maniculatus*). An infection with SNV causes Hantavirus Cardiopulmonary Syndrome (HCPS) which has an average mortality rate of 40%[1]. Hantaviruses have highly conserved Non-coding Terminal Regions (NcTRs) on all three of their genome's segments (small, medium, and large) which produce panhandle structures via complementarity base-pair binding. These panhandle structures interact with the viral Nucleocapsid Protein which is involved in the packing and encapsidation of the viral genome through the formation of a Ribonucleoprotein Complex[2,3]. Our studies on SNV's medium 3' NcTRs (M3'NcTRs) are involved with identifying human host RNA-Binding Proteins (RBPs) that may play a role in SNV transcription and replication through their interactions with the viral NcTRs. A Pull-Down Assay was performed, isolating human host proteins from an A549 lung epithelial cell line and Mass Spectrometry was performed to identify host RBPs that had specific binding interactions with M3'TRs. Of the resulting RBP hits, human proteins Double-stranded RNA-binding protein Staufen homolog 1 (Staufen) and Interferon-inducible double-stranded RNA-dependent protein kinase activator A (PACT) are being investigated to validate potential interaction with M3'NcTRs. Staufen shuttles RNA along the cytoskeleton and has been shown to be involved with other viruses [4-9]. PACT is involved in the immune response, as an activator of PKR, which can result in cell death in response to viral infection [10]. We are characterizing the M3'NcTRs using biophysical techniques such as Small Angle X-ray Scattering (SAXS), and Multi Angle Light Scattering (MALS), to get physical properties of the molecules such as 3D low-resolution structures and the molecular weight. SAXS and MALS are also utilized to obtain those properties for M3'NcTR – RBP complexes, while Microscale Thermophoresis (MST) is utilized to evaluate the binding affinity of such complexes. The results of our studies will open new research avenues into the development of therapeutical drugs designed to intervene in the SNV lifecycle.

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10. https://doi.org/10.1093/emboj/17.15.4379

Abstract # 8

Developing reliable RT-qPCR methods for profiling oligodendrocyte lineage gene expression and alternative splicing.

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Presenting author level: MSc

KEYWORDS: Myelination, Oligodendrocyte, RT-qPCR, Alternative Splicing, Neurodevelopment

In the central nervous system, oligodendrocytes form the myelin sheath, which insulates neuronal axons, facilitates nerve conduction, and provides metabolic support. Oligodendrocyte precursor cells (OPCs) differentiate into mature myelinating glia through defined stages of gene expression. Altered myelin gene expression, and differential expression of variants due to alternative splicing, have been linked to neuropsychiatric disorders[1, 2]. RT-qPCR is commonly used to monitor gene expression changes across cell development, but variations in technical protocols and standardization may lead to inconsistencies in data reproducibility. This study aimed to establish a collection of robust qPCR assays for quantifying oligodendrocyte and myelin gene expression in murine brain tissue. The primer design showed high specificity, minimal temperature dependence and efficiencies in the acceptable 90% - 110% range per the MIQE guidelines[3]. A panel of 8 commonly used reference genes was evaluated for expression stability across brain development: *Gapdh, Sdha, Hmbs, Hprt1,* & *Pgk-1* were consistently ranked as the top choices for normalization, but the order varied depending on the brain region. As proof of concept, we assessed the progression of oligodendrocyte differentiation in murine cortical tissue at P0, P7, P14, P21, P21 and P84. The myelin genes peaked in their expression at postnatal day 21 – the peak of developmental myelination[4–6]. This aligns with previous RNA-seq findings that showed PDGFRα expression highest in OPCs and myelin gene expression at its highest in mature oligodendrocytes[7]. To further investigate the regulation of oligodendrocyte gene expression, qPCR assays are being designed to target the alternative splicing of select myelin genes. For example, proteolipid protein (Plp), one of the major protein components of the myelin membrane, has two known splice variants, Plp-1 and Plp-2/DM-20, which are differentially regulated during neurodevelopment[8, 9]. These validated RT-qPCR assays lay the foundation for reliable gene expression analysis on oligodendrocyte differentiation. Improper coordination of gene expression leads to dysregulation of myelination, which is relevant to mechanisms of neurodevelopment, aging, and neurodegenerative diseases.

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Abstract # 10

Mass spectrometric identification of proteins involved in mRNA translation and RNA binding in bovine sperm

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[3] Arnie Charbonneau Cancer Institute, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
[4] Southern Alberta Genome Sciences Centre (SAGSC), University of Lethbridge, AB, Canada.

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Presenting author level: PhD student

KEYWORDS: Bovine, Capacitation, Fertility, Sperm proteins, Translation

The sustainability of a growing world population demands enhanced livestock productivity for animal-based proteins. Bull fertility is critical as semen from a superior bull can be used to inseminate thousands of cows. However, among bulls considered satisfactory breeders based on traditional evaluations, fertility varies by ~ 20% (1), implicating submicroscopic differences (e.g., proteome, transcriptome, metabolome, etc.) in sperm. Therefore, an enhanced understanding of molecular mechanisms governing male fertility should improve breeding bull selection. Ejaculated sperm were historically considered translationally inactive, as 28s and 18s ribosomal RNA are innately fragmented, and most cytoplasm is excluded during sperm formation (2). However, recent literature indicates *de novo* sperm protein synthesis in several species during capacitation (3-5), a physiological process enabling ejaculated sperm to gain fertilizing ability by undergoing a series of maturational changes in the female reproductive tract. The mechanism underlying translation and its regulation during capacitation has not been elucidated. RNA-binding Proteins (RBPs) are implicated in translational regulation (6); their role is well-established in spermatogenesis but poorly elucidated in sperm. LC-MS/MS was used to generate preliminary data on sperm translational machinery, RBPs, and differentially expressed proteins (DEPs) during capacitation.

Scaffold validated the Mascot-identified proteins, revealing 1319 sperm proteins, of which 1164 proteins common in non-capacitated and capacitated sperm were used for analyzing DEPs. Several proteins involved in translation such as cytoplasmic and mitochondrial translation initiation and elongation factors, ribosomal proteins, tRNA synthetase, ligase, etc. were identified. In addition, some proteins had a functional role in mRNA processing, export, degradation, and binding, indicating their potential to regulate sperm translation. A total of 73 proteins were differentially expressed during capacitation, with 60 and 13 proteins upregulated and downregulated, respectively, in capacitated versus non-capacitated sperm (fold change cutoff: ± 1.2, $p \leq 0.05$, False Detection Rate ≤ 0.01). Gene ontology analysis revealed that DEPs were involved in biological processes of reproduction, capacitation, fertilization, ATP metabolic process, localization, etc. Furthermore, KEGG analysis indicated DEPs to be involved in metabolic pathways, *viz*. Pentose phosphate pathway, glycolysis/gluconeogenesis, insulin signaling pathway, etc. This new knowledge of DEPs and RBPs will assist in elucidating sperm translational machinery, advance understanding of factors regulating fertility, and facilitate the selection of breeding bulls with higher fertility.

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Abstract # 11

Understanding the cycle; Insights into the regulation of flaviviral replication

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KEYWORDS: RNA-Protein Interaction, Biophysics, RNA virus, Viral Replication, RNA-RNA Interactions

The *Flaviviridae* are a family of single-stranded (+) sense RNA viruses responsible for global concerns such as the recent outbreaks of Zika and Dengue. Flaviviruses are highlighted by both the World Health Organization and the Centers for Disease Control as global health threats [1]. As a member of the *Flaviviridae*, JEV has highly structured and functional 5' and 3' Terminal regions (TRs), aiding in viral genome cyclization, viral defence, viral replication, and transcription through the recruitment of non-structural protein 5 (NS5) [2] which is responsible for their replication and transcription. A promoter for this protein has been determined on the 5' TR, known as the stem-loop A (SLA). However, viral transcription is initiated on the 3' TR, and the mechanism of translocation of NS5 from one TR to the other is unknown. There are currently two models by which NS5 interacts with the 3' TR of the RNA. One model suggests that two NS5 proteins bind separately to each TR and dimerize with each other as the genome cyclizes, facilitating one protein at the 3' TR initiation site [3]. A second model proposes that one NS5 protein binds to the 5'TR and, through genome cyclization, arrives in close proximity to the 3' TR to facilitate genome replication in this manner [4]. Using small angle X-ray scattering we have obtained a solution structural model for NS5. In the future we plan to use multi-angle light scattering, to show the stoichiometry of the binding of NS5 when complexed with the terminal regions, and multiscale thermophoresis to show the dissociation constants of NS5 bound to both terminal regions complex and separate.

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Abstract # 12

Reference Gene Recommendations and PACAP Receptor Expression in Murine Sympathetic Ganglia of the Autonomic Nervous System that Innervate Adipose Tissues After Chronic Cold Exposure

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KEYWORDS: PACAP receptors, sympathetic nervous system, stellate ganglia, thermogenesis, real-2me quantitative PCR, reference genes

Pituitary adenylate cyclase-activating polypeptide (PACAP) is an important regulator of the stress response in mammals, influencing both the hypothalamic-pituitary-adrenal (HPA) axis and the sympathe2c nervous system (SNS)[1]. PACAP has been reported to influence energy homeostasis, including adaptive thermogenesis, an energy burning process in adipose tissue regulated by the SNS in response to cold stress and overfeeding. While research suggests PACAP acts centrally at the level of the hypothalamus[2-5], knowledge of PACAP's role within the sympathetic nerves innervating adipose tissues in response to metabolic stressors is limited. This work shows, for the first time, gene expression of PACAP receptors in stellate ganglia and highlights some differen2al expression with housing temperature.

Additionally, we present our dissection protocol, analysis of tyrosine hydroxylase gene expression as a molecular biomarker for catecholamine producing tissue, and recommend three stable reference genes for the normalization of qRT-PCR data when working with this tissue. This work adds to information about neuropeptide receptor expression in peripheral ganglia of the sympathetic nervous system innervating adipose tissue and provides insight into PACAP's role in the regulation of energy metabolism.

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Abstract # 13

RNA Deadenylation Subunit CNOT3 is Required for Hematopoiesis and Maintenance of Hematopoietic Stem Cells

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KEYWORDS: Post-transcriptional regulation; CCR4-NOT complex; Hematopoietic system; Hematopoietic stem cells

Adult mammalian hematopoietic stem cells (HSCs) constitute a heterogeneous population responsible for the generation of various blood cell types throughout adulthood. It is commonly known that gene expression programs underlying the regulation of HSC self-renewal and differentiation are tightly regulated in normal physiological conditions. However, little is known regarding how post-transcriptional regulation of gene expression influences hematopoiesis. It has been shown that mRNA deadenylation pathways affects mRNA half-life, and are key components of post-transcriptional gene regulation. In turn, deadenylation pathways also affects translational efficiency. Amongst the components of the deadenylation pathways, the CCR4-NOT (CNOT) complex is known to be a major deadenylase conserved in eukaryotes. Several components of the complex have previously been shown to maintain pluripotency in embryonic stem cells. However, the extent and mechanisms to which the CNOT complex modulates gene expression programs in HSCs during hematopoiesis is unknown. We surveyed the expression of several subunits of the CNOT complex in the hematopoietic system and identified that CNOT3, a scaffolding subunit, is highly expressed in hematopoietic stem/progenitor cells (HSPCs) and downregulated in differentiated cells. To explore the role of CNOT3 in hematopoiesis, we generated a blood-specific Mx1-Cre Cnot3 conditional knockout mouse. Cnot3 ablation resulted in anemia, reduction in bone marrow (BM) cellularity and spleen enlargement in the mice. We also observed defects in erythroid development in Cnot3 floxed/floxed; Mx1-Cre +ve (KO) animals after induced deletion of Cnot3, which were characterized by reduction in red blood cell counts to half of their Cnot3 floxed/floxed; Mx1-Cre -ve (WT) counterparts and enhanced extramedullary hematopoiesis of blood cell production in the spleen. Multiparameter flow cytometric analysis of BM cells revealed that as compared to WT mice where 38% of the Lin-Sca+cKit+ (LSK) compartment were consisted of HSCs, this is dramatically reduced to less than 5% in KO mice. This is coupled with an expansion of downstream progenitors in Cnot3 KO mice. In order to evaluate the effects of CNOT3 on the functionality BM cells, we conducted in vivo transplantations. We demonstrated that CNOT3-depleted BM cells failed to reconstitute the hematopoietic systems of lethally irradiated mice. Furthermore, cell cycle analysis suggested that Cnot3 deletion leads to increased cycling activity in HSCs and subsequent stem cell exhaustion. Altogether, our results indicate that CNOT3 is essential for normal hematopoiesis and maintenance of potency HSCs.

Abstract # 14

Role of eukaryotic initiation factor 5B (eIF5B) in oral squamous cell carcinoma

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KEYWORDS: eIF5B, OSCC, translation initiation, apoptosis, proliferation, invasion, migration

Oral squamous cell carcinoma (OSCC) is one of the most common malignancies that arise from the mucosal epithelium in the oral cavity, pharynx, and larynx. Despite improvements in therapeutic interventions such as surgery, chemotherapy, and radiotherapy, the prognosis for patients remains poor. As a result, it is imperative that therapeutic targets be identified that increase the sensitivity of OSCC cells to current standard-of-care. eIF5B plays a key role in non-canonical translation initiation and has been suggested to regulate translation of mRNAs encoding certain anti-apoptotic proteins. Bioinformatic analyses have shown that *EIF5B* mRNA is correlated with poorer prognosis for OSCCC patients. The cell viability data suggests that eIF5B depletion significantly increases OSCC cell sensitivity to the pro-apoptotic agents. Depletion of eIF5B has also shown to result in downregulation of the anti-apoptotic proteins B-cell lymphoma extra-large (Bcl-xL), and cellular inhibitor of apoptosis protein 1 (cIAP1), the short isoform of cellular FLICE-like inhibitory protein (cFLIPs), and X-linked inhibitor of apoptosis protein (XIAP). Bromodeoxyuridine (BrdU) incorporation assay and scratch wound assays will be performed to assess the effect of eIF5B depletion on proliferation and migration, respectively. Western blots to assess the effect of eIF5B depletion on pro-angiogenic, pro-growth, and invasion and migration markers such as vascular endothelial growth factor (VEGF), p65 and phosphorylated p65 (p-p65), extracellular signal-regulated kinase (ERK) and phosphorylated ERK (P-ERK), epidermal growth factor receptor (EGFR) and phosphorylated EGFR (P-EGFR), and hypoxia inducible factor 1 (Hif-1a) will also be performed. Polysome profiling experiments will be performed to establish a link between eIF5B and the translation of mRNAs encoding proteins for apoptotic evasion, proliferation, migration and invasion. Therefore, the overarching goal of my study is to establish the pre-clinical rationale for targeting eIF5B as a therapeutic target.

Abstract # 15

Regulation of mRNA translation by tumor suppressor proteins PDCD4 and eIF3F.

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KEYWORDS: Programmed cell death protein 4 (PDCD4), IRES trans-acting factor (ITAF), Eukaryotic initiation factor 3F, co-immunoprecipitation (co-IP), apoptosis.

Programmed cell death protein 4 (PDCD4) is a well-studied tumor suppressor protein and ITAF (IRES trans-acting factor). Although PDCD4 is considered an ITAF, the specific mechanism of PDCD4's role in noncanonical translation initiation remains poorly understood. The cap-dependent mechanism of PDCD4 includes the inhibition of mRNA translation by inhibiting the activity of an RNA helicase, eIF4A. Specifically, PDCD4 interacts with eIF4A's N-terminal Domain (NTD) domain, inhibiting translation initiation and initiating cell death pathways including apoptosis. eIF3 (eukaryotic initiation factor 3) is a 13 sub-unit large initiation factor that significantly regulates translation initiation. Previously, we have reported that eIF3 interacts with the XIAP IRES mRNA to recruit the ribosome, and PDCD4 interacts with the XIAP and Bcl-xL IRES mRNAs, inhibiting translation initiation. Therefore, we hypothesized that eIF3 and PDCD4 interact with each other and have a significant impact on translation initiation. Our most recent findings suggest that PDCD4 forms a complex involving several eIF3 subunits. Using techniques such as co-immunoprecipitation (co-IP), reciprocal co-IP, endogenous IP, and *in vitro* pull-down assays, we have demonstrated that eIF3F (one of the 13 subunits of eIF3) directly interacts with PDCD4 in an RNA-independent manner. Overexpression and knockdown of PDCD4 resulted in enhanced levels of eIF3F and reduction of eIF3F respectively, and *vice versa*. Knockdown of PDCD4 decreased the level of Bcl-xL protein, however, upon knockdown of eIF3F there was a two-fold increase in Bcl-xL levels. We have also recently demonstrated that PDCD4 and eIF3F regulate the IRES translation of Bcl-xL mRNA. This data suggests that PDCD4 and eIF3F possibly regulate each other's levels and regulate the levels of Bcl-xL, although the exact mechanism is unclear. My work focuses on defining this mechanism and examining its effect on non-canonical translation initiation. My work has the potential to understand the molecular mechanism of PDCD4 and eIF3F interaction in non-canonical translation initiation and their activity at the mRNA translation level.

Abstract # 16

Looking beyond *E. coli* bacterial non-coding RNAs: examples in *Methylorubrum, Pseudomonas* and *Burkholderia*, from small RNAs to riboswitches and more.

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KEYWORDS: *Cis*-regulatory RNA, comparative genomics, microbiology, riboswitch, S-adenosylmethionine, ppGpp, ribosome modulation factor (*rmf*)

Model organisms have allowed tremendous progress in biology and, together with comparative genomics, largely contributed to advance from 25 families of RNA structures in Rfam 1.0 [1] to 4108 families in Rfam 14.9 [2] in two decades. In spite of this, a large gap remains between non-coding RNAs (ncRNAs) annotated in models like *Escherichia coli* or *Bacillus subtilis* and less studied bacteria. We have estimated the size of that gap by comparing annotated ncRNAs from ~10,000 genomes and notably found a five fold difference between the most studied bacteria and the majority of other species (and genera, families or orders) for their number of sRNAs [3]. Even among relatively well studied species, for which dozens of strains are sequenced, numerous annotated ncRNAs discovered through transcriptomics or comparative genomics have no assigned function yet. To contribute to fill this gap, and encourage others to do so as well, we turned our attention to *Methylorubrum extorquens* and uncovered new sRNAs found only in *Methylobacteriaceae*. We have also initiated characterization of several annotated cis-regulatory RNAs in the genera *Burkholderia* and *Pseudomonas*, confirming new SAM riboswitches and a regulatory role for several cis-regulatory elements. This highlights the vast diversity of functional RNA structures that remain to be uncovered in the realm of bacteria.

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Abstract # 17

CCR4-NOT transcription complex subunit 4 (CNOT4) is required for survival of myeloid leukemia cells and disease progression

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KEYWORDS: Acute Myeloid Leukemia, translation regulation, protein synthesis, RNA deadenylation, Ccr4-Not complex, codon optimality

Deregulation of normal gene expression control resulted in transformation and malignancy. Post-transcriptional and translation regulations recently emerged to play critical roles in driving oncogenic programs in cancer development. While mutations and aberrant expression of several proteins involved in these processes have been identified in hematological malignancies[1], little is known regarding how post-transcriptional and translational regulation of gene expression influences leukemia development.

Gene expression is influenced by codon usage bias that can affect translation and mRNA decay[2]. Codon optimality, which depends on the availability and decoding rate of particular tRNA, is utilized by cells to regulate gene expression and tailor the proteome under different growth conditions and stress responses[2]. Suboptimal codons can cause translational slowing and ribosomal stalling, leading to decreased mRNA stability and decreased protein synthesis[2,3]. Leukemia cells have been shown to be highly dependent on elevated protein synthesis, and emerging evidence suggests a selective and increased translational rate of oncogenic transcripts[4]. CNOT4 is an E3 ubiquitin ligase that directly ubiquitinates "stalled" ribosomes that do not move efficiently along an mRNA transcript, resulting in disassembly of polysomes and subsequent degradation of both the mRNA and nascent polypeptide chain. Therefore, we hypothesize that CNOT4 drives leukemia development by promoting the translation of malignant gene expression program. We found that the CNOT4 level is elevated in AML patients and cell lines compared to normal cord blood-derived CD34+ cells. To directly explore the role of CNOT4 in leukemia, we performed shRNA-mediated knockdown and CRISPR/Cas9-mediated knockout of CNOT4 in a panel of genetically diverse human leukemia cells (OCI-AML3, KASUMI, MOLM13, MV4-11, and NB4). Efficient depletion of CNOT4 was confirmed using qPCR and immune-blot analysis. Upon CNOT4 knockdown, we observed a strong inhibition of cell growth, increased apoptosis, and significantly delayed leukemia development *in vivo*. Similar results were observed with CRISPR-knockout of CNOT4, confirming the requirement of CNOT4 for survival of AML cells. To explore the downstream pathways mediated by CNOT4 in leukemia, we performed transcriptomic profiling by RNA-sequencing and identified 271 genes downregulated and 327 genes upregulated (FDR □ 0.05) in CNOT4 depleted leukemia cells (vs. control). Gene set enrichment analysis revealed negative enrichment of pathways involved in DNA replication, DNA damage response, and serine metabolism coupled with positive enrichment of gene expression programs connected to IL-6/JAK/STAT3 Signaling, Interferon response, and regulation of immune systems' activities. Overall, our results demonstrated that CNOT4 is required for leukemia survival and development.

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Abstract # 18

Nanopore-based native RNA sequencing of human transcriptomes reveals the complexity of mRNA modifications and crosstalk between RNA regulatory features

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KEYWORDS: Long-read sequencing, Native RNA sequencing, RNA modifications, RNA regulation, Acute Myeloid Leukemia

RNA modifications have recently emerged as an important regulatory layer influencing many facets of an RNA life-cycle from splicing to export, localization to mRNA degradation and translation. Many regulators associated with m^6A RNA methylation, the most prevalent chemical modification within an mRNA, have been implicated in tumorigenesis. However, a comprehensive understanding of RNA methylation selectivity and context-dependent function of individual m^6A marks on specific transcripts has been limited due to the lack of information on the dynamic and stoichiometric nature of modifications within specific RNA molecules. In addition, evaluation of interconnection between RNA methylation and other features of an RNA transcript including mRNA abundance and isoforms is inferred from data acquired by different techniques, potentially introducing technical limitations and bias. To bridge this knowledge gap, we employed Oxford Nanopore Technologies' direct RNA long-read sequencing to obtain an in-depth, high-resolution RNA methylation landscape while gaining comprehensive view of other RNA features in total 15 samples of two myeloid leukemia cell lines. Analysis using Promethion platform generated high-quality ~90 millions reads with an average base-calling quality above 10 and read-length exceeded 1000 bases. Isoform discovery analysis yielded 200,000 transcripts, of which over 860 are novel isoforms. By measuring changes in electrical current flow through the nanopore caused by modified RNA bases, we obtained precise measurements of m^6A methylation stoichiometry at a single site resolution. Using m6Anet [1], we scored 4000-5000 confident sites (confidence score >90%) in different samples. Notably, we observed a decreased m^6A modification rate in the METTL3 knocked down samples, confirming the discovery of the modified sites. These observations are comparable with previously report dataset obtained by miCLIP-seq, the state-of-the-art method for m^6A profiling [2]. Direct RNA long-read sequencing also allowed precise measurement of poly(A) tail lengths at the transcript level. Direct analysis showed that the range of median length of poly(A) tails is around 100 nucleotides. Interestingly, we observed that globally median tail length exhibited a negative correlation with transcript expression. We also observed inter-correlations between m^6A methylation and poly(A) tail length and transcript expression level, this allowed for characterization of their interplays using the same datasets. Overall, our datasets provide a valuable resource while establishing standard analysis for native RNA sequencing data. This has enabled exploration of complexity of RNA modifications and uncover functional crosstalk between RNA regulatory features in cancer cells.

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Abstract # 19

Unravelling the Interactions: Investigating the Role of UTRs in Honey Bee Viral Replication

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KEYWORDS: Honey bee viruses, Biophysics, SAXS, SEC-MALS, biochemistry, host-viral interactions

The western honey bee (*Apis mellifera*) is a critical component of Canadian agriculture. In the past decade, honey bee colony losses have become a serious and widespread issue. The cause of this loss is multifaceted, but major drivers include the use of pesticides, stress, poor nutrition, bacteria, fungi, and viruses (1). Viruses have negative effects on colony productivity traits and can lead to colony collapse. Sacbrood virus (SBV, *Morator aetatulas*) affects honey bee larvae, significantly reducing colony populations and honey production (2). SBV has a single-stranded, positive-sense RNA genome with a single open reading frame surrounded by 5' and 3' untranslated regions (UTRs). Studies have shown that the non-coding regions of viruses can recognize and employ host proteins to facilitate their replication and pathogenicity (3). Based on this, we hypothesize that the UTRs of SBV interact with host proteins of

A. mellifera to aid in the replication and pathogenicity of the virus. To investigate this, pull-down assays with viral UTR RNA and honey bee proteins are being performed, followed by the identification of target proteins using mass spectrometry. Direct interactions will be investigated using isothermal titration calorimetry and microscale thermophoresis. The viral RNA's biophysical properties were also determined using a multi-faceted approach. We first performed size exclusion chromatography multi-angle light scattering (SEC-MALS) to verify the absolute molecular weights of SBV UTRs. Next, we determined the 3D structures of the UTRs using small-angle X-ray scattering (SAXS). Further understanding RNA structure and RNA-protein interactions will provide information on SBV noncoding RNA, and how viral replication is occurring. There are currently no therapeutics available to combat honey bee viral infection, and this study will lay the foundation for the development of inhibitory drugs. Overall, minimizing the loss of honey bee colonies is crucial for maintaining agricultural food production and supply.

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Abstract # 20

Understanding the expression of ARS2 isoforms in arsenic stress response

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KEYWORDS: Arsenic, Stress response, Translation, Cap-independent, Gene expression

Arsenic is a common environmental contaminant and known carcinogen. Paradoxically, arsenic trioxide is an effective treatment for acute promyelocytic leukemia (APL) and is being evaluated as a treatment for other forms of cancer. Despite this we still know little about the precise mechanism of arsenic damage, or how cells cope with arsenic stress. Recent discoveries from our lab suggest that *Ars2*, an essential gene in RNA metabolism, play opposite roles in cellular arsenic response, and is involved in both sensitivity and resistance [1]. The duality in arsenic response is due to the generation of nuclear (ARS2n) and cytoplasmic (ARS2c) isoforms from alternative splicing on intron 5. ARS2n confers arsenic resistance while the ARS2c confers sensitivity. Surprisingly, ARS2c is upregulated during arsenic stress, while ARS2n, is downregulated. This induction of ARS2c is essential for arsenic induce translation induction and cell death.

We have now identified 2 internal ribosomal entry sites (IRES) in intron 5 of *Ars*2 that are responsible for the expression of ARS2c and are working to understand how ARS2c mediates cellular responses to arsenic stress and confers sensitivity to arsenic. This work has implications for arsenic sensitivity in cancer treatment.

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Abstract # 21

Universal tRNA modifications enhance tRNA function in translation and cellular fitness

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KEYWORDS: tRNA, pseudouridine, 5-methyluridine, aminoacylation, protein synthesis, ribosome

All tRNAs are highly modified and harbor 5-methyluridine at position 54 and pseudouridine at position 55 in the T arm, which are generated by the enzymes TrmA and TruB, respectively. *Escherichia coli* TrmA and TruB have both been shown to act as tRNA chaperones, and strains lacking *trmA* or *truB* are outcompeted by wildtype. Here, we investigate how TrmA and TruB contribute to cellular fitness.

Deletion of *trmA* and *truB* in *E. coli* causes a global decrease in aminoacylation and alters other tRNA modification such as acp^{3}U47 and 4-thiouridine. Whereas global protein synthesis is not significantly changed in Δ*trmA* and Δ*truB*, the expression of many specific proteins is altered at the translational level. In conclusion, we demonstrate that universal modifications of the tRNA T arm are critical for global tRNA function by enhancing other tRNA modifications, tRNA folding, tRNA aminoacylation, and translation of specific genes thereby improving cellular fitness and explaining their conservation.

Abstract # 22

Cancer cells release A-to-I edited RNA repeat elements into extracellular vesicles for reprogramming of the tumor microenvironment

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Presenting author level: PhD Candidate

KEYWORDS: Ewing Sarcoma, Extracellular Vesicles, Repeat RNAs, Tumor Microenvironment, Reprogramming

Ewing sarcoma (EwS) is a highly aggressive cancer and the second most common malignant bone tumor in children and young adults with high propensity for metastasis. Patients with metastasis have a poor long-term outcome. Novel targeted therapeutic strategies that are more efficacious and less toxic are therefore desperately needed. Intercellular communication within the tumor microenvironment (TME) is emerging as a crucial mechanism for cancer cells to establish immunosuppressive and cancer-permissive environment. Extracellular vesicles (EVs) offer a candidate mechanism as they are actively released by tumor cells and enriched with proteins and RNAs to communicate with other cells in the TME. In our recent study involving whole transcriptome RNA sequencing, it was found that EVs secreted by EwS cell lines as well as those detected in the plasma of EwS patients are selectively enriched with Adenosine to 3Inosine (A to I) edited RNAs. A high proportion of these A-to-I edited transcripts are derived from diverse long and short interspersed retrotransposon elements (LINEs and SINEs), human endogenous retroviral elements (HERVs) and pericentromeric genomic regions, where their abundance in plasma was associated with metastatic progression. A to I conversion is catalyzed by the ADAR1 enzyme. We therefore performed ADAR1 knock-down (KD) in EwS cells, which accumulated cellular levels of these repeat RNAs in donor cells and limited their packaging into EVs. Moreover, we observed a significant reduction of pro inflammatory response in target cells treated with EwS ADAR1 KD EVs compared to the wild-type cells derived EVs. Notably, ADAR1 KD in EwS cells decreased the potential of their EV mediated monocytes differentiation and T-cell activation and priming. These results suggest that EwS cells secrete EVs enriched with A to I edited RNAs derived from various repeat elements to target the non-tumor host cells, including stromal fibroblasts, monocytes, T cells for dampening and escaping the immune response against them.

Abstract # 23

Regulation of physiological amyloid aggregation by intracellular pH and bicarbonate sensing

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KEYWORDS: amyloid aggregation, pH, bicarbonate, cellular stress, protein folding

Exposure of cells to harsh environmental stimuli can lead to protein misfolding, resulting in amorphous aggregates or highly organized amyloid fibrils. Historically, such amyloid formation has been viewed as pathological since it is implicated in many age-related diseases. However, the formation of amyloid-like aggregates, termed amyloid bodies (A-bodies), is seeded by a family of non-coding RNAs in the nucleoli of cells subjected to heat shock or extracellular acidosis (1). While biophysically similar to irreversible pathological amyloids, A-bodies rapidly disassemble following stress termination, with the constituent proteins returning to their pre-stress localizations (1). Currently, the mechanism of A-body formation during extracellular acidosis and disassembly following stress termination is unclear. Using a

membrane-permeable pH-sensitive fluorescent dye, we show that the intracellular pH lowers as a result of extracellular acidification and this allows for amyloid body formation. During recovery from stress, acidified cells require sodium bicarbonate intracellularly for A-body disaggregation as well as intracellular pH restoration. Investigating an intracellular bicarbonate-dependent signaling pathway, pharmacological inhibition of soluble adenylyl cyclase, a bicarbonate sensor, only prevented A-body disaggregation. This work highlights the central roles of intracellular pH and bicarbonate signaling in regulating A-body formation as well as disassembly, increasing our understanding of the ion-sensing mechanisms and cell biology of this RNA-seeded structure.

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Abstract # 24

Novel IRES-Directed +1 Frame Translation within a Positive-sense RNA Insect Virus

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KEYWORDS: virus; IRES; cap-independent; translation; ORFx

Viruses have evolved mechanisms to subvert cellular processes, specifically to hijack host machinery and prevent immune responses. Canonical cap-dependent translation is a major target of regulation during some virus infections, in which host translation is blocked, thereby liberating translation factors and ribosomes for viral protein synthesis. One such subversive mechanism in positive-sense RNA viruses is the internal ribosome entry site (IRES), a *cis*-acting RNA secondary structure that recruits ribosomes in a 5'-cap independent manner. The most streamlined IRES is the intergenic region (IGR) IRES, derived from dicistroviruses, which resides between two main open reading frames (ORFs). The IGR IRES adopts a triple pseudoknot structure (PKI-III) that can directly recruit ribosomes, and starts translation from a non-AUG codon. Intriguingly, a subset of dicistroviruses produce another open reading frame, dubbed ORFx, that overlaps ORF2 in the +1 frame. Currently, two IRES-mediated ORFx mechanisms are known: ribosomal "skipping" by Cricket paralysis virus (CrPV) IGR IRES[1], and tRNA-directed "ORF selection" by Israeli acute paralysis virus (IAPV) IGR IRES[2]. Here, we suggest a third ORFx mechanism driven by Black queen cell virus (BQCV) IGR IRES. Using bicistronic reporters containing the BQCV IGR IRES and the putative 26-codon ORFx, we show that BQCV IGR IRES can drive +1-frame translation at 40% of the 0 frame in an *in vitro* translation extract, higher than CrPV and IAPV IGR IRESes at 5% and 20%, respectively[1,2]. Mutations within the PKI domain of BQCV IGR IRES disrupt 0- and +1-frame translation, indicating that ORFx translation is IRES- dependent. To map the start site of ORFx, systematic stop codon mutations were inserted in the sequence downstream of the IRES, where stop codons in the +1 frame at positions 1, 4, 7, 10, and 13 did not abolish +1-frame translation. However, inducing stop codons in the 0 frame at positions 1, 4, 7, 10, and 13 disrupted 0-frame, and unexpectedly, +1-frame translation, suggesting that ribosomes recruited to the BQCV IGR IRES start in the 0 frame and subsequently shifts into the +1 frame after the 0 frame 13th codon. Further, a chimeric swap between BQCV and CrPV IGR IRES and downstream sequence demonstrate that robust +1-frame translation is governed by the downstream sequence of the BQCV IGR IRES. We propose that the BQCV IGR IRES directs translation of the +1 frame via frameshift in the IRES downstream sequence after the 0-frame 13th codon, further expanding the repertoire of IRES-mediated +1 frame translation mechanisms.

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Abstract # 25

A comparative exploration of the splicing landscape in three unicellular red algae

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KEYWORDS: Cyanidiales, *Cyanidioschyzon merolae*, *Cyanidiococcus yangmingshanensis*, *Cyanidium caldarium*, pre- mRNA splicing, spliceosome, transcriptomics

The eukaryotic process of pre-mRNA splicing involves the removal of noncoding intron sequences and the fusion of the remaining protein-coding exon sequences [1]. The splicing reaction is catalyzed by the spliceosome, a dynamic multi-megadalton ribonucleoprotein complex [1, 2] that, in humans, is composed of 5 small nuclear RNAs (snRNAs) and over 200 associated proteins [3] acting on more than 200,000 introns present within 25,000 genes [4-6]. The extremophilic unicellular red algal class Cyanidiophyceae is composed of the orders Galdieriales, which contains the genus *Galdieria*, and Cyanidiales, which consists of the genera *Cyanidiococcus*, *Cyanidioschyzon*, and *Cyanidium* [7]. The splicing landscape of *Galdieria sulphuraria* includes 5 snRNAs, 147 splicing proteins, and 13,245 introns spread across 4,655 intron-containing genes [8]. In contrast, *Cyanidioschyzon merolae* possesses a more tractable splicing environment, with only 4 snRNAs and 75 associated proteins [9] interacting with 38 introns found in 37 out of 5,331 genes [10-12]. The ancestral red alga harboured thousands of introns [13-16], indicating that during the course of evolution *C. merolae* has lost nearly all of its introns and many of its splicing proteins. The question of why *C. merolae* has retained a reduced splicing system and only 38 introns is a fascinating one, as is its remarkable lack of the U1 small nuclear ribonucleoprotein (snRNP). To determine whether *C. merolae* is unique in its simplified splicing landscape, we examined the transcriptomes of the closely related species *Cyanidiococcus yangmingshanensis* and *Cyanidium caldarium* [7, 17] using RNA sequencing data. We focused specifically on identifying intron-containing genes and measuring the levels of intron splicing, as well as investigating whether the snRNA and splicing protein complements are as highly reduced as in *C. merolae*. We found that *C. yangmingshanensis* and *C. caldarium* are similarly lacking all components of the U1 snRNP, and have essentially the same complement of splicing proteins as in *C. merolae*. Interestingly, we identified two distinct mechanisms of intron loss in this lineage.

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Abstract # 26

A Unified Protocol for Preparation of Tissues for Multi-Omic Analysis by Transcriptomics and Proteomics

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KEYWORDS: Mass Spectrometry, sample preparation, nucleic acid, protein, quantification

DNA, RNA, and protein each provide different and important information as to cell status. For this reason the popularity of "multi-omic" analysis has grown exponentially over the past 20 years, from just one multi-omic publication on PubMed in 2005 to over 3000 in 2022.[1] Protein and nucleic acid populations from different topographical regions of the same tissue can vary, indicating the importance of a method that can be used for the extraction of both protein and nucleic acid from a single cryosection.[2] Though many protocols and kits have been made available,[3-7] current methods are often focused on nucleic acid recovery and the protein is lacking in terms of quantity, quality, or diversity.[8] This work is directed toward the integration of high quality nucleic acid extraction into the single-pot, solid- phase-enhanced sample preparation for clinical tissue proteomics (SP3-CTP) workflow[9], which is routinely implemented at the BC Cancer Proteomics Platform to quantify 8,000-10,000 proteins from clinical tumour samples. The detergent-based buffer from the SP3-CTP workflow was tested for extraction of nucleic acids as compared to traditional guanidine-thiocyanate (RLT, Qiagen) buffer. This buffer yielded 34 ng RNA/mg of tissue with a RIN of 7.1 as compared to extraction with RLT buffer (15 ng RNA/mg of tissue, RIN of 8.9). Due to the higher RIN value and commercial availability of quality controlled RLT buffer, RLT was selected for tissue homogenization. After removal of an aliquot of supernatant for nucleic acid analysis, detergents were added and the sample re-homogenized to solubilize proteins. Nucleic acid quality and protein extraction efficiency are also impacted by the aggressiveness of sample homogenization. Though protein extraction increases with aggressive homogenization, nucleic acids can be sheared during sample preparation. Three homogenization techniques: pipette mixing, syringe mixing, and bead beating, were applied to tissue from murine lung, liver, skeletal muscle, brain, kidney, and ovary. The homogenization techniques were evaluated for RNA extraction yield, protein yield and nucleic acid quality. In general, syringe homogenization provided the best quality RNA based on RIN values. Though bead beating and syringe homogenization generally improved RNA yield, the use of tough tissue such as skeletal muscle and less cellular tissue such as lung resulted in poor RNA recovery overall. Ongoing work is directed toward replicate analysis of larger amounts of better-preserved tissue as well as standardized commercial homogenization techniques such as the Qiashredder™ prior to protein quantification by SP3-CTP.

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Abstract # 27

Regulation of rIGS-RNA by a Heat-Sensitive Transcription Factor and A-body Formation

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KEYWORDS: non-coding RNA, transcription factor, chaperone, amyloid, stress

Environmental stress triggers multiple protective mechanisms within cells, allowing them to shut down all but their most basic processes in order to survive. One such process is the formation of amyloid bodies (A-bodies) during extreme stresses, such as heat shock or acidosis [1]. A-bodies are subnuclear, amyloid-like structures and exhibit biophysical properties typically associated with disease-related amyloids. They form when cells experience heat shock or acidosis and dissociate rapidly upon stress termination, unlike their disease counterparts. The current model of A-body formation involves long non-coding RNA (lncRNA) transcripts interacting with positively charged domains on misfolded proteins. This interaction allows for the fibrillation of proteins and thus, the assembly of A-bodies, which occurs in the subnuclear compartments occupied by functional nucleoli prior to the stress. The lncRNA transcripts originate from the ribosomal intergenic spacers (rIGS), which are positioned between ribosomal genes. While region is transcriptionally active in physiological conditions, there are select transcripts that accumulate as cells are exposed to heat shock or acidosis and are uniquely associated with either stress [2]. Currently, it is unknown what physiological role these transcripts have or how their transcription is regulated in either normal or stress conditions.

Here, we uncover regulatory mechanisms of rIGS-RNA transcripts associated with exposure to heat shock. We demonstrate that rIGS16-RNA transcripts, associated with heat shock, are upregulated upon inhibition of the molecular chaperone Hsp70. This chaperone-regulated mechanism mirrors HSF1 activation, which is considered to be the quintessential stress-activated transcription factor responsible for inducing the cell heat shock response. However, the inhibition of the heat shock transcription factor failed to prevent upregulation of the rIGS-RNA, indicating that another transcription factor is involved in the regulation of these non-coding transcripts. We thus hypothesize that rIGS-RNAs are under the control of a novel heat-inducible transcription factor that senses its environmental conditions through binding of molecular chaperones.

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Abstract # 28

Role of eukaryotic initiation factor 5B (eIF5B) in the survival and invasion of brain tumor stem cells (BTSCs)

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KEYWORDS: eukaryotic initiation factor 5B (eIF5B), brain tumor stem cells (BTSCs), glioblastoma multiforme (GBM), temozolomide (TMZ), Invasion.

Given the limited success of current glioblastoma (GBM) treatments, there is an urgent need to develop more effective therapies for this disease. Recent studies on mRNA translation and GBM biology suggest that targeting the mRNA translation machinery could be a promising strategy for combating GBM. Targeting the mRNA translation machinery is becoming increasingly recognized as a crucial approach to addressing the clinical challenges of GBM treatment. During stress conditions, certain signaling pathways are activated and cause post-translational modifications to eukaryotic initiation factors (eIFs), which reduce global mRNA translation. Under such stress conditions, eIF5B promotes the translation of mRNAs encoding specific proto-oncogenes and oncogenes through non-canonical modes of translation initiation. Aberrant expression of eIF5B has been observed in several malignancies and implicated in oncogenesis. We have found that reducing eIF5B levels decreases the non-canonical translation of multiple anti-apoptotic proteins and increases GBM cell sensitivity to pro-death cytokine treatment (1). Depletion of eIF5B also inhibits activation of the epidermal growth factor receptor (EGFR), which blunts the pro-survival, pro-growth, and antigenic signaling pathways in eIF5B-depleted GBM cells.

Additionally, the depletion of eIF5B in patient-derived brain tumor stem cells (BTSCs) enhances their sensitivity to temozolomide (TMZ) and inhibits BTSC invasion (2). We also observed that eIF5B depletion significantly reduces the ability of BTSCs cells to invade the extracellular matrix and increases cell death after TMZ treatment. Together, our results indicate that eIF5B plays a critical role in regulating non-canonical translation initiation and is a key regulator of GBM survival and progression. Our ongoing studies utilizing preclinical GBM models have the potential to establish a foundation for targeting eIF5B in the treatment of GBM patients.

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Abstract # 29

Probing the mechanical origin of RNase resistance in exoribonuclease-resistant RNA (xrRNA) from Zika virus

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KEYWORDS: Viral RNA, optical tweezers, non-coding RNA, Zika virus, RNase resistance.

Flaviviruses, the family of RNA viruses that includes Zika, dengue, yellow fever, and West Nile viruses, contain highly-conserved structures in the 3′ untranslated region (3′-UTR) known as xrRNAs that resist degradation by host RNases. In doing so, they generate sub-genomic RNA fragments that accumulate in host cells, enhancing infectivity and pathogenicity. xrRNAs involve an unusual knot-like structure, in which the 5′-end of the RNA is threaded through a ring formed by a 3-helix junction closed by a pseudoknot, and stabilized by tertiary contacts with the ring. It was originally proposed that this unusual 'ring-knot' topology prevents host RNases like Xrn1 from unfolding the xrRNA and digesting the 3′-UTR [1, 2]. However, studying the Zika virus xrRNA, we showed that the ring-knot fold alone is not sufficient to do so—instead, the key requirement appears to be very high mechanical stability [3]. Here, we investigate more fully the origin of mechanical resistance in the Zika virus xrRNA and its connection to RNase resistance. Using optical tweezers to apply force to single xrRNA molecules and repeatedly unfold and refold them mechanically, we examine the extent to which specific mutations weakening the pseudoknot (A37G/U51G and G34C/A37G/U51G) or breaking 5′ tertiary contacts (G3C and U4C) alter both the structure(s) formed by the xrRNA and the force required to unfold the xrRNA, as compared to the wild-type. We find that both mutations at the 5′-end allow the xrRNA to retain a native-like ring-knot topology but lower its unfolding force significantly compared to wild-type, while abolishing RNase resistance completely. Similarly, the triple pseudoknot mutation abolishes both the mechanical resistance and the RNase resistance. In contrast, the double mutation barely lowers the unfolding force but cuts the probability of forming the mechanically resistant ring-knot by half, matching a similar reduction in RNase resistance levels. These results suggest that a rigid connection of the 5′-end to multiple points on the ring through which it is threaded is essential to maintaining sufficient mechanical resistance to prevent digestion by RNases while the strength of the pseudoknot interactions appears somewhat less critical. By identifying which interactions are most important to maintaining the mechanical resistance that confers RNase resistance, this work reveals potential routes to target xrRNAs therapeutically.

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Abstract # 30

PDCD4 and eIF3F regulate each other's protein levels and affects IRES-mediated translation initiation.

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KEYWORDS: PDCD4, eIF3F, mRNA, IRES-mediated translation, regulation

Regulation of protein synthesis plays a crucial role in gene expression. The majority of eukaryotic messenger RNAs (mRNA) are translated in a canonical, cap-dependent mechanism that requires a free 5' cap and a multitude of initiation factors to form a functionally active initiation complex. However, when cells face stresses such as apoptosis, hypoxia, and starvation, there is a downregulation of cap-dependent translation. When cells face such stresses, alternative mechanisms of translation initiation, such as internal ribosome entry site (IRES)-mediated translation initiation, prevail to express proteins required to combat the stresses faced by the cell. There are distinct protein factors, IRES trans-acting factors (ITAFs), known to regulate IRES-mediated translation initiation. Programmed cell death protein 4 PDCD4 is a well-characterized tumor suppressor protein and ITAF that inhibits mRNA translation of certain IRES-containing mRNAs, such as B-cell lymphoma extra-large (Bcl-xL). S6 kinase (S6K)-mediated phosphorylation of PDCD4 leads to its proteasomal degradation and, as a result, translation of Bcl-xL mRNA is enhanced [1]. Most recently, we have shown that eukaryotic initiation factor 3F (eIF3F), and PDCD4 regulate each other's levels and affect the IRES-mediated translation initiation of Bcl-xL. As such, we have shown that siRNA-mediated depletion of eIF3F decreased the levels of PDCD4 in two glioblastoma cell lines. Likewise, the depletion of PDCD4 resulted in a decrease in the levels of eIF3F. We could not observe this effect in non-cancer cell lines. Furthermore, proteasome inhibition by MG132 restored the levels of PDCD4 under the eIF3F depletion condition, and the levels of Bcl-xL remain unaffected. This suggests that eIF3F and PDCD4 regulate each other's levels and together affect the IRES-mediated translation initiation of Bcl-xL mRNA.

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Abstract # 32

Ionic-dependent structural shift in Dengue dumbbell modulates viral replication

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KEYWORDS: DENV, dumbbell, SAXS, NMR, TMPyP4.

Dengue virus (DENV) is the most prevalent arthropod-borne viral disease worldwide. The 11 kb genome encodes 3 structural and 7 non-structural proteins and is flanked by structured 5' and 3' non-coding regions. This diversity of RNA secondary structures modulates viral replication and immune evasion [1]. Our work identified for the first- time putative G-quadruplex (G4) structures in all DENV serotype 2 using Quadruplex-forming G-rich Sequences (QGRS) Mapper and G4RNA screener [2]. Intriguingly, a conserved putative G4 was identified in the dumbbell's (DB) domain in the 3' terminal region. This highly structured RNA domain is responsible for mediating RNA-RNA long- range interactions critical for viral replication [1]. Circular dichroism spectroscopy demonstrated that DB G4 assumes a parallel topology *in vitro* that is affected by increasing concentrations of divalent ions, such as Mg^{2+}, suggesting that canonical (Dumbbell) and non-canonical (G4) structures co-exist in an ionic-dependent manner. Next, we used thioflavin T, a well-established G4-binding partner [3], to demonstrate that in the presence of K^+ and the absence of Mg^{2+}, G4 is predominantly in a folded conformation. NMR also confirmed the RNA structural shifting by four different peaks at the G4 signature (10 to 13 ppm) region in the presence of K^+. An overall three-dimensional structure was determined using Small-angle X-ray Scattering (SAXS) in which a compacted structure was observed in the presence of K^+, and an elongated one in an Mg^{2+} buffer. Finally, we targeted G4s in cells infected with DENV using the cationic porphyrin TMPyP4. Chemical stabilization of G4 by TMPyP4 decreases viral titers and increases viral replication, suggesting that DENV G4s are important in supporting RNA-RNA long-range interactions. Next, we aim to disrupt one tetrad of the DB G4 and re-assess the replication rates of DENV-infected cells.

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Posters

Abstract # 31
A novel RNA IRES-like element from Tombusvirus binds to 80S ribosomes

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KEYWORDS: IRES, Ribosomes, Virus, RNA, Translation

Internal ribosome entry sites (IRESs) are structured RNA elements that some RNA viruses use to recruit ribosomal subunits and initiate non-canonical 5' cap-independent translation using a subset of translation factors . The Dicistrovirus intergenic region IRES (IGR-IRES) uses the most streamlined mechanism for eukaryotic translation. The IRES contains a three-pseudoknot (PKI-III) structure that can recruit ribosomes directly and start translation from a non-AUG codon [1]. PKII/III mediate 40S binding followed by 60S assembly and the PKI adopts a tRNA codon-anticodon mimicry domain that initiates translation at the adjacent non-AUG codon [2]. The origin of this mechanism is not known. We have used metagenomic and bioinformatic based studies to reveal hundreds of dicistrovirus IRES-like elements across a wide range of dicistrovirus genomes. Here, we report an RNA element that resembles a dicistrovirus IRES in a genome of Tombusvirus, a positive sense RNA virus family that typically infects plants. The IRES-like structure is located in the intergenic region upstream of the ORF that encodes for structural proteins. Using radiolabelled RNA:ribosome binding assays, we show that the Tombusvirus IGR-IRES can bind to purified human 40S and 80S ribosomes. Mutations in key structural elements, specifically pseudoknots II and III, abrogate RNA:ribosome binding, in line with the predicted IRES-like structure. The predicted Tombusvirus RNA structure does not support translation in a bicistronic reporter assay in wheat germ, rabbit reticulocyte lysate, or insect Sf21 translation extracts. These results provide a foundation to studying the diversity of RNA elements that bind to ribosomes, and demand further investigation of IRESs in the broader context of translation and infection.

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Abstract # 33

Development of a Split GFP System to Monitor Virus Infection in Cells

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KEYWORDS: Infectious clone, split GFP, reporter, transfection, virus

Cricket Paralysis Virus (CrPV) is a positive-sense, single-stranded RNA virus that has served as a powerful model for studying fundamental virus-host interactions in insect cells [1]. Currently, CrPV infection in *Drosophila* S2 cells can be monitored by assaying viral RNA and protein levels biochemically; however, these methods can be time-consuming and labour-intensive, and is not suitable for quickly and easily visualizing CrPV infection in live cells. In other model virus systems, it has been shown that recombinant virus encoding a fluorescent reporter is an extremely useful tool to monitor infection in real time [2,3]. However, it has been challenging to integrate and maintain a large full-length fluorescent protein tag in the CrPV genome. To overcome this limitation, we developed a split green fluorescent protein (split GFP) system whereby the eleventh strand of the GFP beta barrel (GFP11) is incorporated into the CrPV genome. Flanking 2A peptides allow for co-translational cleavage of the GFP11, to prevent potential interference with viral protein function. Strands one to ten of the GFP (GFP1-10) are stably expressed in S2 cells. Upon viral infection, the virus-encoded GFP11 will complement the stably expressed GFP1-10, thus producing the full-length fluorescent molecule. The resulting fluorescent output can then be easily detected in live cells using a standard fluorescence microscope. Thus far, our preliminary data show that we have successfully cloned the GFP11 fragment into the CrPV infectious clone. Propagation and sequencing of the virus confirmed the stability of the GFP11 integration. We have also successfully selected a polyclonal population of stably-transfected S2 cells expressing GFP1-10. Once optimized, this system will provide a rapid, semi-quantitative readout for viral infection in live cells. It will also enable the selection of the CrPV-infected population by fluorescence activated cell sorting (FACS), thus allowing for targeted downstream assays. Furthermore, this novel viral clone should enable monitoring of virus infection in an organism such as a fruitfly.

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Abstract # 34

Elongation control of mRNA translation drives Group 3 medulloblastoma adaptation to nutrient deprivation

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KEYWORDS: Metabolism, Brain tumor, Medulloblastoma, Messenger RNA translation, Metabolic stress, Nutrient deprivation, Mitochondrial Membrane, Translation Elongation.

BACKGROUND: Group 3 affiliation and *MYC* genetic amplification are associated with poor life expectancy and substantial morbidity in children suffering from medulloblastoma (MB). The high metabolic demand induced by MYC-driven transformation sensitizes MYC-overexpressing MB to cell death under conditions of nutrient deprivation (ND). Additionally, MYC-driven transformation is known to promote mitochondrial oxidative phosphorylation (OXPHOS). We previously reported that eukaryotic Elongation Factor Kinase 2 (eEF2K), the master regulator of mRNA translation elongation, promotes survival of MYC-overexpressing tumors under ND. Interestingly, eEF2K is overexpressed in MYC-driven MB and our preliminary proteomics data highlight large-scale alterations in OXPHOS components affecting eEF2K deficient MB cells. We therefore hypothesized that eEF2K activity is required for the selective translation of mRNAs needed for efficient OXPHOS, and for the progression of MYC-driven MB.

METHODS: Multiplexed enhanced Protein Dynamic Mass Spectrometry performed in eEF2K knockdown MYC-overexpressing D425 MB cells to identify mRNAs selectively translated upon eEF2K activation. Time course experiments under ND were conducted in eEF2K knockout (KO) D425 cells to assess the presence of electron transport chain (ETC) complexes I-IV in their native state (via BN-PAGE), as well as transcript expression of individual ETC complex components (by qPCR). The effects of eEF2K inactivation on oxygen consumption, metabolic fluxes and mitochondrial membrane potential were studied with Seahorse technology and JC1/TMRE staining. The viability of eEF2K KO D425 cells was assessed by Incucyte system. Finally, MB orthotopic xenograft mouse models were used to confirm *in vitro* observations.

RESULTS: Multiple (9 out of 10 detected) components of the mitochondrial OXPHOS pathway are selectively translated upon eEF2K activation. Inactivation of eEF2K by genetic KO leads to the disassembly of ETC complexes I-IV without affecting mRNA levels of their respective components. Consistently, eEF2K KO MB cells display decreased mitochondrial membrane potential and ~20% increased proton leak thorough the mitochondrial membrane. In addition, eEF2K inactivation results in increased D425 cell death under ND and doubles survival of MB bearing mice fed with calorie restricted diets ($p<0.05$).

CONCLUSION: Control of mRNA translation elongation by eEF2K is critical for mitochondrial ETC complex assembly and efficient OXPHOS in MYC-overexpressing MB, likely representing an adaptive response by which MYC-driven MB cells cope with acute metabolic stress. Future therapeutic studies will aim to combine eEF2K inhibition with caloric restriction mimetic drugs as eEF2K activity appears critical under metabolic stress conditions.

Abstract # 35

Vitamin K2 Biosynthesis, Optimization, and Extraction in C. *merolae*

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KEYWORDS: Menaquinone, *Cyanidioschyzon merolae,* Vitamin K2, Pharmaceutical, Stereoselectivity.

Menaquinones, also known as vitamin K2, are essential compounds with diverse functions in microorganisms and humans. *Cyanidioschyzon merolae* (Cm), an extremophilic red alga, shows potential for producing menaquinones, specifically MK-4, the biologically active form of vitamin K2 [1]. However, the biosynthetic pathway and yield of menaquinones in Cm remain unclear. This study aims to investigate menaquinone biosynthesis in Cm and enhance MK-4 production through genetic and environmental manipulation. Menaquinones are synthesized through the O-succinyl benzoate pathway or the futalosine pathway, these pathways are essential in terpenoid backbone synthesis (1). These pathways receive precursor molecules from the MEP, MVA, and glycolysis pathways. Menaquinones aid in phosphorylation and electron transport and RNA sequencing data from the Rader lab, revealed that most of the genes predicted to be in the menaquinone pathway in Cm (CNS227C, CMQ134C, CMJ072C, CMN011C, CMK049C, and CMT582C) showed increased expression under growth conditions involving mercury and nickel. Genes relating to the MEP pathway in Cm, also increase expression levels under the same conditions, thus indicating these conditions can potentially increase menaquinone yeild in Cm. It is expected that menaquinone production will increase under cytotoxic stress, therefore, cultivating Cm in these conditions is anticipated to lead to higher menaquinone yields. A competing pathway of menaquinone biosynthesis is the farnesyl diphosphate conversion to geranyl phosphate, it has been shown that a knockout of a crucial gene to this pathway, HepT, will improve yield of menaquinones [1]. The outcomes of this research will shed light on the biosynthetic pathway of menaquinones in Cm and the factors influencing MK-4 production. Moreover, the study will contribute to the understanding of Cm's genetic engineering potential, given its ease of growth, tractable genetics and simple cellular architecture (a single mitochondrion, chloroplast, and nucleus [2]). Ultimately, this investigation aims to pave the way for efficient biosynthesis of MK-4 in Cm, which holds significant implications for the food and pharmaceutical industries.

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Abstract # 36

RNase MRP Function In *C.merolae*

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KEYWORDS: *C.merolae*, RNase MRP, preribosomal RNA processing, ribosome biogenesis, noncoding RNA

MRP RNA is an abundant, essential noncoding RNA (ncRNA) whose functions have been studied in yeast but are incompletely understood. It has been reported that MRP RNA is required for pre-ribosomal RNA processing into matured ribosomal RNAs involved in protein synthesis. Mutations to the single genomic locus for this noncoding transcript cause inviable yeast (1), embryonic lethality in mice, and a spectrum of severely debilitating human diseases including pleiotropic human diseases such as cartilage hair hypoplasia-CHH (2), harkening to MRP RNA's essential role in biology. MRP is a noncoding RNA with a reported homolog in all eukaryotes studied to date (3). In budding yeast, affinity-purified MRP RNP (ribonucleoprotein) can cleave a fragment of the preribosomal RNA (pre-rRNA) internal transcribed spacer 1 (ITS1) at a specific site to generate the two forms of 5.8S rRNA (4), and this site exhibits reduced cleavage at a nonpermissive temperature in temperature-sensitive mutants of the MRP RNA (5). In Humans, the role of the RNA component of RNase MRP complex (RMRP) in pre-rRNA processing has long been established, but the precise role was not understood until the generation of CRISPR/Cas9- mediated deletions of the RMRP gene revealed that RMRP directs the cleavage at site 2 in ITS1 of human pre-rRNA (6). In Drosophila, the expression of the Drosophila ortholog of MRP RNA (CR33682) which was predicted by a bioinformatics screen for MRP RNA sequences (7) has been reported and the characterization of a mutant strain shows that Drosophila MRP (dMRP) mutant displays a defect in processing 5.8S rRNA that has been associated with human and *S. cerevisiae* RNase MRP mutants, as well as a defect in early rRNA processing similar to a defect reported in *S. cerevisiae*. The characterization of this activity in *Saccharomyces cerevisiae* and subsequent cloning of the gene coding for the RNA subunit of the yeast enzyme has enabled a genetic approach to the identification of a nuclear role for RNase MRP. Although RNase MRP has been extensively studied in a wide range of organisms, relatively little is known about this complex in *C. merolae*. However, studies have identified a putative MRP RNA gene in the *C. merolae* genome, suggesting that this organism also possesses an RNase MRP complex. It is not yet clear how the MRP RNA gene in *C. merolae* is processed or how the MRP RNA complex functions in this organism. However, given the conservation of MRP RNA in other eukaryotes and the importance of RNase MRP for ribosome biogenesis, it seems likely that the MRP RNA complex in *C. merolae* plays a similar role in processing rRNA and maintaining cell growth and proliferation as reported in other organisms. In *C. merolae*, the MRP RNA is located in the intronic region of the non-coding CMK142T gene, and this intron turns out to be the most accumulated intron when exposed to heat stress at 57°C and it houses MRP RNA (our unpublished data). This research investigates the effect of heat stress on rRNA processing by measuring ribosome biogenesis in *C. merolae* and the proteins associated with the accumulated CMK142T intron by biochemically isolating the intron and performing mass spectrometry.

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Abstract # 37
Intron accumulation: an intriguing consequence of heat stress in *Cyanidioschyzon merolae*

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KEYWORDS: Intron, accumulation, RNA, splicing, C. merolae

Pre-mRNA splicing, catalyzed by the spliceosome, is an essential step in eukaryotic gene expression as it allows organisms to remove otherwise deleterious introns that interrupt the gene. Exons encode protein segments, while intronic regions have many functions, including gene regulation and maintaining protein diversity[1], but must be removed prior to translation. The red alga *Cyanidioschyzon merolae* has a highly reduced spliceosome, which lacks the U1 snRNP normally required for 5' splice site recognition[2] and has lost all but 38 remaining introns. The last common eukaryotic ancestor contained an intron-rich genome[3]. This dramatic loss of introns may indicate that the remaining introns are essential for survival. Recent evidence in yeast has shown that introns can play a key role in adapting to stress sometimes due to non-coding RNAs (ncRNAs) embedded within the intronic regions[4]. We aim to analyze the effect of heat stress on *C. merolae* by carefully measuring intron accumulation in four distinct genes.

Short-read transcriptomic data suggested that, in addition to widespread changes in gene expression generally, introns in 19 genes accumulated at 55 ℃ after one hour compared to 42 ℃. However, at 60 ℃ for 15 and 30 minutes, only the intron within CMK142T accumulated. Read coverage plateaued only in the middle of the introns indicating that only part of the introns accumulated. To validate these results and get a more quantitative measure of the accumulation, we have used RT-qPCR to measure changes in four target genes under heat stress. We have separately monitored levels in the intron, the pre-mRNA, and all transcripts. We confirmed the increase in expression at 57 ℃ (ten-fold) of the accumulated plateau in CMJ129C. We are also using Northern blotting to validate and extend these results. Intriguingly, all of the accumulated regions are predicted to be highly structured, which may reflect unknown functions. Intron accumulation was first reported in yeast subject to starvation. In that case, the accumulation may have led to regulation of ribosome biogenesis via inhibition of splicing. We are investigating this possibility in *C. merolae*.

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Abstract # 38

Unraveling the Functional Significance of Introns in *Cyanidioschyzon merolae*

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Submission type: Poster presentation

KEYWORDS: Splicing, intron, Cyanidioschyzon merolae, homologous recombination, and intron-mediated enhancement

Eukaryotic genes consist of exon sequences interrupted by introns. Upon transcription, introns are removed from pre-mRNA through the splicing process, and the exons are ligated together to form mature mRNA. Initially, introns were considered genetic junk because they have no function on their own and simply need to be removed. Subsequent research has shown that some introns contain essential information, such as non-coding RNAs, and may even have their own functions. They are also direct regulators of multiple stress-response genes during nutrient depletion. Introns can also have a more active role in regulating alternative splicing, and nonsense-mediated decay (NMD) mechanism as well as promoting gene expression through a process called intron-mediated enhancement (IME).

Cyanidioschyzon merolae 10 D (Cm) is an interesting organism as it only has 38 introns, but the closest ancestor of Cyanidiales had around 2,000 introns. Our research aims to determine whether the maintenance of the small number of introns in Cm is due to some essential function or whether they simply have not yet been removed. A first reasonable step toward answering this question is to examine whether deleting Cm introns affects its growth.

We used homologous recombination to delete introns in Cm. Linear constructs containing an intronless version of the target gene and a selectable marker were transformed into algal cells. Colonies were screened by PCR and Southern blotting to ensure that the intron was removed and that additional copies of the gene were not introduced non-specifically into the genome. To date, we have made 13 individual Δi (intron-deleted) strains, as well as one strain with eight introns deleted simultaneously.

Growth measurements comparing the Δi and parent (T1) strains showed, surprisingly, that some Δi strains grow faster than T1. For others, there was no difference in growth rate observed between the tested strains in normal media. However, some Δi strains grew slower in phosphorous-depleted media, which may explain the importance of introns in adapting to stress such as nutritional depletion. We are testing other growth conditions to investigate further the effect of these deletions on Cm and to get a global view of whether Cm's minimal pool of remaining introns are biologically important.

Abstract # 39

Unraveling the Mystery of 5’ Splice Site Recognition: Insights from Pre-mRNA Splicing in Cyanidioschyzon merolae

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KEYWORDS: Genetic mutations, 5' splice site recognition, Pre-mRNA splicing, U5 snRNA, Cyanidioschyzon merolae, Nanoluciferase splicing reporter

ABSTRACT

Splicing is a vital process in mRNA maturation, facilitated by the spliceosome complex consisting of small nuclear RNAs (snRNAs) and associated proteins. The spliceosome initiates splicing through the binding of U1 snRNA and U2 snRNA to the 5' splice site (5'ss) and branchpoint (BP), respectively. *Cyanidioschyzon merolae*, a unicellular red alga with a reduced genome and spliceosome, lacks U1 snRNA and its associated proteins. This intriguing characteristic raises the question of how the 5’ss is recognized. One possibility is that U5 snRNA, which contains a complementary sequence to the 5'ss, may substitute for U1 snRNA.

To explore this hypothesis, we made a plasmid incorporating the CMC053 gene fused with a nanoluciferase gene to form a splicing reporter, along with a second copy of U5 snRNA in addition to the endogenous one. Genetic mutations were introduced to the 5'ss to assess the reporter's activity. Complementary mutations were also introduced to the U5 snRNA to determine if they could restore reporter function, which would provide evidence for U5 acting in place of U1.

The Nluc assay results showed a significant reduction in splicing activity with a single mutation at the 5'ss, resulting in a two-thirds loss of activity. Furthermore, three mutations led to a 95% decrease, indicating a 26-fold drop in splicing efficiency. While no rescue was seen for the single-mutant 5’ss, the introduction of compensatory U5 snRNA, capable of interacting with the triple-mutant 5'ss, significantly but only partially increased nanoluciferase activity compared to the absence of compensatory U5 snRNA. Despite this partial rescue, previous studies suggest that 5'ss recognition is not solely dependent on U1 snRNA base pairing, as U6 snRNA also plays a role in 5'ss interaction. The triple mutation may have eliminated some of the base pairing with U6 snRNA, contributing to the partial rescue observed. Additionally, measuring the expression level of compensatory U5 snRNA through fluorescence northern blot analysis revealed an average fluorescence intensity three times higher than the wild type, confirming that the plasmid-borne U5 is properly expressed.

In conclusion, this study provides evidence supporting the role of U5 snRNA in 5'ss recognition during splicing in C. merolae. The Nluc assay demonstrated the impact of 5'ss mutations on splicing activity and the partial rescue by compensatory U5 snRNA. The fluorescence northern blot analysis confirmed the increased expression of compensatory U5 snRNA. These findings shed light on the complex mechanisms of splicing and its evolution, highlighting the need for further investigations to fully elucidate the role of U5 snRNA in splicing.

Abstract # 40

Uncovering the regulatory pathways associated with functional and reversible amyloid aggregation

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KEYWORDS: Amyloid bodies, functional amyloid aggregation, long non-coding RNA, high throughput imaged based screening, PI3K.

In response to environmental stress, human cells have been shown to form reversible amyloid aggregates within the nucleus, termed amyloid bodies (A-bodies). These structures share many of the biophysical characteristics of the irreversible pathological amyloid aggregates observed in neurodegenerative diseases; however, A-bodies are rapidly disassembled after the stressor is removed. Functionally, A-body formation induces a state of cellular dormancy by sequestering proteins away from their downstream effectors to conserve energy during periods of cell stress. As a newly identified subcellular domain, the regulation of the A-body aggregation process remains uncharacterized, with the only regulatory factor identified to date being a class of long non-coding RNAs that seed the formation of these structures. This functional and readily inducible amyloid-like domain provides a unique opportunity to study cell-environment communication pathways, as well as the molecular interactions that regulate amyloid aggregation in normal physiology. As no candidate pathways have been identified to regulate A-body formation, an unbiased image-based high-throughput screening approach was used to search for small molecules capable of impairing A-body formation. By screening a diverse library of over 4000 small molecules, the PI3K/mTORC2/AKT signaling pathway was identified as a potential regulator of A-body formation. An analysis of the A-body associated long noncoding RNA expression following AKT1 depletions revealed that the PI3K/mTORC2/AKT pathway may be regulating A-body formation through the expression of these seeding RNAs. This identifies a novel role for the predominantly growth associated PI3K/mTORC2/AKT signaling pathway, in inducing cellular dormancy through functional amyloid aggregation.

Abstract # 41

Developing a Reliable qPCR and Western Blot Workflow for Detection of Myelin Genes in Murine Brain Tissue

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KEYWORDS: Neurodevelopment, Myelination, Gene Expression, qPCR, Western Blot

Mature oligodendrocytes facilitate efficient conduction of electrical signals in the brain by insulating the neuronal axon with a myelin sheath[1]. During neurodevelopment in mouse model, oligodendrocytes undergo maturation from progenitor cells within the first three postnatal weeks which is marked by the production of myelin-specific genes. Cell differentiation is highly regulated at the both the transcriptional and translational level, and there is often a large discrepancy in the magnitude of change between mRNA and protein expression[2]. While this has biological relevance in allowing precise timing of cell differentiation, the limitations in conventional Western Blot (WB) techniques such as narrow dynamic range and low sensitivity as compared to qPCR may exaggerate these differential expression levels[3,4]. Our aim is two-fold: (i) validate qPCR assays for quantification of myelin mRNA while following MIQE guidelines and (ii) optimize WB workflows for quantification of protein to better reflect the expression of myelin genes in murine neurodevelopment[5]. Validated qPCR assays showed changes in developmental mouse myelin mRNA expression that correspond with a previously published RNA-seq data on oligodendrocyte lineage cells[6]. To address limitations in WB, the following variables were examined: sample solubilization, transfer conditions, protein load, detection and imaging parameters, and data normalization. A proof of principle study with the optimized WB protocol showed greater association between myelin oligodendrocyte glycoprotein (MOG) mRNA and protein expression over the course of postnatal neurodevelopment. The combined qPCR and WB compendium offers updated information on materials and methods to aid in reliability and reproducibility when quantifying myelin gene expression. The methodology will allow for accurate assessment on the extent of myelination in mouse models of neurodegenerative disease, such as multiple sclerosis, and facilitate screening of therapeutic interventions that mitigate myelin damage or promote myelin repair.

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Abstract # 42

Up-regulation of membrane transport genes in overwintering adult mountain pine beetles (*Dendroctonus ponderosae*).

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KEYWORDS: differential gene expression, *Dendroctonus ponderosae*, bark beetles, overwintering, cold acclimation.

With mountain pine beetle populations undergoing range expansion into the boreal forests east of the Rocky Mountains, where temperatures are typically colder, investigations into cold acclimation abilities of mountain pine beetles (*Dendroctonus ponderosa*) have increased. Though many investigations focus on larvae as the main overwintering stage, a recent study noted overwintering new adults [1]. Similar to overwintering larva, this study found an increase in critical metabolite concentrations associated with cold tolerance [1]. To further study cold acclimation, we examined changes in gene expression within the overwintering adults. A preliminary Tagseq analysis showed a list of upregulated and downregulated genes, which included genes associated with the production and transportation of metabolites associated with cold tolerance. Using digital droplet polymerase chain reaction (ddPCR), changes in mRNA transcripts of the identified membrane transporters were quantified over the study period, late autumn to early winter. We found a significant increase in gene expression in four of the five genes examined: facilitated trehalose transporter, long-chain fatty acid transporter, sodium-coupled amino acid transporter, and proton-coupled amino acid transporter. This analysis increases our understanding of cold acclimation processes in bark beetles by confirming the upregulation of four membrane transport genes associated with the increased metabolite concentrations.

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Abstract # 43

Developing RNA interference (RNAi) as a biopesticide for mountain pine beetle (*Dendroctonus ponderosae*)

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KEYWORDS: RNA interference, gene expression, Dendroctonus ponderosae, mountain pine beetle, forest pest management

In western North America, the mountain pine beetle (*Dendroctonus ponderosae*, MPB) is the most destructive pest of pine trees, consequently causing ecological, economic, and socio-ecological impacts. British Columbia is currently in a recovery phase following the MPB outbreaks from the early 2000s; however, with favourable conditions in regenerating pine and climate conditions, it is likely that large-scale MPB outbreaks will recur. Previous and current management techniques are ecologically and economically costly, creating a need for a low-cost, ecologically safe method for MPB population management. A naturally occurring pathway, ribonucleic acid interference (RNAi), can be used to silence targeted genes in insects by disrupting cellular function. Developing RNAi to use in ecologically safe trap trees can create a novel tool for managing MPB populations. This research will lay the groundwork leading to use of RNAi as a real-world application for MPB management. The aim is to develop existing and new gene targets, and combinations thereof, for causing species-specific MPB mortality via RNAi. Lab based studies will investigate feeding MPB both dsRNA-treated phloem (dsRNA translocated throughout phloem) and heat-inactivated, dsRNA-expressing yeast to test for gene silencing effects and efficient delivery systems. To look for potential cascading effects of silencing the targeted gene, transcriptomics will be used to measure the total RNA expression within the cells of treated adult MPB.

Abstract # 44
SF3B4 and Regulation of pre-mRNA Splicing During Starvation in Yeast

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KEYWORDS: RNA, splicing, intron, yeast, regulation, growth, starvation

ABSTRACT
Splicing of precursor-messenger RNAs (pre-mRNAs) through the removal of introns is an essential part of the maturation of protein-coding RNAs in eukaryotic cells.[1] Recently, it has been shown that introns mediate the starvation response in yeast, downstream of the TORC1 and PKA pathways.[2,3] Both pre-mRNA and intron accumulation under starvation conditions result in a downregulation of splicing of RNAs coding for ribosomal proteins with a resulting decrease in ribosome synthesis and enhanced cell survival. Intron deletion has been shown to negatively affect cell survival in stationary phase; cell viability can be restored by reintroduction of the deleted introns. Here, we show that significant enhancement of splicing by mutation of an essential splicing factor also negatively affects cell survival, further demonstrating the importance of the splicing pathway in response to changing growth conditions in yeast.

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Abstract # 45

Stress Granules in ex-vivo cultures of primary cancer cells or tissue induced by chemotherapeutic treatment

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KEYWORDS: integrated stress response, colorectal cancer, liver metastasis, stress granules, explant cultures, organoids

Background: Stress Granules are accumulations of RNA-binding proteins, parts of the translation initiation complex and mRNA. If cells encounter certain stressful environments, they can form SGs via phosphorylation of eukaryotic initiation factor 2, this leads to restructuring in translation habits, with favoring stress-related proteins. The mRNA that is included into SGs is halted in its translational process, however once stress is released they can quickly be translated again. SGs have been discovered in various cells including brain and heart cells. Recently they have been described to play a crucial role in cancer research. SGs are involved in cancer progression, metastasis and possibly also drug resistance.

Materials and Methods: Patient samples from colorectal cancer and liver metastasis were cultured as either explant slices or organoids. Samples were treated with oxidative stress inducer Sodium Arsenite (positive SG control) or with chemotherapeutics oxaliplatin/5-fluorouracil. Stress Granules were visualized using co-Immunofluorescence staining of Y-box binding protein 1 (YB-1) and Ras GTPase-activating protein-binding protein 1 (G3BP1). Quantification of them was done using the HALO® software, which has an integrated AI.

Results: SGs could be induced in both model systems via sodium arsenite as well as chemotherapy treatment. SGs are significantly more prominent in tumor tissue than in the surrounding stroma.

Conclusion and future directions: SGs are inducible in explant cultures as well as in organoids. Currently clinically used chemotherapeutic combinations could activate SG formation, which opens doors for future long-term experiments. Preliminary data on cell lines proposed the HDAC inhibitor Entinostat as a good solution to inhibit SG formation in cell culture. Whether it works in ex-vivo systems has yet to be determined.

Abstract # 46

mRNA Expression Levels of HDACs, HATs, and BETs in Cortical Brain Tissue of Mice at Different Stages of Postnatal Development

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Presentation type: Poster

KEYWORDS: Brain, Neurodevelopment, Epigenetics, Oligodendrocytes, Myelination

Oligodendrocytes produce myelin, which is a protective sheath that wraps nerve projections for efficient communication between brain cells[1]. In the developing brain, neural progenitor cells undergo specific stages of differentiation to become mature oligodendrocytes[2]. Neural differentiation is governed by epigenetic regulations such as histone post-transitional modifications (PTMs)[3]. One important modification is acetylation, which is regulated by histone acetyltransferases (HATs), histone deacetylases (HDACs) and bromodomain / extra-terminal domain proteins (BETs)[3]. HATs transfer acetyl groups to lysine residues, thus they are known as the "writers"[3]. HDACs remove acetyl groups from and are known as the "erasers"[3]. BETs play the role of "readers" as they recognize acetyl groups to promote the transcriptional activation of genes[4]. To further understand the functional roles of these epigenetic modifiers, we measured their mRNA expression profiles in the developing murine brain using real-time quantitative PCR. Primer pairs for amplification of HDACs, HATs, and BETs (35 acetylation-related genes in total) were designed using NCBI primer blast and empirical validation was performed to determine specificity, temperature dependence, and efficiency. The validated qPCR assays for HDACs, HATs, and BETs were used to quantify mRNA expression in mouse cortical brain tissue across different postnatal stages (P0, P7, P14, P21, P28 and P84) that span the period of oligodendrocyte differentiation and maturation. Five general patterns of gene expression across development were identified for the acetylation-related genes: (i) decline of expression followed by plateau, (ii) decline followed by a return to P0 expression, (iii) decline followed by an incline that plateaued below P0 expression, (iv) increase followed by plateau, and (v) increase followed by a return to P0 expression. Most genes of interest (25/35) followed the first pattern, a decrease of expression. Three HDACs (Sirt3, Hdac9, and Hdac11) increased across developmental stages. Two HDACs and two HATs (Hdac4, Hdac5, Pcaf, and Myst4) declined and returned to P0 expression. Two HATs (Cbp and P300) declined and returned to a plateau below P0 levels. Lastly, Sirt2 uniquely increased to peak at P21 and then returned to P0 levels. These patterns will help provide insight into complex interactions between epigenetic modifications that regulate gene expression in neural development, but given that protein acetylation is not limited to histones, the results may also have implications beyond epigenetics in the regulation of cell differentiation.

References: [1] DOI: 10.1038/nn1620 [2] DOI: 10.1161/STROKEAHA.109.570325

[3] DOI: 10.1016/j.cell.2009.06.049 [4] DOI: 10.1038/ncomms13855

Abstract # 47

Exploring the impact of intron sequence on splicing and expression in *C. merolae*

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KEYWORDS: intron- mediated enhancement, splicing, expression, luciferase, *C. merolae*

Intron-containing genes in the unicellular red alga *Cyanidioschyzon merolae* exhibit a surprisingly broad range of splicing. In this project, we aim to determine whether the amount of splicing of any particular gene is specified by its introns. Another intriguing aspect of my research is to investigate the potential influence of introns on gene expression levels. Introns were initially considered non-functional elements that required an energy-consuming process to remove them prior to translation. Today, they are known to be crucial elements in both intron-rich and intron-poor lineages. Recent evidence indicates that Intron-rich genomes can benefit their host species through improved gene expression, proteomic diversity, and increased genetic stability. One of the most interesting functions of introns that is observed in some eukaryotic organisms is the phenomenon known as intron-mediated enhancement (IME) in which gene expression is increased by the presence of introns. Intronic splicing enhancers and inhibitors, cis-acting sequences found in many introns, are another important example of intronic function that have been well documented in metazoans. Yet, whether introns play essential roles in intron-poor lineages has been less investigated. C. *merolae*, is a reduced eukaryotic organism with 39 introns and is considered an intron-poor lineage. It also displays a wide range of splicing from about 10 to 85%, yet we have not been able to find anything that correlates with the splicing level. By focusing on this organism, which has retained such a small number of introns and a dramatically reduced spliceosome, this study is aims to understand the effect of intronic sequences on gene splicing and expression efficiency and identify these essential regulatory sequences. To conduct this experiment, an intron swapping construct has been designed. This construct designates CMC053, a highly spliced and highly expressed gene of *C. merolae*, as the host gene which is under control of its own natural promotor and is fused to the nanoluciferase gene, the product of which is readily detected. To determine the impact of intron sequence, introns from genes with a range of splicing levels will be swapped into CMC053 to replace its intron. The level of splicing will be assessed whit a luciferase assay, while the changes in expression level will be investigated using Northern blot analysis. This research could significantly advance our understanding of the evolutionary aspects of introns and their functions, which can be useful in different biotechnological aspects.

Abstract # 48

Translational efficiency of N1-methylpseudouridine-modified mRNAs in *Drosophila melanogaster* Schneider 2 cells

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Presenting author level: Undergraduate

KEYWORDS: mRNA nucleoside modifications, N1-methylpseudouridine, translation efficiency, immunogenicity, invertebrate systems

Modifications in mRNA nucleosides occur naturally and can be introduced in *in vitro* transcribed mRNA, which is used in therapeutic applications [1]. One notable example of a modified nucleobase is N1-methylpseudouridine (m1Ψ), which is incorporated in the Pfizer/BioNTech and Moderna COVID-19 mRNA vaccines [2]. Such nucleoside modifications improve translation efficiency and reduce immunogenicity both *in vitro* and *in vivo* in mammalian systems [3]. However, the effect of mRNA nucleoside modifications in non-mammalian systems remains largely unexplored. In this study, we examine the translation of m1Ψ-modified mRNA in *Drosophila melanogaster* Schneider 2 (S2) cells. We in *vitro* transcribed a reporter RNA containing Nano-luciferase (Nluc) that contains unmodified ribonucleotides or m1Ψ (100%). mRNAs were then transfected into S2 cells, and Nluc activity was measured at 3-, 6-, 9- and 24-hours post-transfection. We found that at 24-hours, m1Ψ-modified mRNA samples exhibited a significantly higher level of Nluc activity compared to its unmodified counterpart while no discernible differences were observed between the m1Ψ-modified and unmodified mRNAs samples at other transfection timepoints. These results suggest that m1Ψ-modified mRNA can induce higher translation levels than unmodified mRNA in *Drosophila melanogaster* cells. Taken together, these results provide valuable insights into the effect of mRNA nucleoside modifications in invertebrate systems and serves as a foundation for further investigation into the innate immune pathways of *Drosophila melanogaster* involved in the recognition of and immunogenic response against mRNAs.

REFERENCES

[1] https://doi.org/10.1016/j.biopha.2021.111953

[2] https://doi.org/10.1021/acscentsci.1c00197

[3] https://doi.org/10.1016/j.jconrel.2015.08.051

Abstract # 49
Is splicing essential in *Cyanidioschyzon merolae?*

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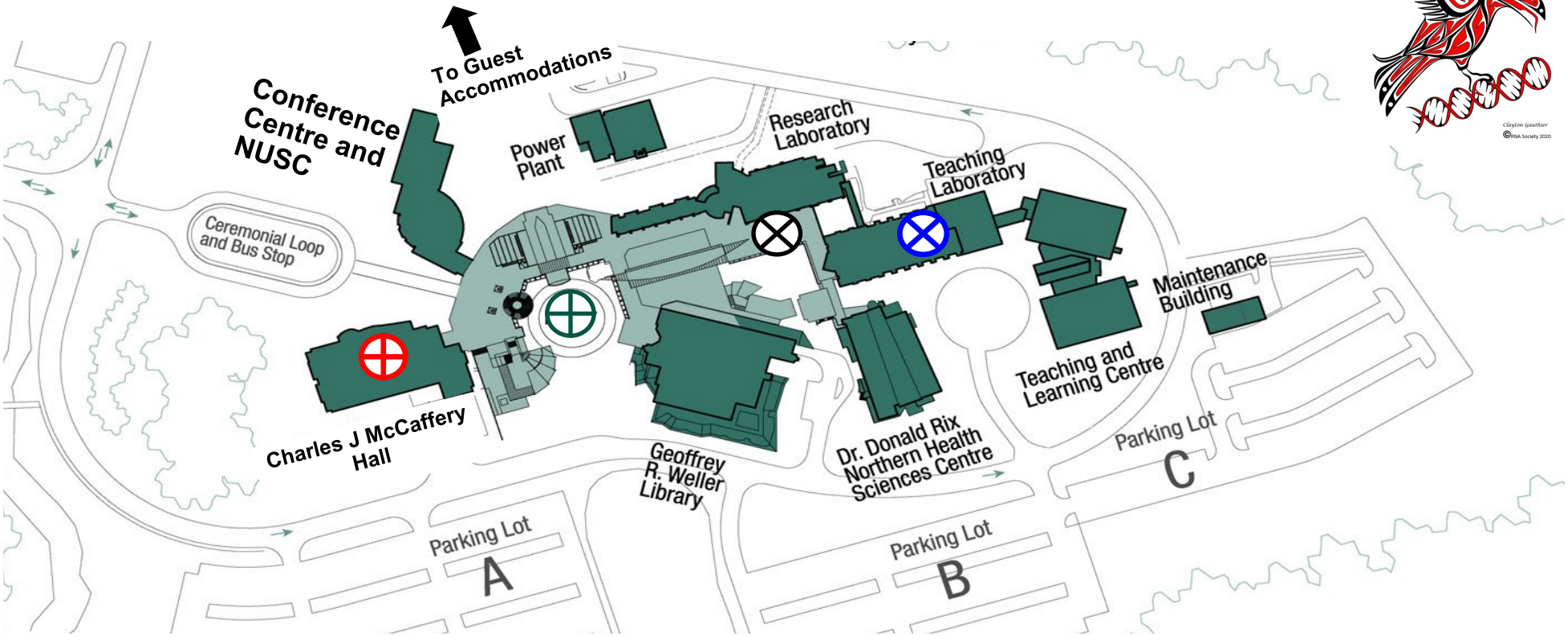
Presenting author level: M.Sc

KEYWORDS: splicing inhibition, snRNAs, antisense DNA, degron, transient gene expression, inducible gene expression.

Protein-coding sequences of eukaryotic genes are often interrupted by non-coding sequences called introns. Introns must be removed from mRNA precursors, and protein-coding segments known as exons are then ligated together to form mature messenger RNA. This essential process in eukaryotic gene expression is called mRNA splicing. Splicing is carried out by a large complex that contains small, nuclear RNAs (snRNAs) and many proteins. Due to the complexity of the spliceosomal machinery in other eukaryotes, studying splicing has been very challenging. *Cyanidioschyzon merolae* is a unicellular red alga with only 38 introns in its genome and a much simpler set of splicing machinery than in humans. This simplicity allows us to do experiments that would be impossible in humans. It has been estimated that the ancestral red alga contained ~1700 introns from which we can infer that *C. merolae* has lost almost all of its introns. This raises the possibility that splicing is no longer essential for this organism.

I am addressing the question of whether this vestigial splicing system is biologically important by inhibiting splicing in various ways. If splicing is not required for the survival of this organism, inhibiting this process should not impact cell survival. In contrast if splicing is essential, a deleterious phenotype and cell death is expected. The attempt to inhibit splicing was performed using antisense RNA and degron techniques. In the first approach, we transiently express the antisense version of U2 and U4 snRNAs, and Cef1 mRNA, under the control of a nitrate inducible promoter by transforming an engineered plasmid with a selectable marker into the cells. The antisense RNA should bind the snRNAs leading to their degradation. The nitrogen source for *C.merolae* in rich media is ammonium, in which the promoter will be off. By shifting cells to nitrate media, we activated antisense expression, after which I expect splicing to be inhibited and cell death to occur. In the second approach we implemented an inducible degron system to degrade splicing proteins. Degrons are motifs that target proteins for degradation, and they can be fused to target genes to allow encoded proteins to be degraded by addition of rapamycin, a small molecule that turns on the degradation system. We targeted Prp8 and Clf1, both core spliceosomal proteins. Cell growth was monitored for both techniques as well as antisense RNA expression (Northern Blotting) and protein degradation (Western Blotting) and levels of splicing will be measured by qRT-PCR on a panel of five intron-containing genes. While these experiments have so far been unsuccessful, we have been able to over-express U5 snRNA from a plasmid, so I am currently generating an antisense version of this plasmid, as well as trying induction with a stronger promoter. Despite numerous attempts with morpholino oligos, gene deletion, splicing inhibitors, etc, our only experiment to date that is consistent with Cm splicing being essential was our failure to delete the gene for the splicing protein Cef1.

RiboWest 2023



⊗ Bentley Centre: Welcome Reception, Registration, Information, Poster Sessions, Breakfasts, Lunches

⊕ Atrium, Charles McCaffery Hall (Administration Building): Banquet

⊗ 8-164 Teaching Lab Building: Sessions

⊕ Agora Courtyard: Group Photo and BBQ

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RIBOWEST 2023

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  - Monday through Friday 8am – 3:30pm

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Ribowest 2023 Speaker Schedule
All trainee talks are 12 minutes + 3 mins for questions
PI talks 25minutes + 5 mins for questions

	Abstract #	Speaker		Lab	Institution	Title
Session I-Monday June 26th 1:00-2:30pm						
1:00 - 1:15	4	Zhen, Jin	PDF	Vu	UBC	*Lnc35682/PAN3-AS1* : A De Novo Discovered Conserved Long Noncoding RNA Fueling the Progression of Acute Myeloid Leukemia
1:15 - 1:30	5	Zhong, Jenny	PhD Student	Chen	UBC	Application of an integrated Illumina RNA-seq and shotgun proteomics workflow for identifying alternative proteoforms in a leukemia cell line with a recurrent point mutation in SF3B1
1:30 - 1:45	6	Hay, Brenna	PhD student	Jan	UBC	Investigating the role of ribosome biogenesis in the translation of the interferon response
1:45 - 2:00	7	Geeraert, Mitchell	MSc Student	Patel	ULeth	Characterization of the North American Sin Nombre orthohantavirus (SNV) 3' Medium Non-coding Terminal Regions and their Interactions with Human RNA-Binding Proteins
2:00 - 2:15	8	Smith, Samantha	MSc Student	Furber	UNBC	Developing reliable RT-qPCR methods for profiling oligodendrocyte lineage gene expression and alternative splicing
2:15 - 2:30	9	***Providence Therapeutics***				
Session II-Monday June 26th 3:30-5:00pm						
3:30 - 3:45	10	Tiwari, Saurabh	PhD Student	Thundathil	U Calgary	Mass spectrometric identification of proteins involved in mRNA translation and RNA binding in bovine sperm
3:45 - 4:00	11	Tetrsteeg, Scott	MSc Student	Patel	ULeth	Understanding the cycle; Insights into the regulation of flaviviral replication
4:00 - 4:15	12	Pandher, Parleen	MSc Student	Gray	UNBC	Reference Gene Recommendatons and PACAP Receptor Expression in Murine Sympathe2c Ganglia of the Autonomic Nervous System that Innervate Adipose Tissues AEer Chronic Cold Exposure
4:15 - 4:30	13	Yuen, Katie	UG	Vu	UBC	RNA Deadenylation Subunit CNOT3 is Required for Hematopoiesis and Maintenance of Hematopoietic Stem Cells
4:30 - 4:45	14	Patel, Jinay	MSc Student	Thakor	Uleth	Role of eukaryotic initiation factor 5B (eIF5B) in oral squamous cell carcinoma
4:45 - 5:00	15	Hegde, Veda	PhD Student	Thakor	ULeth	Regulation of mRNA translation by tumor suppressor proteins PDCD4 and eIF3F
Session III-Tuesday June 27th 8:30-10:00am						
8:30 - 9:00	16	Perreault, Jonathan	PI	Perreault	INRS, Laval	Looking beyond *E. coli* bacterial non-coding RNAs: examples in *Methylorubrum, Pseudomonas* and *Burkholderia* , from small RNAs to riboswitches and more.
9:00 - 9:15	17	Liu, Yilin	PhD Student	Vu	UBC	CCR4-NOT transcription complex subunit 4 (CNOT4) is required for survival of myeloid leukemia cells and disease progression
9:15 - 9:30	18	Kim, Yerin	MSc Student	Vu	UBC	Nanopore-based native RNA sequencing of human transcriptomes reveals the complexity of mRNA modifications and crosstalk between RNA regulatory features
9:30 - 9:45	19	Letain, Jenna	MSc Student	Patel	ULeth	Unravelling the Interac□ons: Inves□ga□ng the Role of UTRs in Honey Bee Viral Replica□on
9:45 - 10:00	20	Ngo, Loc	MSc Student	Howard	UVic	Understanding the expression of ARS2 isoforms in arsenic stress response
Session IV--Tuesday June 27th 10:30-12:00pm						
10:30 - 11:00	21	Kothe, Ute	PI	Kothe	Uman	Universal tRNA modifications enhance tRNA function in translation and cellular fitness
11:00 - 11:15	22	Pachva, Manideep	PhD Student	Sorensen	UBC	Cancer cells release A-to-I edited RNA repeat elements into extracellular vesicles for reprogramming of the tumor microenvironment
11:15 - 11:30	23	Pereira, Lionel	MSc Student	Audas	SFU	Regulation of physiological amyloid aggregation by intracellular pH and bicarbonate sensing
11:30 - 11:45	24	Young, Christina	MSc Student	Jan	UBC	Novel IRES-Directed +1 Frame Translation within a Positive-sense RNA Insect Virus
11:45 - 12:00	25	Slat, Viktor	PhD Student	Rader	UNBC	A comparative exploration of the splicing landscape in three unicellular red algae
Session V--Tuesday June 27th 1:00-2:30pm						
1:00 - 1:15	26	Spencer, Sandra	Staff Scientist	Morin	BC Cancer	A Unified Protocol for Preparation of Tissues for Multi-Omic Analysis by Transcriptomics and Proteomics
1:15 - 1:30	27	Momchilova, Evgenia	PhD Student	Audas	SFU	Regulation of rIGS-RNA by a Heat-Sensitive Transcription Factor and A-body Formation
1:30 - 1:45	28	Narasimha, Pavan	PhD Student	Thakor	Uleth	Role of eukaryotic initiation factor 5B (eIF5B) in the survival and invasion of brain tumor stem cells (BTSCs)
1:45 - 2:00	29	Zhaguparov, Daniiar	PhD Student	Woodside	UAlberta	Probing the mechanical origin of RNase resistance in exoribonuclease-resistant RNA (xrRNA) from Zika virus
2:00 - 2:15	30	Luddu, Jason	UG	Thakor	Uleth	PDCD4 and eIF3F regulate each other's protein levels and affects IRES-mediated translation initiation
2:15 - 2:30	32	Classen, Curtis	MSc Student	Patel	ULeth	Ionic-dependent structural shift in Dengue dumbbell modulates viral replication

Abstract #	Presenter		Lab	Insituition	Title
Poster Session I - Monday, June 26th 2:30-3:30pm					
M	33 DaSilva, Rachel	RT	Jan	UBC	Development of a Split GFP System to Monitor Virus Infection in Cells
M	34 de Santis, Jessica Oliveira	PhD	Sorensen	UBC	Elongation control of mRNA translation drives Group 3 medulloblastoma adaptation to nutrient deprivation
M	36 Fumador, Sebastian	MSc	Rader	UNBC	RNase MRP Function In C.merolae
M	37 Geertz, Patrick	UG	Rader	UNBC	Intron accumulation: an intriguing consequence of heat stress in Cyanidioschyzon merolae
M	40 Lacroix, Emma	PhD	Audas	SFU	Uncovering the regulatory pathways associated with functional and reversible amyloid aggregation
M	42 Murray, Brent W.	PI	Murray	UNBC	Up-regulation of membrane transport genes in overwintering adult mountain pine beetles (Dendroctonus ponderosae)
M	46 Sanghera, Serena.	UG	Furber	UNBC	mRNA Expression Levels of HDACs, HATs, and BETs in Cortical Brain Tissue of Mice at Different Stages of Postnatal Development
M	47 Soleimani, Tayebe	PhD	Rader	UNBC	Exploring the impact of intron sequence on splicing and expression in C. merolae
M	49 Zumarraga, Begoña	MSc	Rader	UNBC	Is splicing essential in Cyanidioschyzon merolae?
Poster Session II - Tuesday, June 27th 2:30-3:30pm					
T	31 Chapagain, Subash	PhD	Jan	UBC	A novel RNA IRES-like element from Tombusvirus binds to 80S ribosomes
T	35 Fast, Callie	UG	Rader	UNBC	Vitamin K2 Biosynthesis, Optimization, and Extraction in C. merolae
T	38 Ghaffarzadeh, Maryam	PhD	Rader	UNBC	Unraveling the Functional Significance of Introns in Cyanidioschyzon merolae
T	39 Kazemi, Faegheh	PDF	Rader	UNBC	Unraveling the Mystery of 5' Splice Site Recognition: Insights from Pre-mRNA Splicing in Cyanidioschyzon merolae
T	41 Liu, Victor P.	RT	Furber	UNBC	Developing a Reliable qPCR and Western Blot Workflow for Detection of Myelin Genes in Murine Brain Tissue
T	14 Patel, Jinay	MSc	Thakor	ULeth	Role of eukaryotic initiation factor 5B (eIF5B) in oral squamous cell carcinoma
T	43 Penfold, Cassandra D.	MSc	Murray	UNBC	Developing RNA interference (RNAi) as a biopesticide for mountain pine beetle (Dendroctonus ponderosae)
T	44 Poholka, Andrew	MSc	MacMillan	UAlberta	SF3B4 and Regulation of pre-mRNA Splicing During Starvation in Yeast
T	45 Renner, Annalena	PhD	Bergmann	UBC/Vienn	Stress Granules in ex-vivo cultures of primary cancer cells or tissue induced by chemotherapeutic treatment
T	48 Tong, Michelle	UG	Jan	UBC	Translational efficiency of N1-methylpseudouridine-modified mRNAs in Drosophila melanogaster Schneider 2 cells