The Inaugural RNA Biology
and Biotechnology Conference
A-RNA
Thredbo, NSW 2022

A-RNA Conference

Abstract Book

2022

we acknowledge and celebrate the traditional owners of the Snowy High Country land on which we gather and we pay our respects to their Elders past and present

we extend our respect to Aboriginal and Torres Strait Islander peoples

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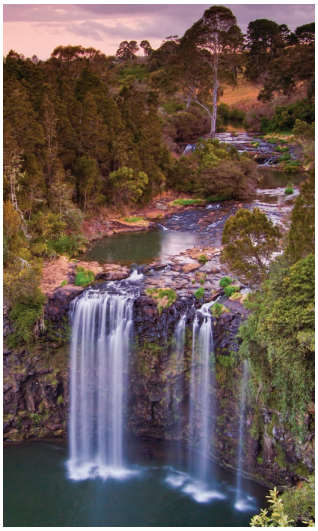


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PROGRAM

A-RNA Conference Program

Conference program with live updates:
https://a-rna.org/program/

Sunday 15/05/2022

12.00 pm – 2:45 pm Arrivals, check-in and welcome
Alpine Hotel foyer and reception (hotel check-in); Lounge Bar (Conference check-in)

Session 1: RNA therapeutics and RNA in disease Chair: Archa Fox
Session co-sponsored by Abacus DX https://www.abacusdx.com/

2.30 pm – 2.45 pm Welcome to the Country: **Aunty Sandra Patten**
Kosciuszko room; video feed to Townsend & Tate rooms
Indigenous Welcome Ceremony
Kosciuszko room; video feed to Townsend & Tate rooms
2.45 pm – 3.00 pm Conference open
Kosciuszko room; video feed to Townsend & Tate rooms
3.00 pm – 3.45 pm Opening Keynote Talk: **Dan Peer** "RNA Therapeutics Going Beyond the Liver: From Gene Silencing to Gene Editing"
Kosciuszko room; video feed to Townsend & Tate rooms
3.45 pm – 4.15 pm Tea and coffee break
Keller Bar, Townsend Room
4.15 pm – 5.00 pm Opening Plenary Lecture: **Damian Purcell**
Kosciuszko room; video feed to Townsend & Tate rooms
5.00 pm – 5.05 pm Abacus DX introduction: **Manuel Valle** "Abacus dx supports Australasian nucleic acid research"
Kosciuszko room; video feed to Townsend & Tate rooms
5.05 pm – 6.05 pm Session talks
Kosciuszko room; video feed to Townsend & Tate rooms

5.05 pm	5.20 pm	Michael P. Gantier	Taming the immunomodulatory effects of RNA therapeutics
5.20 pm	5.35 pm	Mélodie Migault	Combination of small inhibitory RNAs as an efficient strategy for targeting oncogenic pathway
5.35 pm	5.50 pm	Raman K. Sharma	Splice-Switching Oligonucleotide-mediated correction of TIMMDC1 Deep Intronic Splice-Variant in cells of Patients with Fatal Early Onset Neurodegenerative Disorder: A Step Closer to Patient Treatments
5.50 pm	6.05 pm	Nigel McMillan	RNA Therapies targeting infectious diseases

6.05 pm – 6.45 pm Free time
Explore Thredbo and surroundings

6.45 pm – 8.00 pm Social Time & welcome BBQ dinner
Lounge Bar (Overflow to Alpine Bar and BBQ area)

8.00 pm – 10.00 pm **Poster Session 1:** odd numbers
Townsend room, Kosciuszko room, Crackenback & Keller rooms (Sponsors), Lounge Bar (Drinks)

Monday 16/05/2022

6.30 am – 8.20 am Breakfast
The Local Pub

Session 2: RNA technologies Chair: John Mattick
Session co-sponsored by New England Biolabs https://www.nebiolabs.com.au/

8.20 am – 8.30 am New England Biolabs introduction
Kosciuszko room; video feed to Townsend & Tate rooms

Andrew Boslem, New England Biolabs
Tiffany Pang, New England Biolabs
"Have You Heard The Message?"

8.30 am – 9.15 am Plenary Lecture: **Howard Chang** "Genome regulation by long noncoding RNAs"
Kosciuszko room; video feed to Townsend & Tate rooms
9.15 am – 10.30 am Session talks
Kosciuszko room; video feed to Townsend & Tate rooms

9.15 am	9.30 am	Simon A. Hardwick	Single-nuclei isoform RNA sequencing unlocks barcoded exon connectivity in frozen brain tissue
9.30 am	9.45 am	Samantha Bryen	Prevalence, parameters, and pathogenic mechanisms for splice-altering acceptor variants that disrupt the AG-exclusion zone
9.45 am	10.00 am	Maina Bitar	Assessing the Landscape of Long Noncoding Transcripts in Breast Cell Populations
10.00 am	10.15 am	Attila Horvath	Tracking disomes by translation complex profiling to reveal diffusional variance and define stochastic translation efficiency
10.15 am	10.30 am	Pall Thordarson	Decoding RNA-peptide interactions

10.30 am – 11.00 am Morning Tea
Townsend room, Keller Bar, Crackenback room

Session 3: RNA in development and epigenetics Chair: Nikolay Shirokikh
Session co-sponsored by Oxford Nanopore Technologies https://nanoporetech.com/

11.00 am – 11.45 am Plenary talk: **Ling-Ling Chen** "Circular RNA in innate immunity and its potential application"
Kosciuszko room; video feed to Townsend & Tate rooms
11.45 am – 1.00 pm Session talks
Kosciuszko room; video feed to Townsend & Tate rooms

11.45 am	12.00 pm	Wei Cao	Functional recovery of the germ line following splicing collapse
12.00 pm	12.15 pm	Scott Berry	Mechanisms of cellular mRNA concentration homeostasis

12.15 pm	12.30 pm	Stacey Edwards	Breast cancer-associated lncRNA, BRIAR, regulates chromatin looping between a breast cancer-specific super-enhancer and BHLHE40
12.30 pm	12.45 pm	Dawei Liu	Regulation, biogenesis and function of circDOCK1(exon2-27), an abundant epithelial-specific circRNA
12.45 pm	1.00 pm	Michael Uckelmann	RNA-mediated regulation of repressive chromatin hubs

1.00 pm – 2.30 pm Oxford Nanopore Lunch: **Eduardo Eyras** "Exploring hidden aspects of the transcriptome with Nanopore sequencing"
Cascades Restaurant (banquet with overflow in The Local Pub)

2.30 pm – 3.30 pm Oxford Nanopore Technologies Workshop
Cascades Restaurant (banquet with overflow in The Local Pub)

2.30 pm	2.35 pm	Ross Napoli	Welcome address
2.35 pm	2.50 pm	Warren Bach	Through the Nanopore: Latest Developments and RNA Overview
2.50 pm	3.05 pm	Rachel Thijssen	Single-cell long-read RNA-seq reveal complex heterogeneity in leukaemia
3.05 pm	3.20 pm	Paul Marshall	Experience-dependent accumulation of G-quadruplex DNA serves as a transcriptional control device to regulate the consolidation and stability of fear-related memories
3.20 pm	3.30 pm	Warren Bach	Closing remarks

3.30 pm – 3.50 pm Tea and coffee break
Keller Bar, Townsend room, Crackenback room

<u>Session 4: RNA in neuroscience</u> Chair: Minni Änkö
Session co-sponsored by Gene Target Solutions https://www.genetargetsolutions.com.au/

3.50 pm – 4.00 pm Gene Target Solutions introduction: **Andrew Szentirmay** "Simple. Fast. Elegant. Directional RNA-Seq Library Preparation with Integrated Ribo-globin Depletion"
Kosciuszko room; video feed to Townsend & Tate rooms

4.00 pm – 4.45 pm Plenary Talk: **Timothy Bredy** "RNA in Neuroscience"
Kosciuszko room; video feed to Townsend & Tate rooms
4.45 pm – 6.00 pm Session talks
Kosciuszko room; video feed to Townsend & Tate rooms

4.45 pm	5.00 pm	Cecile King	The retrotransposon Lx9 puts a brake on the immune response to virus infection
5.00 pm	5.15 pm	Chengcheng Zhong	G:U base-paired hpRNA is a potent small RNA sponge in plants
5.15 pm	5.30 pm	Laura Leighton	C/D box small nucleolar RNAs methylate neuronal mRNAs and modulate memory-related processes
5.30 pm	5.45 pm	Gavin Sutton	Functional screening of regulatory enhancers in human primary astrocytes

5.45 pm	6.00 pm	Mike Clark	Characterising neuropsychiatric risk genes in human brain with long-read sequencing

6.00 pm – 8.00 pm Dinner
The Local Pub (Buffet style)

8.00 pm – 10.00 pm **Poster Session 2:** even numbers
Townsend room, Kosciuszko room, Crackenback & Keller rooms (Sponsors), Lounge Bar (Drinks)

Tuesday 17/05/2021

6.30 am – 8.30 am Breakfast
The Local Pub

Session 5: RNA processing and modifications Chair: Erin Heyer

8.30 am – 9.15 am Keynote Lecture: **Melissa Moore** "mRNA as Medicine"
Kosciuszko room; video feed to Townsend & Tate rooms
9.15 am – 10.00 am Plenary Talk: **Amy Gladfelter** "RNA sequence code in SARS CoV-2 condensates"
Kosciuszko room; video feed to Townsend & Tate rooms
10.00 am – 10.30 am Morning Tea
Keller Bar, Townsend room, Crackenback room
10.30 am – 11.30 am Session talks
Kosciuszko room; video feed to Townsend & Tate rooms

10.30 am	10.45 am	Justin Wong	The virilizer-like methyltransferase VIRMA overexpression determines the aggressiveness and outcome of breast cancer in an RNA m6A-dependent manner
10.45 am	11.00 am	Marco Guarnacci	The lack of NSUN2 methyltransferase enzyme affects the role of UPF1 RBP
11.00 am	11.15 am	Ulf Schmitz	Multi-omics data analysis identifies epigenetic regulators of alternative splicing
11.15 am	11.30 am	Madara Ratnadiwakara	Selective processing of clustered miRNAs in cancer: structure is the key

Session 6: RNA structure, functions and interactions Chair: Robert Weatheritt

11.30 am – 12.45 pm Session talks
Kosciuszko room; video feed to Townsend & Tate rooms

11.30 am	11.45 am	Jai Tree	Treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections is dependent on the efficacy of last-line antibiotics including vancomycin
11.45 am	12.00 pm	Fionna E. Loughlin	Keeping RNA unstuck: modulating RNA structure with RNA binding proteins
12.00 pm	12.15 pm	Seth Cheetham	Coding and noncoding mechanisms of human pseudogene function
12.15 pm	12.30 pm	Sandro Ataide	Structural characterization of the ANTAR antiterminator domain bound to RNA
12.30 pm	12.45 pm	Tamas Fischer	Structural and mechanistic insights into the role of the canonical poly(A) polymerase Pla1 in RNA surveillance by the fission yeast MTREC complex.

12.45 pm – 1.30 pm Oxford Nanopore Plenary Talk: **Yue Wan** "Detecting RNA structures using nanopore sequencing"
Kosciuszko room; video feed to Townsend & Tate rooms
1.30 pm – 2.45 pm Lunch Talk: **Sue Fletcher** "Antisense therapeutics for rare diseases; delivering the potential"
Cascades Restaurant (banquet with overflow in the Local Pub)
2.45 pm – 4.00 pm Social Time: Sponsored Thredbo Walks
Thredbo and surroundings

Session 7: RNA in cell biology Chair: John Mattick

4.00 pm – 4.45 pm Plenary Talk: **Narry Kim** "Molecular basis of pre-microRNA processing by DICER"
Kosciuszko room; video feed to Townsend & Tate rooms
4.45 pm – 6.15 pm Session talks
Kosciuszko room; video feed to Townsend & Tate rooms

4.45 pm	5.00 pm	Lithin Louis	Metabolic enzymes moonlight as RNA-binding proteins
5.00 pm	5.15 pm	Jacki Heraud-Farlow	Defining novel components of endogenous dsRNA sensing pathways
5.15 pm	5.30 pm	Sang Huynh	The molecular action mode of restorer-of-fertility-like proteins in plant mitochondria
5.30 pm	5.45 pm	Toby Williams	A cell-cycle mediated checkpoint that regulates mRNA export in response to replication stress
5.45 pm	6.00 pm	Javier Fernandez-Chamorro	P-bodies are key regulators of the cell cycle and their depletion induces a cancer cell stem-like transition
6.00 pm	6.15 pm	Ulrike Schumann	Pinpointing spatial transcriptional changes driving retinal degeneration

Drinks and Awards

6.15 pm – 6.30 pm Conference close
Kosciuszko room
6.30 pm – 7.30 pm Free time

7.30 pm – 9.30 pm Dinner and Poster and Talk Award announcements
Kosciuszko room
9.30 pm – 11.45 pm Social time
Kosciuszko room, Keller Bar, Crackenback Room

Wednesday 18/05/2022

6.30 am – 8.30 am Breakfast
Cascades restaurant
8.30 am – 10.00 am Farewell and departure
Alpine Hotel foyer and reception (hotel check-out); Lounge Bar (conference check-out)

TALKS

Session 1

1 RNA Therapeutics Going Beyond the Liver: From Gene Silencing to Gene Editing

Dan Peer[1]
[1] Laboratory of Precision NanoMedicine,Tel Aviv University

Accumulating work points out relevant genes and signaling pathways hampered in human disorders as potential candidates for therapeutics**.** Developing nucleic acid-based tools to manipulate gene expression, such as siRNAs, mRNA and genome editing strategies, open up opportunities for personalized medicine. Yet, although major progress was achieved in developing RNA targeted delivery carriers, mainly by utilizing monoclonal antibodies (mAbs) for targeting, their clinical translation has not occurred. In part because of massive development and production requirements and high batch-to-batch variability of current technologies, which relies on chemical conjugation. Here we present a self-assembled modular platform that enables to construct theoretically unlimited repertoire of RNA targeted carriers. The platform self-assembly is based on a membrane-anchored lipoprotein, incorporated into RNA-loaded lipid nanoparticles that interact with the antibody Fc domain. We show that a simple switch of 8 different mAbs, redirects specific uptake of siRNAs by diverse leukocyte subsets *in vivo.* The platform therapeutic potential is demonstrated in an inflammatory bowel disease model, by targeting colon macrophages to reduce inflammatory symptoms, and in Mantle Cell Lymphoma xenograft model, by targeting cancer cells to induce cell death and improve survival. In addition, I will discuss novel approach for delivering modified mRNA to specific cell types *in vivo* utilizing this platform. I will also share some data on mRNA vaccines for COVID19 and Finally, I will share new data showing very high efficiency genome editing in glioma and metastatic ovarian cancer. This modular delivery platform can serve as a milestone in turning precision medicine feasible.

2 TBA

Damian Purcell[1]
[1] University of Melbourne

3 Taming the immunomodulatory effects of RNA therapeutics

Michael P. Gantier[1,2], Arwaf Alharbi[1,2], Sunil Sapkota[2,3], Mary Speir[4,5], Olivier Laczka[4,5]

[1] Hudson Institute of Medical Research; [2] Monash University; [3] Hudson Institute of Medical Research; [4] Pharmorage Pty Ltd; [5] Noxopharm Ltd

While RNA therapeutics, from mRNA vaccines to siRNAs, have the ability to revolutionize clinical treatment across a broad spectrum of conditions and diseases, to-date, their efficacy and tolerability have been constrained by engagement of innate immune sensors that drives potent immune activation. While existing strategies to limit such immune responses have largely been focused on backbone and/or nucleotide modification of RNA therapeutics, little is still understood about how these approaches function to impair innate immune sensing.

We have recently described a broad, sequence-dependent immune-suppressive effect of 2'OMe-modified antisense oligonucleotides on key innate immune sensors (Alharbi et al., Nucleic Acids Res., 2020; Valentin et al., Nucleic Acids Res., 2021). Given the widespread use of 2'OMe modifications in RNA therapeutics, our findings indicate a risk for unknown immunosuppression, which may sensitize patients to intercurrent viral and bacterial infections.

Our on-going research has now defined the precise molecular determinants of these immune-suppressive activities, which are not restricted to 2'OMe-modified bases. This work has critical implications for the widespread roll-out of RNA therapeutics, including the improvement of current mRNA vaccines, while also presenting opportunities to create new classes of oligonucleotide-based anti-inflammatory agents.

4 Combination of small inhibitory RNAs as an efficient strategy for targeting oncogenic pathway

Mélodie Migault[1,2], Julie Bracken[3,4], Katherine A. Pillman[5,6], Gregory J. Goodall[7,8], Cameron P. Bracken[9,10]

[1] Centre for Cancer Biology, an Alliance of SA Pathology and University of South Australia, North Terrace, Adelaide, SA, Australia; [2] ; [3] Centre for Cancer Biology, an Alliance of SA Pathology and University of South Australia, North Terrace, Adelaide, SA, Australia; [4] ; [5] Centre for Cancer Biology, an Alliance of SA Pathology and University of South Australia, North Terrace, Adelaide, SA, Australia; [6] ACRF Cancer Genomics Facility, Centre for Cancer Biology, SA Pathology, Adelaide, Australia; [7] Centre for Cancer Biology, an Alliance of SA Pathology and University of South Australia, North Terrace, Adelaide, SA, Australia; [8] School of Medicine, Discipline of Medicine, University of Adelaide, SA, Australia; [9] Centre for Cancer Biology, an Alliance of SA Pathology and University of South Australia, North Terrace, Adelaide, SA, Australia; [10] School of Medicine, Discipline of Medicine, University of Adelaide, SA, Australia

Despite continuing advances in the identification of new targets for cancer therapy, many approaches show modest clinical benefits, induce a response only in a portion of the patients, and/or become inefficient after patients develop drug resistance. New approaches are therefore needed to overcome these effects. There is tremendous optimism regarding the potential of RNA-based therapies where oncogenes could be suppressed by small non-coding RNAs (specifically, siRNAs and miRNAs). Many challenges, including delivery and toxicity, are being solved however the problem of off-target effects persist. One option to avoid this issue is to combine these small RNAs at low concentrations, ensuring that genuine targets are inhibited and that unintended effects on other genes are minimal and widely distributed.

Here we select small RNAs to target simultaneously the main components of the RAS-MAPK pathway, a cell signalling cascade that governs cell survival and is often dysregulated in cancer through genetic alteration. To evaluate the small RNAs efficiency to inhibit their target expression, RT-qPCR and western-blot were used for measuring RNA and protein levels of the RAS-MAPK effectors, respectively. The RAS-MAPK pathway activity was measured through the quantification of phospho-ERK level using an automated western-blot-like system and live-cell imaging was used to assess cell survival.

We show that combination of these small RNAs at sub-nanomolar level efficiently reduces the activity of the RAS-MAPK pathway and significantly decrease cell survival in cancer cells resistant to front-line therapy. Future work aims to confirm the reduction of off-targets effects and verify the efficiency of this strategy on tumour growth in vivo.

This work will lead to the validation of multiplexing small RNAs as a potential therapeutic strategy with high specificity and less toxicity to treat drug-resistant cancer cells.

5 Splice-Switching Oligonucleotide-mediated correction of TIMMDC1 Deep Intronic Splice-Variant in cells of Patients with Fatal Early Onset Neurodegenerative Disorder: A Step Closer to Patient Treatments

Raman K Sharma[1,2]
[1] University of Adelaide; [2] The Robinson Research Institute

Combined analysis of genome and RNA sequencing data from patient-derived cells or disease-relevant tissues have been particularly effective in identifying disease variants with splicing or gene-regulatory effects. Splicing variants represent up to 13% of the known pathogenic variants and offer great opportunity for the development of treatments. We used genome and RNA sequencing (fibroblasts) analyses of two children and their consanguineous parents and identified a deep-intronic, homozygous TIMMDC1 c.596+2146A>G, cryptic splice-site activating variant (allele frequency 1/5,000) in patients with severe early-onset, progressive, neurodegenerative disorder. TIMMDC1 encodes the Translocase of Inner Mitochondrial Membrane Domain-Containing protein 1 subunit of complex I of the electron transport chain responsible for ATP production. We showed that TIMMDC1 c.596+2146A>G enhances aberrant splicing, leading to an insertion of a poison exon, undetectable TIMMDC1 protein, altered complex I proteome and compromised mitochondrial function in the patient fibroblasts. Using two different Splice-Switching Oligonucleotides (SSOs) targeting the TIMMDC1 c.596+2146A>G variant, we completely restored normal TIMMDC1 mRNA, protein, complex I proteome and mitochondrial function in patients' cells, thus providing proof of principle correction of the molecular defect underlying this severe and fatal neurological disorder. To better understand various aspects of the disorder and have a suitable pre-clinical model, we have generated a humanized Timmdc1 mouse that carries a 398bp of the human TIMMDC1 c.596+2146A>G variant-containing intron. RNA analyses of F1 and F2 Timmdc1 heterozygous mice showed that the human variant-containing intronic sequence in mice impacts aberrant mRNA splicing and insertion of poison exon into the mouse Timmdc1 mRNA identical to the TIMMDC1 mRNA in human patient fibroblasts. Importantly, we have identified at least one surviving international patient for whom the clinical teams are considering our SSOs as a possible therapeutic option. This aspect of our studies will be critical for treating other patients in the future.

6 RNA Therapies targeting infectious diseases

Nigel McMillan[1], Adi Idris[1], Alicia Davis[2], Aroon Supramaniam[1], Dhruba Acharya[1], Gabrielle Kelly[1], Yaman Tayyar[1], Nic West[1], Ping Zhang[1], Christopher L.D. McMillan[3],
Citradewi Soemardy[2], Roslyn Ray[2], Denis O'Meally[2], Tristan A. Scott[2], Kevin V. Morris[1]
[1] Griffith University; [2] City of Hope; [3] University of Queensland

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in humans. Despite several emerging vaccines, there remains no verifiable therapeutic targeted specifically to the virus. Here we present a highly effective small interfering RNA (siRNA) therapeutic against SARS-CoV-2 infection using a novel lipid nanoparticle (LNP) delivery system. Multiple siRNAs targeting highly conserved regions of the SARS-CoV-2 virus were screened, and three candidate siRNAs emerged that effectively inhibit the virus by greater than 90% either alone or in combination with one another. We simultaneously developed and screened two novel LNP formulations for the delivery of these candidate siRNA therapeutics to the lungs, an organ that incurs immense damage during SARS-CoV-2 infection. Encapsulation of siRNAs in these LNPs followed by in vivo injection demonstrated robust repression of virus in the lungs and a pronounced survival advantage to the treated mice. Our LNP-siRNA approaches are scalable and can be administered upon the first sign of SARS-CoV-2 infection in humans. We suggest that an siRNA-LNP therapeutic approach could prove highly useful in treating COVID-19 disease as an adjunctive therapy to current vaccine strategies. We have used simiair approaches to RSV, Hendravirus, HMV, and HPV.

Session 2

7 Have You Heard The Message?

Andrew Boslem[1] & Tiffany Pang[1]
[1] New England Biolabs

For over 45 years, NEB® has been a world leader in the discovery and production of reagents for the life science industry. Our enzymology expertise effectively positions us to supply reagents for RNA research and synthesis of high-quality mRNA – from detection & sequencing, template generation & transcription, capping & tailing, clean-up & purification. We will describe how NEB's portfolio of reagents are based on decades of molecular biology experience, so that you can be confident they will work for your application. When it is time to scale up and optimize, our standalone reagents are readily available in research-grade and GMP-grade* formats, enabling a seamless transition to large-scale therapeutic mRNA manufacturing.

8 Genome Regulation by Long Noncoding RNAs

Howard Y Chang[1]
[1] Howard Hughes Medical Institute, Stanford University School of Medicine

The discovery of extensive transcription of long noncoding RNAs (lncRNAs) provide an important new perspective on the centrality of RNA in gene regulation. I will discuss genome-scale strategies to discover and characterize lncRNAs, notably the impact of lncRNAs on gene memory over time. LncRNAs form extensive networks of ribonucleoprotein (RNP) complexes with numerous chromatin regulators, and target these enzymatic activities to appropriate locations in the genome. Consistent with this notion, long noncoding RNAs can function as modular scaffolds to specify higher order organization in RNP complexes and in chromatin states. An emerging theme is the intersection between lncRNA biology and immunity. Self vs. foreign identity of lncRNA impacts innate and adaptive immunity. The importance of these modes of regulation is underscored by the newly recognized roles of long RNAs in human diseases.

9 Single-nuclei isoform RNA sequencing unlocks barcoded exon connectivity in frozen brain tissue

Simon A Hardwick[1,2], Wen Hu[1,2], Anoushka Joglekar[1,2], Li Fan[1,3], Paul G Collier[1,2], Careen Foord[1], Jennifer Balacco[4], Samantha Lanjewar[5], Maureen McGuirk Sampson[5], Frank Koopmans[6], Andrey Prjibelski[7], Alla Mikheenko[7], Natan Belchikov[1,8], Julien Jarroux[1,2], Anne Bergstrom Lucas[9], Miklós Palkovits[10], Wenjie Luo[1,3], Teresa A Milner[1], Lishomwa C Ndhlovu[1,11], August B Smit[6], John Q Trojanowski[12], Virginia MY Lee[12], Olivier Fedrigo[4], Steven A Sloan[5], Dóra Tombácz[13], M Elizabeth Ross[1,2], Erich Jarvis[4], Zsolt Boldogkői[13], Li Gan[1,3], Hagen U Tilgner[1,2]

[1] Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York, NY, USA; [2] Center for Neurogenetics, Weill Cornell Medicine, New York, NY, USA.; [3] ; [4] The Rockefeller University, New York, NY, USA; [5] Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA; [6] Department of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, VU University, Amsterdam, The Netherlands; [7] Center for Algorithmic Biotechnology, Institute of Translational Biomedicine, St. Petersburg State University, St. Petersburg, Russia; [8] Physiology, Biophysics & Systems Biology Program, Weill Cornell Medicine, New York, NY, USA; [9] Agilent Technologies, Santa Clara, CA, USA; [10] Human Brain Tissue Bank, Semmelweis University, Budapest, Hungary; [11] Department of Medicine, Division of Infectious Diseases, Weill Cornell Medicine, New York, NY, USA; [12] Center for Neurodegenerative Disease Research, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; [13] Department of Medical Biology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary

Single-nuclei RNA sequencing characterizes cell types at the gene level. However, compared to single-cell approaches, many single-nuclei cDNAs are purely intronic, lack barcodes and hinder the study of isoforms. Here we present single-nuclei isoform RNA sequencing (SnISOr-Seq). Using microfluidics, PCR-based artifact removal, target enrichment and long-read sequencing, SnISOr-Seq increased barcoded, exon-spanning long reads 7.5-fold compared to naive long-read single-nuclei sequencing. We applied SnISOr-Seq to adult human frontal cortex and found that exons associated with autism exhibit coordinated and highly cell-type-specific inclusion. We found two distinct combination patterns: those distinguishing neural cell types, enriched in TSS-exon, exon-polyadenylation-site and non-adjacent exon pairs, and those with multiple configurations within one cell type, enriched in adjacent exon pairs. Finally, we observed that human-specific exons are almost as tightly coordinated as conserved exons, implying that coordination can be rapidly established during evolution. SnISOr-Seq enables cell-type-specific long-read isoform analysis in human brain and in any frozen or hard-to-dissociate sample.

10 Prevalence, parameters, and pathogenic mechanisms for splice-altering acceptor variants that disrupt the AG-exclusion zone

Samantha Bryen[1], Michaela Yuen[1,2], Himanshu Joshi[1,3], Ruebena Dawes[1,2], Katharine Zhang[1,3], Jessica K. Lu[1,2], Kristi J. Jones[2,4], Christina Liang[5,6], Wui-Kwan Wong[1,2], Anthony J. Peduto[7], Leigh B. Waddell[1,2], Frances J. Evesson[1-3], Sandra T. Cooper[1-3]

[1] Kids Neuroscience Centre, Kids Research, The Children's Hospital at Westmead, Westmead, NSW, 2145, Australia; [2] Discipline of Child and Adolescent Health, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW, 2145, Australia; [3] Functional Neuromics, Children's Medical Research Institute, The University of Sydney, Westmead, NSW, 2145, Australia; [4] Department of Clinical Genetics, Children's Hospital at Westmead, Westmead, NSW, 2145, Australia; [5] Department of Neurology, Royal North Shore Hospital, St Leonards, NSW, 2065, Australia; [6] Department of Neurogenetics, Northern Clinical School, Kolling Institute, University of Sydney, NSW, 2065, Australia; [7] Department of Radiology, Westmead Hospital, Western Clinical School, University of Sydney, Westmead, NSW, 2145, Australia

Predicting the pathogenicity of variants outside the essential 'AG' of the acceptor splice site is challenging due to high sequence diversity of the extended splice site region. Critical analysis of 24,445 intronic extended acceptor splice site variants reported in ClinVar and LOVD demonstrates 41.9% of pathogenic variants create an AG dinucleotide between the predicted branchpoint and acceptor splice site, 28.4% result in loss of a pyrimidine at the -3 position and 15.1% result in the loss of one or more pyrimidines in the polypyrimidine tract. Pathogenicity of AGs created in the AG-exclusion zone (AGEZ) was highly influenced by their position. 93.1% of pathogenic AGs were created > 6 nucleotides (nt) downstream from the predicted branchpoint or > 5 nt upstream from the acceptor splice site – hereafter defined as the 'high-risk zone'. In contrast, only 44.9% of benign AGs arise in the high-risk zone - concordant with evolutionary intolerance for naturally occurring AG dinucleotides in the polypyrimidine tract of human introns (only 5.8% of natural AGs lie within this region). We highlight clinical examples showing contrasting mechanisms for mis-splicing arising from AG variants in the AGEZ; 1) Cryptic acceptor created; 2) Splicing Silencer created: an introduced AG dinucleotide silences the acceptor splice site, resulting in exon skipping, intron retention and/or use of an alternative existing cryptic splice site, and 3) Splicing Silencer disrupted: loss of a deep intronic AG activates inclusion of a pseudo-exon. In conclusion, we highlight prevalence of pathogenic AG-creating variants in the AGEZ, elucidate mechanisms for pathogenic disruption of pre-mRNA splicing and outline factors conferring critical risk for splice-altering outcomes from creation or loss of an AG in the AGEZ.

11 Assessing the Landscape of Long Noncoding Transcripts in Breast Cell Populations

Maina Bitar[1], Wei Shi[1], Stacey Edwards[1], Juliet French[1]
[1] QIMR Berghofer

Single-cell transcriptomics (scRNAseq) has emerged as a powerful tool to assess the transcriptome of individual cells, revealing new and rare cell types and improving the reconstruction of lineage hierarchies. However, a major limitation of current studies is that they only quantify sequencing reads that map to annotated isoforms, which represent roughly one third of all human transcripts. Since the vast majority of unannotated genes are long noncoding RNAs (lncRNAs), these remain largely unexplored. The human breast is a complex organ that harbours different cell populations. Interestingly, different mammary cell populations give rise to different breast tumour subtypes and the cell-of-origin determines the tumour molecular characteristics and clinical outcomes. Using deep bulk RNAseq of normal breast epithelial cells we discovered >13,000 lncRNAs (being 95% unannotated) and mapped their expression levels in scRNAseq, showing they perform better than protein-coding genes at clustering the different cell types. On average, each cell expressed 900 lncRNAs and 4,000 protein-coding genes. LncRNAs had significantly higher cluster specificity levels and were expressed in less cells than their protein-coding counterpart (on average ~30 cells/lncRNA compared with ~150 cells/mRNA), which is in line with the view of lncRNAs being highly cell type-specific and their apparent lower expression levels being a result of bulk RNAseq estimates. Indeed, when investigating the expression of lncRNAs at cellular level, we confirmed it to be comparable to that of protein-coding genes. We conducted a thorough assessment of these lncRNAs, their expression levels in individual cells and across populations and their correlation with breast cancer subtypes. On average, each cell population has nearly 300 lncRNA markers, from which at least 30 (10%) can be used to classify breast cancer tumours in their different subtypes. Notably, using their predicted protein-coding targets or annotation, we were able to link several specific lncRNAs to tumour subtypes and cancer

12 Tracking disomes by translation complex profiling to reveal diffusional variance and define stochastic translation efficiency

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Full-transcriptome methods have brought versatile power to protein biosynthesis research, but remain difficult to apply for the quantification of absolute translation rates. Here we propose and, using enhanced translation complex profile sequencing, confirm co-localisation of ribosomes on messenger(m)RNA resulting from the translational dynamics. We demonstrate that the co-localised ribosomes (such as disomes) can be of a different origin. Some co-localised ribosomes are related to the well-accepted translation elongation delays on mRNA. Others are reflective of the mRNA spatial arrangement in polysomes and descend from individual molecular co-localisation events.

The stochastically co-localised ribosomes are linked to the translation initiation efficiency and provide a robust variable to model specific protein output from mRNA. Employing unbiased machine learning and the stochastic signal, together with other variables derived from translation complex profile sequencing (TCP-seq; Archer et al. 2016 Nature), we demonstrate accurate prediction of the absolute translation output in the form of Stochastic Translation Efficiency (STE) measure (Horvath et al. 2022 bioRxiv). Using STE, it is possible to rank mRNAs by the absolute protein output and thus, characterise the ‘power’ of translation control elements across transcripts in a single setting or between different conditions. STE does not use bias-inducing normalisation to the RNA abundance or signals of different types and relies on self-normalised signal pairs.

Applying STE to the prototypical example of translational control during yeast response to glucose depletion, we find that glucose stress results in a response that is more complex than previously thought, exhibiting a high degree of selective translational control that acts towards both, suppression and activation of different mRNAs. Unexpectedly, we uncover elevated initiation rate for the many of ‘mid-power’ mRNAs under the stress. We suggest STE use for dissecting finer dynamics of translation and elucidation of very rapid cell responses inaccessible to the other approaches.

13 Decoding RNA-peptide interactions

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We know now that (long) non-coding RNA plays a major role as a structural and organisational element in cell biology and development. The dynamic interactions between non-coding RNA and various intrinsically disordered proteins (IDP – effectively very long unfolded peptide chains) leads to the formation of condensates that are often referred to as liquid-liquid phase separated (LLPS) droplets. As a chemistry-research group, we entered this field, noting the similarities between these RNA-protein droplets/LLPS and peptide-gels – materials that we have been studying for a considerable time.

Building on our peptide work, we therefore hypothesised that even very short RNA and peptide could under the right conditions form gel-like aggregates. More importantly, these studies should give us a detailed molecular level understanding of how RNA and peptides/proteins interact to form liquid-liquid phase separated structures and other condensates. This includes investigating: condensates formed between short peptide such as triglycine-X-triglycines and pentanucleotide RNA oligo's. e.g., AAXAA (X = variable amino acid or nucleotide). These initial studies are already providing valuable new insight into the factors that at the (sub)-molecular level control RNA-peptide interactions, and in doing so take us a step closer to decoding the language that controls RNA-peptide/protein interactions.

Session 3

14 Circular RNA in innate immunity and its potential application

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Circular RNAs are composed of different types of covalently closed, single-stranded RNA molecules. Recent transcriptomic studies have uncovered the widespread expression of circular RNAs (circRNAs) produced from back-splicing of exon(s) of pre-mRNAs. I will discuss the characterization of this type of RNA circles, including their production, structure and turnover. All these have allowed the appreciation of their unique cellular functions and potential biomedical applications related to innate immunity

15 Functional recovery of the germ line following splicing collapse

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Pre-mRNA splicing is a conserved process that is essential for generating mature mRNAs and subsequent translation of proteins. As such, disruption of splicing causes cell cycle arrest in yeast, Drosophila and cultured human cells. Due to essential function of pre-mRNA splicing, conditional depletion of splicing factors is required to study their function in a multicellular organism. Here, we use Caenorhabditis elegans as a model to study pre-mRNA splicing in the germ line through spatio-temporal protein depletion. In an on-going RNAi screen, we found that knockdown of the previously uncharacterized mog-7 gene causes sterility. Using CRISPR-Cas9, we fused a plant degron sequence to endogenous MOG-7 to enable germline-specific depletion of MOG-7 induced by the plant hormone auxin. We found that loss of MOG-7 impedes the cell cycle and germ cell sex determination - causing sterility. Using in vivo mass spectrometry, we found that MOG-7 is a spliceosome factor. Further, RNA sequencing revealed that acute depletion of MOG-7 causes intron retention in the majority of germline-expressed genes. Interestingly, we found that the germline can recover from the catastrophic effect caused by MOG-7 depletion once MOG-7 expression is restored in adults. By removing auxin, germ lines restore MOG-7 expression, and recover their function to produce viable and fertile progeny. Functional recovery of the germ line depends on apoptosis, which removes defective germ cells, and proliferation to replenish the germ line. In conclusion, our study found that MOG-7 is essential for germline development, and the germ line has remarkable plasticity to enable recovery from a collapse in RNA splicing.

16 Mechanisms of cellular mRNA concentration homeostasis

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Cells maintain constant concentrations of cellular mRNAs, which requires that mRNA synthesis or mRNA degradation rates are regulated according to cell volume. Moreover, there is evidence that rates of mRNA synthesis and degradation are coupled, so that perturbation of one of these rates results in adaptation of the other. Our recent work using genetic screening, single-molecule FISH and RNA metabolic labelling in human cells suggests that these two mechanisms converge on the regulation of the abundance and activity of RNA polymerase II and, moreover, that this process involves fast-acting feedback from nuclear mRNA concentration on transcription itself. Based on these data, we present a model for the coordination of mRNA production with cell volume that underlies mRNA concentration homeostasis.

17 Breast cancer-associated lncRNA, BRIAR, regulates chromatin looping between a breast cancer-specific super-enhancer and BHLHE40

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Breast cancer genome-wide association studies (GWAS) have identified one signal at 3p26 containing four genetic variants associated with increased risk. The risk variants fall in non-coding regions but no protein-coding eQTLs were identified. Importantly, our recent targeted RNA-sequencing identified a novel lncRNA (we named BRIAR) at 3p26. BRIAR is an eQTL that colocalised with the GWAS signal, suggesting it is a target gene, with reduced expression associated with risk. BRIAR is a 1.1 kb, three exon, polyadenylated transcript which localises to the nucleus and cytoplasm. BRIAR expression is restricted to estrogen receptor positive (ER+) breast cancer cell lines and tumours and it is induced in breast cells treated with estrogen. The BRIAR promoter sits at the 5` end of an 11 kb super-enhancer exhibiting high H3K27ac signal, with binding sites for breast-specific transcription factors. Accessible chromatin (ATACseq) peaks called in 410 TCGA samples from 23 cancer types, showed the super-enhancer is also largely restricted to ER+ breast tumours. Some lncRNAs function close to their sites of synthesis to regulate the expression of nearby protein-coding genes. We used promoter capture HiC to show that BRIAR physically loops to BHLHE40, a transcriptional regulator located ~125 kb away. CRISPR-interference of the BRIAR promoter ablated the 11 kb super-enhancer, reduced chromatin looping between BRIAR and BHLHE40, resulting in decreased BHLHE40. RNAseq on BRIAR repressed cells identified two main pathways; activated proliferation and suppressed interferon response, mainly through repression of STAT1. Consistent with the eQTL data, BRIAR repression significantly increased 2D and 3D ER+ breast cancer cell growth. Moreover, previous studies showed that STAT1 deficient mice spontaneously develop ER+ breast cancer, suggesting that early modulation of the interferon response is a major contributor to breast cancer risk.

18 Regulation, biogenesis and function of circDOCK1(exon2-27), an abundant epithelial-specific circRNA

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We describe here the regulation, biogenesis and potential function of a large circular RNA that is highly epithelial-specific in expression. Formed from exons 2 to 27 of the DOCK1 gene, this unusually large circRNA is strongly downregulated during epithelial to mesenchymal transition (EMT), despite the DOCK1 mRNA being increased in level during EMT. Formation of the circRNA is dependent on the splicing regulator ESRP1, which promotes circRNA formation by inhibiting splicing of exon 1 to exon 2, thereby holding the intron 1 acceptor site unspliced and available while Pol II completes its 157 kb journey from exon 2 to exon 27. We found by HITS-CLIP analysis that ESRP1 binds to a GU-rich motif in a tandem repeat region in intron 1, and we solved the crystal structure of ESRP1 RRM2 domain bound to the repeat motif, revealing that the DOCK1 RRM2 domain binds a GGU motif, with the guanines embeded in clamp-like aromatic pockets in the protein. Binding of ESRP1 to DOCK1 intron 1 is necessary and sufficient for high efficiency formation of the circDOCK1(2-27), to the extent that splicing of the DOCK1 pre-mRNA is diverted to circRNA formation, limiting the production of DOCK1 mRNA and protein. Not only does its biosynthesis reduce levels of DOCK1 protein, but the circRNA itself also reduces the migratory capacity of cells if introduced ectopically into mesenchymal cells.

19 RNA-mediated regulation of repressive chromatin hubs

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Chromatin modifiers are required for the regulation of transcription and commonly bind to RNA, although the functional consequences of the RNA interactions are often elusive. While investigating the molecular mechanism dictating the regulation of a chromatin modifier we discovered an accessory subunit that bridges its function and RNA interaction. We describe a critical role for the RNA interaction in the organization of chromatin structure. We show that the identified protein-RNA interactions are required for RNA-mediated regulation of chromatin compaction. In vitro, the identified RNA-binding protein is required to organize compacted chromatin hubs, composed of both compacted chromatin and a repressive chromatin modifier complex. These chromatin hubs are characterised by a distinct boundary and tightly packed nucleosome organisation. Structural and biophysical analysis suggests that the structural state of chromatin, in the compacted chromatin hubs, is more akin to an amorphous solid than to a previously proposed liquid state. The non-histone protein-component of the chromatin hub remains highly mobile. RNA dissolves the compacted-chromatin hubs and counteracts compaction, leading to a drastically more relaxed chromatin structure. In cells, the identified RNA-binding determinants are required for gene repression.

In summary, we discovered repressive chromatin hubs that are composed of a solid-like chromatin phase and a liquid-like RNA-binding protein component. Importantly, we show that protein-RNA interactions control the formation and size of these repressive hubs. Our data support a model where an accessible chromatin architecture is maintained around active genes by the transcribed RNA, which sequester repressive chromatin modifiers and directly interfere with their chromatin-compaction activity.

20 Exploring hidden aspects of the transcriptome with Nanopore sequencing

Eduardo Eyras[1]
[1] Australian National University

The expanding field of epitranscriptomics might rival the epigenome in the diversity of the biological processes impacted. However, the identification of modifications in individual RNA molecules remains challenging. We present CHEUI (CH3 (methylation) Estimation Using Ionic current), a new computational approach that uses a two-stage deep learning algorithm to enable for the first time the detection of N6-methyladenosine (m6A) and 5-methylcytidine (m5C) at single-nucleotide and single-molecule resolution from Nanopore signals from any sample. Using extensive benchmarking with Nanopore data derived from synthetic and natural RNA, CHEUI achieves higher accuracy than other existing methods in detecting m6A and m5C sites and quantifying the site stoichiometry levels, while maintaining a lower proportion of false positives. As further validation, applying CHEUI to cell models with and without the deletion of m6A or m5C modifying enzymes, we recover the sequence and structural properties of methylated sites expected from previous studies. We applied CHEUI to Nanopore RNA data derived from mouse embryonic prefrontal cortex and uncovered isoform-specific modifications that change stoichiometry during neuronal development. In a mouse model of autism spectrum disorder (ASD), we identified significant differences in isoform-specific modifications in genes associated with neuronal development, highlighting the potential biological relevance of the isoform specificity of RNA modifications in neurodevelopmental disorders. CHEUI's ability to detect RNA modifications with high accuracy and resolution can be expanded to other modifications to unveil the full span of the epitranscriptome in normal and disease conditions.

21 Through the Nanopore: Latest Developments and RNA Overview

Warren Bach[1]
[1] Oxford Nanopore Technologies

Nanopore sequencing technology is constantly developing and adding new functionality. This presentation will touch on some of the most recent developments including several for RNA analysis. Comprehensive validation studies have been published recently which demonstrate the effectiveness of both cDNA and dRNA assays in analysing gene, isoform and RNA modifications. Finally the exciting area of single cell and spatial isoform analysis is possible with optimised kits, workflows and analysis tools being released.

22 Single-cell long-read RNA-seq reveal complex heterogeneity in leukaemia

Rachel Thijssen[1]
[1] Walter and Eliza Hall Institute of Medical Research

While introduction of novel targeted inhibitors has markedly altered the therapeutic options for treating patients with blood cancers, relapses due to acquired resistance after initial response remain a major problem. To delineate drug resistance, we applied a novel single-cell omics approach on samples from patients with progressive leukaemia who failed therapy with a targeted agent. Combining short-read with long-read targeted and whole transcriptome sequencing identified mutations and alternative transcripts in specific sub-clones of the tumour at relapse. Thus, our single-cell integration of short-read and full-length RNA-seq provides novel insights into how complex tumour heterogeneity evolves upon acquisition of drug resistance.

23 Experience-dependent accumulation of G-quadruplex DNA serves as a transcriptional control device to regulate the consolidation and stability of fear-related memories

Paul Marshall[1]
[1] Queensland Brain Institute

DNA can adopt more than 20 different conformational states; however, beyond the right-handed double helix, little is known about the dynamic nature of DNA and whether altered DNA structure states contribute to experience-dependent transcriptional activity in the adult brain. A genome-wide analysis of G-quadruplex DNA (G4-DNA) in neurons activated by behavioural experience revealed a transient, cell-specific, increase in the accumulation of G4-DNA following fear extinction learning. Critically, knockdown of the G4-specific helicase DHX36 led to a global increase in G4-DNA, which was associated with Pol II stalling, an overall reduction in transcriptional activity, and impaired memory. Targeted resolution of G4-DNA using site-directed dCas9-DHX36 at the Gephyrin locus caused a reduction in G4-DNA-associated RNA expression and impaired the formation fear extinction memory. In contrast, the same manipulation within the cell-adhesion like-1 locus resulted in an increase in G4-DNA-associated RNA expression and destabilization of the original fear memory trace. These findings reveal a causal relationship between dynamic G4-DNA structure and experience-dependent gene expression, with variable gene-specific effects on the consolidation and stability of fear-related memories.

One sentence summary: Dynamic G4-DNA structure states are molecular switches that enable temporeal regulation of gene expression underlying memory.

Session 4

24 Simple. Fast. Elegant. Directional RNA-Seq Library Preparation with Integrated Ribo-globin Depletion

Andrew Szentirmay[1]
[1] Gene Target Solutions

sparQ RNA-Seq HMR Kit simultaneously depletes rRNA and globin mRNA while generating stranded RNA-seq libraries for Illumina® NGS platforms in 5 hours. In a single step and tube, RNA fragmentation and depletion of abundant ribosomal and globin transcripts (human, mouse, and rat) are integrated. The proprietary, highly optimized enzymes and streamlined workflow generate high quality, directional transcriptome NGS libraries from either intact or degraded RNA samples, with key improvements for low input and FFPE samples.

25 RNA in Neuroscience

Timothy Bredy[1]
[1] University of Queensland

RNA, once thought to simply be an intermediate step in the transition from DNA code to the proteome, is increasingly being recognised as a critical feature information processing in the brain. This is due, in part, to the discovery that the majority of our genes do not code for protein but instead generate a diverse population of regulatory RNAs that function in a cell-type and state-dependent manner. Indeed, dysregulated RNA metabolism is involved in a variety of brain disorders, including neuropsychiatric conditions such as like phobia, PTSD, schizophrenia and depression, as well as neurodegenerative diseases, including ALS and epilepsy. RNA therefore represents a new frontier in the quest to design new treatment approaches for brain disorders. This talk will focus on my labs journey through the world of RNA in neuroscience over the a past 15 years, highlighting new avenues for exploration along the way.

26 The retrotransposon Lx9 puts a brake on the immune response to virus infection

Cecile King[1]
[1] University of New South Wales

The mammalian genome has an abundance of transposable elements (TEs) but their contribution to complex biological systems remains poorly understood. Here, we report that genetic deletion of a LINE-1 element (Lx9c11) in mice led to an exaggerated and lethal immune response to virus infection. Lx9c11 was critical for the neogenesis of a non-coding RNA (Lx9c11-RegoS) and negatively influenced the expression of virus response genes and LINE-1 elements across the Schlafen gene family locus. Lx9c11 appears to operate through the expression of natural anti-sense transcripts and replacement of Lx9c11-RegoS reduced the hyperinflammatory phenotype and rescued lethality in virus infected Lx9c11-/- mice. These findings provide the first evidence that a transposable element can control the immune system to favour host survival during virus infection.

27 G:U base-paired hpRNA is a potent small RNA sponge in plants

Chengcheng Zhong[1], Nei Smith[1], Daai Zhang[1], Ian Greaves[1], Anthony Millar[2], Tom Wash[1], Weixing Shan[3], Ming-Bo Wang[1]
[1] CSIRO; [2] Australian National University; [3] Northwest A&F University

Small noncoding RNAs, namely 20-24 nt microRNAs (miRNAs) and small interfering RNAs (siRNAs), play essential roles in plant development and defence. Currently the most useful approach for analysis of sRNA function is the sRNA sponge technologies developed based on the property of naturally occurring miRNA target mimicry RNAs in plants and animals. Here we report a new and robust sRNA sponge technology based on G:U base-paired hairpin RNA. We found that G:U hpRNA, unlike the perfectly base-paired hpRNA, accumulate as full-length molecules in the cytoplasm when transcribed in plant cells. We further demonstrated that transgenically expressed G:U hpRNA containing miRNA or siRNA target mimicry sequences in the loop region is highly effective at reducing miRNA or siRNA accumulation and inhibiting their function. We showed that this G:U hpRNA design inhibits miRNA functions causing strong miRNA-repressed phenotypes in 60~100% of the transgenic plant lines. Furthermore, we showed that the G:U hpRNA design is effective at inhibiting the function of a highly abundant siRNA of cucumber mosaic virus satellite RNA, preventing the yellowing disease symptoms in tobacco caused by the viral siRNA. We propose that the G:U hpRNA design will be a robust tool for studying the functions of small RNAs in plants as well as for engineering plants for improved agronomical traits.

28 C/D box small nucleolar RNAs methylate neuronal mRNAs and modulate memory-related processes

Laura J. Leighton[1], Qiongyi Zhao[1], Paul R. Marshall[1], Sachithrani U. Madugalle[1], Mason R. B. Musgrove[1], Haobin Ren[1], Wei-Siang Liau[1], Esmi L. Zajaczkowski[1], Ambika Periyakaruppiah[1], Hao Gong[1], Joshua Davies[1], Bryan C. Dickinson[1], Lisheng Zhang[1], Chuan He[1], Timothy W. Bredy[1]
[1] Queensland Brain Institute

C/D box small nucleolar RNAs (snoRNAs) guide the RNA modification 2'-O-methylation primarily to pre-ribosomal and small nuclear RNAs. However, many 'orphan' C/D box snoRNAs lack complementarity to rRNA or snRNA. To date, few studies have investigated their potential targets and functions, nor has the function of C/D box snoRNAs in dynamic cellular signalling processes been fully revealed. We hypothesised that orphan snoRNAs, several of which are known to be brain-specific, are key regulators of learning and memory via the modification of plasticity-related mRNAs. Using a ligation-based small RNA library preparation method, we sequenced C/D box snoRNAs from the mouse prefrontal cortex and detected more than 150 C/D box snoRNAs, of which two are novel, and 35 are orphans without known target RNAs. Additionally, we observed rapid upregulation of 31 C/D box snoRNAs in response to learning. Using a candidate gene approach, we investigated the interaction between the orphan snoRNA snord64 and its predicted target, an mRNA encoding the ubiquitin ligase Rnf146. We found that this mRNA is 2'-O-methylated and that this modification is proportionally reduced following snord64 knockdown. The same manipulation of snord64 in the prefrontal cortex attenuated forgetting of conditioned fear, and enhanced memory updating after weak fear extinction learning. These findings provide new insight into the biogenesis, processing and function of snoRNAs in the brain and highlight their role in regulating mRNA targets associated with learning and memory.

29 Functional screening of regulatory enhancers in human primary astrocytes

Gavin Sutton[1], Nicole Green[1], Irina Voineagu[1]
[1] University of New South Wales

Enhancers are non-coding regulatory regions, important for gene expression regulation during development, cell-type specification, and synaptic activity. Genetic variants associated autism, schizophrenia, major depressive disorder, Alzheimer's disease and brain cancers are enriched in enhancers. The function of enhancers in the human brain, and how it is impacted by genetic variants remains poorly understood. While thousands of candidate enhancers have been predicted based on chromatin marks, it remains unclear (i) which of these predicted enhancers are functional in glial and neuronal cells, and (ii) which genes they regulate.

To functionally identify enhancers and characterize their target genes in human primary astrocytes, we have carried out a large-scale screen of ~ 1000 enhancers in their native genomic context using CRISPRi screening, which combines epigenome editing with single-cell RNA-seq. We describe the genomic properties of functional enhancers, identify their downstream genes, and link them to disease-associated variants. To our knowledge, this is the first CRISPRi screen of enhancer function in primary human cells.

30 Characterising neuropsychiatric risk genes in human brain with long-read sequencing

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Neuropsychiatric disorders are a spectrum of complex and highly debilitating conditions. Genome-wide association studies (GWAS) have led to the discovery of hundreds of risk genes as well as non-coding or intergenic genomic regions associated with disease risk. The underlying mechanisms behind disease risk are poorly understood but generally involve changes in the gene expression and splicing. Standard short-read RNA sequencing (RNA-seq) methodologies have limitations in identifying splice isoforms, while both short and long-read RNA-seq lack the sensitivity to comprehensively profile expression from non-coding genomic regions. Using a combination of long-read targeted RNA-seq and long-read amplicon sequencing we have profiled the expression and splicing of neuropsychiatric risk genes and loci in multiple regions of adult human brain. Investigation of >30 neuropsychiatric risk genes by amplicon sequencing identified hundreds of novel isoforms, including many genes where most expression was from previously-undiscovered isoforms. Targeted RNA sequencing across >3000 genomic regions identified thousands of novel isoforms including hundreds of previously unannotated intergenic lncRNAs in risk regions. Additional comparisons to matched short-read targeted RNA-seq further demonstrated the superior performance of targeted long-read RNA-seq in isoform identification. These novel genes and isoforms can now be functionally characterised and tested for association with disease. In summary the accurate characterisation of RNA isoforms enabled by long-read RNA-seq will help enable the translation of genomic findings into a pathophysiological understanding of neuropsychiatric disease.

Session 5

31 mRNA as Medicine

Melissa J Moore[1]
[1] Moderna Therapeutics

With synthetic mRNA now fully validated as a platform for the rapid creation and distribution of highly effective vaccines, the age of mRNA medicines is upon us. Because mRNAs can program the body to produce any desired protein (e.g., cytoplasmic, intraorganelle, membrane-bound, secreted) or set of proteins (e.g., multiprotein complexes) in their native state, possible applications are nearly infinite. In addition to a plethora of new vaccines (both prophylactic and therapeutic), experimental mRNA medicines already in the clinic include pro-inflammatory cytokines as anticancer agents, an angiogenic promoting blood vessel regrowth in damaged heart muscle, and protein replacement therapies for inborn metabolic diseases. I will discuss Moderna's overall process for production of mRNA medicines and our new "mRNA Access" initiative designed get Moderna's formulated mRNAs into the hands of academics interested in helping create the mRNA medicines of the future.

32 RNA sequence code in SARS CoV-2 condensates

Amy Susanne Gladfelter[1]
[1] The University of North Carolina at Chapel Hill

The nucleocapsid protein of SARS CoV-2 performs many critical steps in viral replication and has been shown to have a high propensity to condense with RNA. Here, I will discuss the role of RNA primary sequence, secondary structure and RNA-RNA interaction motifs in impacting N-protein condensation, including the temperature at which the protein phase separates. These studies reveal the way in which RNA can encode the properties of biomolecular condensates to generate an array of distinct compartments with a single protein component.

33 The virilizer-like methyltransferase VIRMA overexpression determines the aggressiveness and outcome of breast cancer in an RNA m6A-dependent manner

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Methylation of N6-adenosine (m6A) residues is the most prevalent modification on messenger RNAs (mRNAs). It regulates mRNA transcription, splicing, export, turnover and translation, thereby affecting key cellular processes. m6A methylation is deposited co-transcriptionally by m6A methylases or writers. Aberrant expression of core writers including METTL3 and METTL14 confers oncogenic potentials in myriad cancers. Other auxiliary proteins are important to maintain m6A in the cells; however, the impact of aberrant expression of these proteins in cancer development, maintenance and outcome remains elusive. One of these auxiliary proteins is the Virilizer-like m6A methyltransferase-associated protein (VIRMA), a large 202kD scaffolding protein that holds together all the components of the m6A writer complex. By analysing large datasets from the TCGA and METABRIC consortiums, we observed amplification and overexpression of VIRMA in 15% of breast cancers including the aggressive triple negative subtype. Amplification of VIRMA is associated with poorer overall survival in these cohorts. By ectopically expressing the full-length VIRMA and a shorter N-terminal isoform, we found that only full-length VIRMA promotes cell proliferation and tumour growth in breast cancer cell lines and xenografts. Consistent with the co-transcriptional deposition of m6A, only full-length VIRMA is enriched in the nucleus and enhances transcriptome-wide m6A levels in breast cancer cells. Using m6A RNA immunoprecipitation sequencing, we identified the enrichment of transcripts involved in the unfolded protein response (UPR) signalling consequent to full-length VIRMA overexpression in breast cancer cells. These m6A-enriched UPR-regulating transcripts also increased in expression and are rapidly translated under conditions that mimic tumour microenvironment including hypoxia and endoplasmic reticulum stress. Compared to controls, VIRMA-overexpressing cancer cells are also less viable when UPR is enhanced. Thus VIRMA overexpression promotes tumour growth in an m6A-dependent manner but appears to be an “Achilles Heel” that may be exploited for cancer therapy that targets UPR.

34 The lack of NSUN2 methyltransferase enzyme affects the role of UPF1 RBP

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5-methylcytosine (m5C) is an abundant RNA modification decorating diverse species of RNA. The re-discovery of m5C as an mRNA modification came to light by coupling bisulfite conversion of RNA with transcriptome-wide RNA sequencing (bisRNA-seq). Subsequent studies revealed prominence of deposition of m5C modification in regulating gene expression.

Presently, bisRNA-seq experiments carried out in different cell types have generated several lists for m5C-decorated mRNAs that are often different from each other. This disparity could be partially explained by the variation in the expression levels of the very methyltransferase enzymes (NSUN family of proteins) responsible for the deposition of m5C modification (‘m5C writers’).

In an attempt to make this coherent, we generated a comprehensive, transcriptome-wide human dataset of m5C sites by re-analysing existing bisRNA-seq datasets from 10 different human cell lines and 7 tissues, with a stringent pipeline for m5C site calling. This allowed us to compile a union list of 9,903 high-confidence m5C sites. This union list was then employed to investigate the relationship between m5C modification and RNA-binding proteins (RBPs) binding to mRNA. Therefore, we overlapped the exonic m5C sites with publicly available eCLIP data for >100 RBPs, identifying several proteins whose footprint significantly overlaps with m5C modification. We further generated a knockout HeLa cell line for NSUN2, the main m5C writer protein, to investigate the effect of mRNA methylation level and its influence on RBPs function.

Knockdown experiments in WT vs NSUN2 knockout HeLa cells showed that the lack of NSUN2 enzyme reduces the effect of UPF1 in regulating the steady state of a subgroup of its methylated mRNA targets. Overall, our data suggests a novel role for UPF1 as m5C reader proteins.

35 Multi-omics data analysis identifies epigenetic regulators of alternative splicing

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Introduction
The phenomenon of widespread and dynamic intron retention (IR) programs in cells of vertebrate species has recently gained increasing attention. It has been shown that IR is involved in a multitude of cell-physiological processes, while aberrant IR profiles have been associated with numerous human diseases including several cancers.
Despite consistent reports about intrinsic sequence features that predispose introns to being retained, conflicting findings about cell type or condition-specific IR regulation by trans-regulatory and epigenetic mechanisms demand an unbiased and systematic analysis of IR in a controlled experimental setting.

Methods
We integrated matched mRNA sequencing (RNA-seq), whole genome bisulfite sequencing (WGBS), nucleosome occupancy methylome sequencing (NOMe-Seq), and chromatin immunoprecipitation sequencing (ChIP-seq) data from primary human myeloid and lymphoid cells. Using these multi-omics data and machine learning we trained two complementary models to determine the role of epigenetic factors in the regulation of IR in cells of the innate immune system.

Results
Our results suggest that intrinsic characteristics are key for introns to evade splicing and that epigenetic marks can modulate IR levels. However, cell type-specific IR profiles are largely caused by changes in chromatin accessibility, whereby predisposed introns in nucleosome free regions are more likely to be retained.

Discussion
This study is the first to demonstrate the important role of nucleosome occupancy in IR regulation. Our results have profound implications for the analysis of other forms of alternative splicing as well. Since an increasing number of studies describe pathogenic alterations in splicing regulation and therapeutic approaches targeting aberrant splicing, our findings will inform novel epigenetic therapy development.

36 Selective processing of clustered miRNAs in cancer: structure is the key

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Almost a half of microRNAs (miRNAs) in mammalian cells are generated from polycistronic primary transcripts encoding more than one miRNA. How the processing of individual miRNAs within the clusters is controlled to give rise to distinct miRNA levels in vivo is not fully understood. Using miR17-92 cluster as a model system, we demonstrate SRSF3 (Serine-Arginine Rich Splicing Factor 3) selectively enhances the processing of two paralog miRNAs, miR-17 and miR-20a, targeting overlapping mRNAs including the cell cycle inhibitor CDKN1A/p21. We propose that when bound to the miR17-92 transcript, SRSF3 rearranges the RNA structure leading to differential processing of miRNAs. SRSF3 binding site context, not merely the distance from the stem loop, within primary transcript is a critical determinant of the processing efficiency of distinct miRNAs derived from polycistronic miRNA clusters. Functional analysis demonstrated that SRSF3 inhibits CDKN1A expression and promotes cell cycle and self-renewal through the miRNA processing pathway both in normal pluripotent stem cells and cancer cells. Strikingly, analysis of colorectal cancer tumour-normal pairs demonstrated that the SRSF3-regulated miRNA processing pathway is present in a large proportion of colorectal cancer patients and distinguishes poorly differentiated high-grade tumours. Our research uncovers a critical role of SRSF3 in selective processing of miR-17-92 miRNAs, which mechanistically and functionally links SRSF3 to hallmark features of cancer.

Session 6

37 Treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections is dependent on the efficacy of last-line antibiotics including vancomycin

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Treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections is dependent on the efficacy of last-line antibiotics including vancomycin. Treatment failure is most commonly linked to the emergence of vancomycin-intermediate tolerance in clinical isolates (termed VISA). These isolates have not acquired resistance genes but accumulate varying single nucleotide polymorphisms that collectively reduce susceptibility to vancomycin. Cell wall thickening is common among VISA isolates and is thought to decrease vancomycin permeability. Changes in regulatory small RNA (sRNA) expression have been correlated with antibiotic stress responses in VISA isolates however the functions of the vast majority of these RNA regulators is unknown. Here we have used the endoribonuclease RNase III to capture RNA-RNA interactions using an RNA proximity-dependant ligation technique termed CLASH. RNase III-CLASH uncovered hundreds of novel RNA-RNA interactions in vivo allowing functional characterisation of many sRNAs for the first time. Surprisingly, many mRNA-mRNA interactions were recovered and we find that an mRNA encoding an unusually long 3' untranslated region (UTR) (here termed vigR 3'UTR) functions as a regulatory 'hub' within the RNA-RNA interaction network. We demonstrate that the vigR 3'UTR promotes expression of folD and the cell wall lytic transglycosylase isaA through direct mRNA-mRNA base-pairing interactions. Deletion of the vigR 3'UTR re-sensitised VISA to glycopeptide treatment and both isaA and vigR 3'UTR deletions impact cell wall thickness. Our results demonstrate the utility of RNase III-CLASH for identifying new regulatory RNA functions and indicate that S. aureus uses mRNA-mRNA interactions to co-ordinate gene expression more widely than previously appreciated.

38 Keeping RNA unstuck: modulating RNA structure with RNA binding proteins

Fionna E Loughlin[1], Jackie Wilce[1]

RNA performs a multitude of functions throughout the cell. These range from protein coding mRNAs, catalytic RNA at the heart of machines such as the ribosome, to gene regulatory functions of small non-coding RNAs. The single stranded nature of RNA has a fundamental influence on the structure and function of RNA. Exposed bases enable RNA to form both complex secondary structures essential for many biological functions and encode powerful RNA targeting potential through intermolecular base pairing such as mRNA-miRNA pairs. However, this same property renders RNA susceptible to misfolding in kinetic traps or into stable inactive conformations. These structures can slow down translation of mRNAs or alter gene expression by burying key regulatory sequences. One way in which this problem of RNA folding has been solved is through the action of RNA binding proteins which can chaperone RNA, reducing misfolding and thus ensuring optimal function. We are using NMR spectroscopy to investigate the molecular mechanisms by which RNA binding proteins influence RNA structure with emphasis on defining the unique contributions of both globular and intrinsically disordered domains. Here we present investigations of Cellular nucleic acid binding protein (CNBP) and Fused in Sarcoma (FUS) with short RNA binding motifs, structured G quadruplexes and long non-coding RNAs.

39 Coding and noncoding mechanisms of human pseudogene function

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Pseudogenes are mutant copies of genes that have been thought of as functionless relics of evolution. Several pseudogenes have been shown to regulate tumourigenesis (PTENP1, BRAFP1), inflammation (Lethe) and diabetes (HMGA1-p) through RNA-intrinsic functions or encoded peptides. The impact of pseudogenes in human biology has been understudied due to technical limitations that preclude accurate identification of pseudogene transcripts. Most short RNA-seq reads do not align uniquely to pseudogenes and cannot confidently distinguish the highly similar pseudogene and parent gene transcripts. PacBio Iso-Seq cDNA reads harbour enough sequence differences to accurately quantify pseudogene and parent gene transcription. Using deep full-length cDNA sequencing of normal human tissues and cancer cell lines, we identified hundreds of novel pseudogene transcripts. Many pseudogenes exhibit complex splicing patterns and are often incorporated as exons into protein-coding genes. We identified a nuclear-enriched pseudogene-derived lncRNA, PDCL3P4 which is transcribed for an endogenous retrovirus promoter. Genetic ablation of PDCL3P4 disrupts the cellular transcriptome independent of its parent gene. We also identified a pseudogene called MSL3P1 that has a large intact open reading frame encoding the MRG domain, but not chromo domain of its parent gene MSL3. MSL3P1 is overexpressed in cancer and its expression is correlates with poor prognosis. MSL3P1 physically interacts with a H4K16 acetyltransferase complex to module chromatin state. CRISPR/Cas9-mediated genetic ablation and overexpression revealed that MSL3P1 controls key cell cycle genes including cyclin D, to drive cellular proliferation. This study identifies a complex, dynamic human pseudogene transcriptome that impacts human biology through coding and noncoding mechanisms.

40 Structural characterization of the ANTAR antiterminator domain bound to RNA

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Regulated transcription termination provides an efficient and responsive means to control gene expression. In bacteria, rho-independent termination occurs through the formation of an intrinsic RNA terminator loop, which disrupts the RNA polymerase elongation complex, resulting in its dissociation from the DNA template. Bacteria have a number of pathways for overriding termination, one of which is the formation of mutually exclusive RNA motifs. ANTAR domains are a class of antiterminator that bind and stabilize
dual hexaloop RNA motifs within the nascent RNA chain to prevent terminator loop formation. We have determined the structures of the dimeric ANTAR domain protein EutV, from Enterococcus faecialis, in the absence of and in complex with the dual hexaloop RNA target. The structures illustrate conformational changes that occur upon RNA binding and
reveal that the molecular interactions between the ANTAR domains and RNA are restricted to a single hexaloop of the motif. An ANTAR domain dimer must contact each hexaloop of the dual hexaloop motif individually to prevent termination in eubacteria. Our findings thereby redefine the minimal ANTAR domain binding motif to a single hexaloop and revise the current model for ANTAR-mediated antitermination. These insights will inform and facilitate the discovery of novel ANTAR domain RNA targets.

41 Structural and mechanistic insights into the role of the canonical poly(A) polymerase Pla1 in RNA surveillance by the fission yeast MTREC complex.

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Mtl1-Red1 core (MTREC), the S. pombe orthologue of the human PAXT complex, is an eleven-subunit complex which targets cryptic unstable transcripts (CUTs) to the nuclear RNA exosome for degradation. It also encompasses the canonical poly(A) polymerase Pla1, responsible for polyadenylation of nascent RNA transcripts as part of the cleavage and polyadenylation factor (CPF/CPSF). In this study we identified and characterised the interaction between Pla1 and the MTREC complex core component Red1 and analysed the functional relevance of this interaction in vivo. Our crystal structure of the Pla1-Red1 complex showed that a 58-residue fragment in Red1 binds to the RNA recognition motif domain of Pla1 and tethers to it the MTREC complex. Structure-based Pla1-Red1 interaction mutations showed that Pla1, as part of MTREC complex, hyper-adenylates CUTs for their efficient degradation. Interestingly, the Red1-Pla1 interaction was also required for the efficient assembly of the fission yeast facultative heterochromatic islands. Together, our data suggest a complex interplay between the RNA surveillance and 3’-end processing machineries.

42 Detecting RNA structures using nanopore sequencing

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RNA structures are important in regulating almost every step of an RNA's lifecycle. While high throughput structure probing typically involves identifying chemical modifications along an RNA by reverse transcription and deep sequencing, recent developments in direct RNA sequencing enable us to detect RNA structure modifications directly using nanopores. In this talk, we describe our strategy to identify structure modifications on long RNA molecules to identify isoform-specific RNA structures in human ES and SARS-CoV-2 transcriptomes. As direct RNA sequencing allows us to obtain structure signals at a single-molecule level, I will also describe our efforts in studying single-molecule structural heterogeneity using direct RNA sequencing.

43 Antisense therapeutics for rare diseases; delivering the potential

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Treatment options for rare diseases have been limited, although novel gene and molecular therapeutics are now demonstrating significant potential in the treatment of both inherited and acquired conditions. RNA therapeutics in particular hold unique promise in these diseases; although achieving safe and efficient delivery of molecular drugs to deep target tissues such as heart, skeletal muscle, central nervous system and the retina remains a significant obstacle to clinical application. Antisense oligomers are a well-established class of RNA therapeutic whose potential is yet to be fully realised due to this delivery challenge, and to recurrent efficacy and tolerability failures.

Evolving oligonucleotide chemistries, conjugates and delivery modalities are addressing these challenges and delivering safer drug molecules. However, we are working in an environment beset by recent failures in the clinic, a poor appetite for biotech investment and a shrinking biopharmaceutical sector. Despite this currently unfavourable ecosystem, opportunities exist to deliver patient impact and demonstrate the utility of antisense drugs, particularly in rare monogenic diseases.

Session 7

44 Molecular basis of pre-microRNA processing by DICER

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DICER, a multi-domain ribonuclease III protein, is a key enzyme in the production of microRNAs (miRNAs) and small interfering RNAs (siRNAs). Yet, the lack of the structure of vertebrate DICER in a catalytic state has limited our mechanistic understanding of the small RNA biogenesis. Here we report the cryo-electron microscopy structure of the human DICER bound to pre-let-7a-1, uncovering the structural rearrangement from the apo to the dicing states, which involve large conformational changes in the helicase domain, double-stranded RNA-binding domain (dsRBD), and PAZ domain. The helicase domain becomes largely flexible in a dicing state, with no visible RNA interaction, consistent with its autoinhibitory role as a gatekeeper between the closed and open conformations. In contrast, the C-terminal dsRBD moves to anchor a specific position in the pre-miRNA and mediates both sequence-independent and sequence-specific recognition of the substrate, highlighting the importance of dsRBD in the cleavage step. The DICER-specific helix in the PAZ domain is also re-oriented to accommodate the substrate tightly in an angle compatible with catalysis. In line with this, mutating the PAZ helix impairs processing *in vitro* and in cells. Furthermore, the structure reveals how the 5′ end of pre-miRNA is anchored by the basic residues in the 5′ pocket in the platform domain, which plays a critical role in cleavage site decision. Our study explains, in atomic detail, how human DICER interacts with pre-miRNAs during the catalytic step with stringent specificity.

45 Metabolic enzymes moonlight as RNA-binding proteins

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Conventional RNA-binding proteins (RBPs) regulate the post-transcriptional gene expression forming dynamic ribonucleoprotein complexes via their globular RNA-binding domains (RBDs). Unbiased cardiomyocyte interactome capture using HL-1 cells detected multiple unconventional RBPs, notably a high representation of metabolic enzymes.

To understand the relevance of such unconventional interactions, an unbiased methodology employing the principles of crosslinking and immuno-precipitation followed by sequencing (CLIP-seq) was set up for selected moonlighting metabolic enzymes and SERCA2a. CLIP-seq when validated using a positive control PUM2 identified 60% of its known RNA targets indicating the efficiency of the methodology. Preliminary screening for Aldolase A (ALDOA), Enolase 1 (ENO1), Phosphoglycerate Kinase 1 (PGK1) and Glucose-6-phosphate isomerase (GPI1) identified multiple RNAs exhibiting interactions. Interestingly, ALDOA and PGK1 exhibited enrichment for two common RNA targets ANKRD40 and RHOQP1. indicating the possible existence of RNA scaffolds facilitating the formation of oligomers or metabolons.

Our preliminary results indicate that metabolic enzymes interact with multiple potential RNA targets exhibiting a common functional theme indicative of their likely functional relevance. The common RNA targets identified across the metabolic enzymes suggest the possibility of RNAs facilitating the formation of metabolons enhancing the metabolic output. The RNA targets identified would provide a platform to explore the sequence motifs and protein domains that determine the interaction and their modulation without affecting the enzymatic activity would enable us to determine the biological relevance of such unconventional interactions.

46 Defining novel components of endogenous dsRNA sensing pathways

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Adenosine-to-inosine (A-to-I) editing of double-stranded RNA (dsRNA) by ADAR proteins is a highly prevalent form of RNA base modification that is essential for self/non-self discrimination of dsRNA. The primary physiological function of ADAR1 is to edit dsRNA structures in endogenous RNAs, resulting in a change in secondary structure. Non-edited endogenous dsRNA would otherwise be recognised as non-self/foreign dsRNA by the innate immune system. In the absence of editing the cytosolic dsRNA sensor MDA5 oligomerises on endogenous dsRNAs and signals to the mitochondrial protein MAVS leading to the ongoing production of interferon-stimulated genes (ISGs) and a permanent antiviral state that is extremely detrimental. Causative mutations have been identified in both ADAR (ADAR1) and IFIH1 (MDA5) in individuals with the rare auto-inflammatory disease, Aicardi-Goutieres syndrome (AGS). We have developed a cell culture model for Adar1-editing deficiency which recapitulates the in vivo biology in order to perform forward genetic screens for loss of function alleles that modify the requirement for A-to-I editing by ADAR1. I will present data describing the screening approach and the results from the screen where we have identified novel genes and pathways required for the recognition of endogenous unedited dsRNA.

47 The molecular action mode of restorer-of-fertility-like proteins in plant mitochondria

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The interaction between nuclear restorer of fertility (RF) genes and mitochondrial cytoplasmic male sterility (CMS) causing genes has important application in hybrid seed production. Most RF and their homologous Restorer-of-fertility-like (RFL) proteins belongs to P-class pentatricopeptide repeat protein (PPRs) family. Although, it is documented the majority of characterised RF and RFL protein genes can bind and induce cleavage in their mitochondrial targeting transcripts, the molecular mechanism by which the cleavage is performed, remains unknown. Comparative genomics studies revealed presence of a conserved C-terminal domain (CTD) of 60-70 amino acids in majority of those proteins. In planta truncation experiments indicated that CTD is important for Arabidopsis thaliana RFL6 to bind and induce cleavage in 5'UTR cox3 most likely by recruiting mitochondrial endonucleases. Reversely, CTD does not play essential role for function of A. thaliana RFL2 which binds and induces the cleavage in CDS of orf291. Immunoprecipitation assays followed by mass spectrometry and yeast-interactome assays were conducted to characterised interactome of the RFL6. Lastly, five variants of synthetic PPR added with natural RFL6-CTD were designed and demonstrated in deep the role of CTD. This research by bringing new insights into the molecular mode of action of RFL proteins in plants is expanding our understanding of the mechanisms underlying CMS and fertility restoration in plants.

48 A cell-cycle mediated checkpoint that regulates mRNA export in response to replication stress

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Nuclear export of mRNA is extensively coupled to transcription and processing of mRNA throughout the cell cycle. Little is known about how cells regulate mRNA export during DNA replication in S-phase. Here, we uncover a checkpoint mediated by WEE1, CDK1 and PLK1 that regulates mRNA export in response to replication stress. We show that mRNA export complexes are recruited to sites of replication stress in S-phase. WEE1 inhibition prematurely activates CDK1 and PLK1, leading to accumulation of R-loop associated mRNA in large nuclear speckles, with late markers of replication stress present around their periphery. This recruitment is dependent on CDK1 activity. mRNA export factors including ALYREF are mis-localised from these speckles, preventing nuclear export of R-loop associated mRNA. Phosphorylation of ALYREF by CDK1 and PLK1 regulates nuclear speckle accumulation of mRNA following replication stress. Thus, WEE1, CDK1 and PLK1 enforce a cell cycle checkpoint that serves to protect the cell from major sources of genome instability by ensuring that R-loop associated mature mRNA is not exported to the cytoplasm.

49 P-bodies are key regulators of the cell cycle and their depletion induces a cancer cell stem-like transition

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Processing bodies (P-bodies) are cytoplasmic ribonucleoprotein granules, which are highly conserved among eukaryotes and involved in the storage and degradation of mRNAs. However, the roles of P-bodies in influencing cellular phenotype are not fully understood. Previous studies have shown that P-bodies are cell cycle-regulated and dissipate during M-phase. Using a combination of cell cycle optical sensor (FUCCI(CA)) to detect cell cycle stages (G1, S, and G2/M) and an inducible shRNA knockdown targeting DDX6, a core P-body protein, in HeLa cell lines, we found that upon P-body depletion cells display an extended M-G1 transition. This extended transition was concomitant with irregular nuclei shape and reduced levels of Lamin A/C but no increase in cell death. RNA mapping of P-bodies with APEX-seq identified an enrichment of M-phase regulators suggesting a potential role in mRNA storage and release. Extension of M-G1 transition and low levels of Lamin A/C are associated with a stem-like phenotype. To investigate if P-body depletion can produce a similar phenotype, we depleted P-bodies in triple-negative breast cancer model cells. This promoted a transition of cancer cells into a cancer stem cell state, which shows a higher capacity to form primary tumours, metastasize, and resist to chemotherapy. Together this study reveals an important role for P-bodies in cell cycle regulation and cell fate decisions.

50 Pinpointing spatial transcriptional changes driving retinal degeneration

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Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world, affecting 1 in 7 Australians over the age of 50 and costing the Australian economy $16.6 billion annually (2009). AMD patients are seriously impacted by the loss of central vision, due to the death of the light-sensing photoreceptor cells and underlying retinal pigment epithelium. While environmental, lifestyle and genetic risk factors are well known to lead to the disease, at the molecular level inflammation has been identified as a central driver of disease progression and reducing inflammatory processes has been shown to ameliorate retinal degeneration. However, precisely how and where inflammation is first established and how this drives disease progression remains unclear. The advent of spatial transcriptomics technologies now allows us to answer these questions and identify key genes and pathways as well as the cell types involved. In this study we utilised the Visium spatial gene expression technology to examine the spatial and temporal gene expression changes within the retina using a mouse model of retinal degeneration. We analysed parasagittal cross sections of the eye, focussing specifically on the retina and identified highly localised induction of inflammatory genes and pathways within key retinal tissue layers prior to the onset of retinal function impairment. We further provide evidence that over time gene expression changes radiate from the initial site of dysregulation, causing disruptions to retinal homeostasis in adjacent region, ultimately leading to photoreceptor cell death in the periphery. Although the Visium spatial resolution is limited, we can identify specific cell types that likely drive this dysregulation. Our study provides new insights into the role of inflammation in onset and progression of degeneration and offers novel cellular and gene targets to combat inflammation to prevent retinal damage.

POSTERS

51 Novel Approach to Authenticating Australian Honey

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Honey adulteration is a problem that affects the global honey industry and specifically, has been discovered in the Australian market. Common methods of adulteration include dilution with sugar syrup substitutes and the mislabelling of the floral and geographic origin(s) of honey. Current authentication tools rely on the molecular variability between different honeys, identifying unique chemical profiles and/or DNA signatures characteristic of a particular honey.

Honey is known to contain plant miRNAs derived from its floral source. To explore the composition and variability of honey RNA molecules, this is the first study to catalogue the small RNA content of Australian polyfloral table honey and New Zealand Leptospermum scoparium honey using next generation sequencing. The data shows that in addition to miRNAs, honey contains a variety of small non-coding RNAs including tRNA-derived fragments. Moreover, the honey small RNAs are derived from a range of phylogenetic sources, including from plant, invertebrate, and prokaryotic species. The data indicates that different honeys contain unique small RNA profiles, which suggests a novel avenue in developing molecular-based honey authentication tools.

52 A two-step approach for detecting and phasing variant associated mis-splicing.

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Short-read RNA sequencing (srRNA-seq) has proven utility to extend the diagnostic yield from genomic testing in rare disease. However, due to short read length, srRNA-seq struggles to align aberrant transcripts resulting in loss of diagnostically important information. We present a two-step approach using reverse RT-PCR to validate splice aberrations identified by srRNA-seq and phase correctly spliced transcripts for accurate variant interpretation.

Total RNA from whole blood of an individual affected with Aicardi-Goutières syndrome and compound heterozygous variants in RNASEH2B (NM_024570.3:c.[321+287C>G];[529G>A]) was prepared using Illumina Stranded Total RNA Prep with Ribo-Zero Plus rRNA Depletion to yield ~216M 150 bp paired-end reads. Reads were aligned to GRCh38 reference genome using STAR aligner. Gel-extracted RT-PCR amplicons were analysed by Sanger sequencing.

srRNA-seq identified exon 4 skipping and activation of a cryptic acceptor within intron 4 of RNASEH2B associated with paternal variant c.321+287C>G. Manual inspection of reads revealed ectopic inclusion of a pseudoexon (PE) within intron 4. STAR aligner could not align split reads splicing from the PE donor and were instead soft clipped or misaligned as exon 4 skipping. Custom alignment allowed split reads to be aligned to the PE donor, however, misalignment to exon 4 skipping persisted. RT-PCR confirmed PE inclusion and exon 4 skipping was not detected. Sanger sequencing of an amplicon encompassing the c.529G>A variant in trans showed correctly spliced transcripts were detected exclusively from the maternal allele.

Misaligned and/or soft clipped reads remain a significant caveat of srRNA-seq and we recommend custom alignment followed by RT-PCR validation for increased diagnostic confidence. Apparent levels of aberrant transcripts are often misleading as it is difficult to distinguish partial mis-splicing from transcripts degraded by nonsense mediated decay. Use of a distal coding variant to phase correctly spliced transcripts by RT-PCR confirmed complete mis-splicing from the paternal allele, providing clarity for variant interpretation.

53 Rules of engagement for microRNAs targeting protein coding regions

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It is well established that microRNAs (miRNAs) target the 3’ untranslated regions (3’UTRs) of mRNAs to suppress gene expression, but the extent to which miRNAs regulate genes via interaction within protein coding regions is contentious. Although miRNAs frequently interact with coding regions, the minimal effect this has upon the level of the target transcript means these interactions are rarely considered. However, a new class of regulation has been reported whereby miRNAs bind to coding regions in a manner that is dependent upon base-pairing of the 3’ end of the miRNA and suppress gene expression exclusively at the translational level (Zhang et al, Nat Struct Mol Biol 2018). It is therefore possible that miRNAs may exert much broader regulation than previously thought, given the non-canonical mode of binding and because the regulatory effects are not observable at the transcript level. Using an extensive reporter gene approach, we found that miRNAs can indeed regulate genes through interaction with coding regions, but in contrast to the results of Zhang et al., we find that the inhibition requires seed pairing, does not require base pairing of the 3' terminal bases, and acts through reducing mRNA levels. We conclude that suppression of endogenous genes can occur through miRNAs binding to protein coding regions, but the requirement for extensive base-pairing will limit the regulatory impact to a select subset of targets.

54 Improving response of oral cancer to cisplatin chemotherapy with microRNA-7-5p

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Despite recent advances, platinum-based chemotherapy remains the mainstay treatment for a wide range of cancers. Although many patients may respond initially, drug resistance is common leading disease progression and death. Overcoming resistance to cisplatin chemotherapy is therefore, a major challenge for improving outcomes for cancer patients. Using preclinical models of oral squamous cell carcinoma (OSCC) with acquired cisplatin resistance, we investigated the therapeutic potential of a microRNA, miR-7-5p, and its utility in combination with cisplatin. Using both 2D and 3D cell culture systems we found that ectopic expression of miR-7-5p significantly reduced the growth, migration and invasion of OSCC that were both sensitive or resistant to cisplatin. Functional assays determined that the reduced growth was a result of cell cycle arrest (G0/G1 phase) and cellular senescence. Pre-treatment of cells with cisplatin prior to transfection with miR-7-5p mimic led to synergistic growth inhibition, which was further evident when these tumour cells were implanted in immunocompromised mice. Mechanistically, RAF1 proto-oncogene, aka c-RAF, was identified as an important direct target of miR-7-5p and siRNA-mediated inhibition of RAF1 was able to recapitulate the functional effects observed with miR-7-5p in vitro. The development of a novel miR-7-5p mimic with stabilized chemistry now holds promise for improving OSCC patients' response to cisplatin chemotherapy.

55 Expression of cancer-related microRNAs in tumour and extracellular vesicles from early-onset colorectal cancer patients

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New Zealand has one of the highest rates of colorectal cancer (CRC) in the world, with over 1200 deaths per year. Recent trends have identified an increasing number of individuals presenting with sporadic CRC under 55 years of age, considered early-onset CRC. This increasing early incidence is not well understood and it is possible that there is a unique molecular phenotype in this population that is distinct to normal-onset CRC. Previous targeted DNA sequencing in sporadic early-onset (E-CRC, aged 65) CRC patients identified key differences in cancer-related gene mutations. Since microRNAs (miRNAs) are key regulators of cancer and are dysregulated in pathological states, this study examined expression of miRNAs between normal and tumour tissue, and in extracellular vesicles (EVs) obtained from sporadic E-CRC and N-CRC patients, and age-matched healthy controls (HCs). RNA was extracted from E-CRC (n=18) and N-CRC (n=18) tumour and normal tissue, and from plasma EVs of the same cohort and HCs (n=35). Quantitative real-time PCR (RT-qPCR) was performed for expression of miR-21-5p, miR-23a-3p and miR-25-3p. miR-21-5p was upregulated in tumour relative to normal tissue, only in E-CRC. In both groups, EVs were upregulated compared to HCs, and significantly downregulated relative to tumour.
Interestingly, differential expression of miR-25-3p was only observed in E-CRC patients. We found downregulation in tumour relative to normal tissue, and in EVs compared to both HCs and matched tissue. miR-23a-3p was upregulated in tumour tissue relative to normal mucosa in both groups and upregulated in EVs compared to controls in N-CRC patients.
In conjunction with targeted sequencing results, these differences in miRNA expression
indicate that despite similar clinical profiles, E-CRC patients may have a different molecular profile to N-CRC patients. Ongoing elucidation of the pathophysiology of E-CRC may help
guide surveillance and treatment strategies for this population.

56 Identification of colorectal cancer-specific transcripts and alternative splicing events using full-length isoform sequencing

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The recent development of long-read isoform sequencing allows identification of full-length single-molecule transcript sequences, overcoming the limitations of short-read RNA-seq for transcript discovery. Cancer-specific transcript isoforms may contribute to mechanisms of cancer development, progression and therapy response. They may also have potential as disease biomarkers

Here, we used PacBio Iso-seq and whole-genome sequencing of primary tumour, metastases and matched normal samples from four colorectal cancer patients to identify cancer-specific transcripts and investigate the genetic association of somatic variants on alternative splicing. Approximately half of all transcripts identified were different from known GENCODE transcripts at least one splice boundary and more than half of transcripts across the cancer samples were cancer specific. We found a shared pattern of cancer specific alternative transcription in 30 CRC associated genes across at least three patients. Analysis of somatic variants identified six alternatively spliced transcripts associated with somatic splice site variants at conserved donor/acceptor positions resulting in cancer-specific exon skipping and intron retention. Our findings indicate the utility of long-read sequencing to fully investigate cancer transcriptomes.

57 SpliceVault: predicting the precise nature of variant-associated mis-splicing.

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Clinical interpretation of splicing variants depends critically upon the nature of variant-associated mis-splicing and consequence(s) for the encoded gene product. Arrestingly, ranking the four most common unannotated splicing events across 335,663 reference RNA-sequencing samples (300K-RNA Top-4), identifies the nature of variant-associated mis-splicing with remarkable prescience. 300K-RNA Top-4 correctly identifies 96% of exon-skipping events and 86% of cryptic splice-sites induced by 88 variants across 74 genes and 140 affected individuals or heterozygotes subject to RNA Diagnostics. 300K-RNA shows higher sensitivity and positive predictive value than SpliceAI in predicting exon-skipping and cryptic-activation events. Importantly, RNA re-analyses showed we had missed 300K-RNA Top-4 events for several clinical cases tested prior to 300K-RNA. In conclusion, 300K-RNA provides an evidence-based method that predicts with high sensitivity the nature of variant-associated mis-splicing. The SpliceVault web portal allows users easy access to 300K-RNA, to augment both pathology consideration of PVS1 and RNA diagnostic investigations.

58 CRISPR-Cas13d-based screens identify lncRNAs implicated in breast cancer risk

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The combination of GWAS and genetic fine-mapping has identified >5000 genetic variants associated with breast cancer risk. We recently reported the discovery of thousands of lncRNAs transcribed from breast cancer GWAS regions in a range of breast cell types, the majority of which not found in existing databases. Notably, based solely on the expression of these lncRNAs, we can discriminate breast cancer subtypes and normal breast tissue from tumours. We showed that breast cancer GWAS variants are enriched in lncRNA exons, but not their promoters or introns, suggesting modulation of lncRNAs is a predominant mechanism underlying breast cancer development. We identified 874 lncRNAs as candidate breast cancer risk genes, based on risk-associated variants that fall in their exons, promoters or distal regulatory elements.

To identify which of the 874 novel lncRNAs are functionally implicated in breast cancer, we performed the first ever CRISPR-Cas13 screen for lncRNAs. We screened two breast cancer cell lines, identifying 18 and 10 lncRNAs whose knockdown promotes proliferation and 33 and 17 which inhibit proliferation. Our hits feature novel lncRNAs that regulate MYC, TERT and the estrogen receptor (ER) gene ESR1. Another lncRNA hit from the screen, which we named KLNC, harbours a risk-associated variant that we demonstrate reduces its half-life from 11h to 6h. Consistently, we observed a significant (P=1.95x10-10) association between the risk variant and decreased KLNC expression in breast tumours (n=700). KLNC is an intron-derived lncRNA induced by estrogen and expressed higher in ER+ breast cancers. Overexpression of KLNC in breast cancer cell lines either by CRISPR-based activation or lentiviral overexpression reduces cell proliferation and promotes apoptosis. This is consistent with a role for KLNC in breast cancer development and was not observed in experiments with normal cell lines. Together, our results demonstrate a novel mechanism by which GWAS variants alter disease risk.

59 Exploring the role of circRNAs in learning and memory

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Recently, a new class of RNA known as circular RNA (circRNA) has been identified in the brain. CircRNAs are closed-loop, single-stranded RNA molecules that are generated by back-splicing between the downstream 5' splice site and the upstream 3' splice site. In previous work, we found that circRNAs accumulate in the synaptic compartment, and play a critical role in the formation of fear extinction memory. In this study, we are exploring the
functional relevance of the RNA modification N6-methyladenosine (m6A) in a subpopulation
of synapse enriched circRNAs. Preliminary evidence suggests that this RNA modification may be crucial for circRNA function in the brain by promoting the translation of m6A-modified circRNAs. We are currently investigating the functional role of specific m6A-modified circRNAs in the synaptic compartment following fear extinction learning.

60 Circulating SncRNAs as Biomarkers in Colorectal Cancer

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Colorectal cancer (CRC) is the second leading cause of cancer death in New Zealand. Diagnostic delay and inaccurate risk assessments for treatment contribute significantly to negative outcomes for CRC patients. Small non-coding RNAs (sncRNAs) have been posited as circulating, non-invasive biomarkers that may improve diagnostic and predictive strategies in CRC. Dysregulated sncRNAs are released from the tumour microenvironment and are potential markers of tumour burden and an immune response. The aim of this study was to discover and explore origins of circulating sncRNA biomarker candidates for the early detection of CRC and predictors of response to pre-operative long course chemoradiation (LCCR). Small RNA sequencing was performed on baseline plasma samples of healthy controls (n=20), stage I CRC patients (n=15), responders to LCCR (n=9) and non-responders to LCCR (n=11). A selected panel of seven differentially expressed miRNAs from each comparison was validated using RT-qPCR in the same cohort. The miRNA panel was further profiled in post-surgery plasma samples and tumour tissue. An average of 10 million sequencing reads per sample were generated. Pairwise comparisons yielded 28 differentially expressed miRNAs between healthy controls vs stage I patients (-0.5 ≥ LogFC ≥ 0.5, unadj p ≤ 0.002) and 17 between responders and non-responders to LCCR (-0.5 ≥ LogFC ≥ 0.5, unadj p ≤ 0.01). RT-qPCR validated miR-21, miR-23a and miR-375 as significantly upregulated in stage I vs controls (FC ≥ 1.48, p ≤ 0.05) and miR-25, was downregulated (FC < 0.76, p ≤ 0.03). These combined miRNAs revealed an area under the curve of 0.91 from ROC analysis. Additionally, miR-23a trended towards upregulation in non-responders compared to responders of LCCR. Tumoural expression was dysregulated compared to matched normal mucousa of these top markers and the dysregulation of miR-23a and miR-25 in post-surgical plasma samples was significantly reduced compared to baseline.

61 A novel RNA binding protein (ZCCHC24) as a regulator of cancer cell plasticity

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Cancer cell plasticity (commonly termed epithelial-mesenchymal transition or EMT) is a major facilitator of cancer cell invasion, tumour metastasis and therapy resistance. Accordingly, there is great interest in understanding the molecular events leading to EMT. In a screen for novel regulators of EMT, we uncovered a zinc finger protein (ZCCHC24) that appears to coordinate cellular and metabolic plasticity. ZCCHC24 is repressed by miR-200 in epithelial cells and its translation is potently induced during EMT. Unlike well-known EMT-driving transcription factors, we identified ZCCHC24 as a cytoplasmic RNA binding protein that influences cancer cell plasticity, cell migration and metabolic capacity through post-transcriptional modes of action. Using CLIP-seq we identify many RNA targets of ZCCHC24. We propose that ZCCHC24 coordinates distinct gene expression outputs that drive cellular and metabolic plasticity.

62 Novel mechanisms of piRNA biogenesis in Drosophila

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Piwi-interacting RNA (piRNA) is an evolutionarily conserved class of small RNA that targets transposable elements (transposons) in animal gonadal tissues. Like viruses today, transposons change their sequences over millions of years, so do piRNAs to counteract them. Therefore, piRNA production is flexible in that, unlike microRNAs and siRNAs, it does not rely on specific RNA structures or any sequence motifs for its production. On the other hand, piRNAs have a potential to silence any RNA, including host mRNAs. Hence, organisms have evolved mechanisms to generate only the piRNAs that silence transposons, but not other RNAs.

I will introduce mechanisms to solve this apparent conundrum in diverse systems, then move into our recent findings in Drosophila.

63 The Emerging Role of a Natural Antisense to Brain-Derived Neurotrophic Factor in Drug Addiction

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Natural antisense transcripts (NATs) are a pervasive feature of mammalian genomes and are potent regulators of overlapping sense genes. Aberrant expression of NATs has been implicated in many diseases, including neurological disorders. We recently discovered a long non-coding RNA that is antisense to the neurotrophic growth factor BDNF (AS-Bdnf) in rats. Nicotine self-administration upregulates the expression of AS-Bdnf and knockdown reduces relapse of nicotine seeking. This suggests a key role for AS-Bdnf in addiction presumably by modulating the expression of sense Bdnf. To investigate this, we performed high-throughput strand-specific RNA-sequencing on the medial prefrontal cortex of rats that had self-administered nicotine or cocaine followed by extinction training. Analysis of the transcriptional profiles revealed Bdnf upregulation following nicotine and cocaine administration. Furthermore, drug-induced differential splicing of exon iv and exon vi was detected. We performed a de novo transcriptome assembly to generate a draft annotation of AS-Bdnf and identified 42 putative exons and several splice variants. Despite being expressed at levels 50- to 100- fold lower than sense Bdnf, AS-Bdnf expression was increased upon nicotine and cocaine self-administration. Using RNA immunoprecipitation followed by mass spectrometry we identified several protein partners of AS-Bdnf that may offer a mechanistic explanation for its action. Collectively, this study revealed a concordant relationship between the antisense and sense Bdnf pair upon nicotine and cocaine self-administration that may play a role in the maintenance of drug-seeking during addition.

64 The Regulation of miRNAs by the OncomiR miR-21 in Cancer Cells

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MicroRNAs (miRNAs) control messenger RNA (mRNA) and have an important role in disease development. Normally, miRNAs bind to the 3´ untranslated region (UTR) of mRNA. However, several studies have demonstrated that miRNAs are capable of regulating other miRNAs, known as a miRNA-miRNA interaction. This is a relatively new area of research and little is known about the impact of these interactions in cancer cells.

To explore this idea, we investigated the role of miR-21, a major oncomiR, in initiating miRNA:miRNA interactions. In cancer cells, miR-21 was over-expressed and KO using an ASO. Cells were then sequenced to understand which miRNAs could be altered in these conditions.

We identified over 100 miRNAs that could be potentially regulated by miR-21. Interestingly, the 17-92a cluster and its members appear to be under the control of miR-21. This observation was evident in a number of different cancer cell lines. Bioinformatic analysis indicates several miR-21 binding sites within the 17-92a precursor sequence. We believe that regulation is post-transcriptional, and miR-21 inhibits miRNA processing. On the premise that other miRNAs can also regulate miRNAs, we examined the frequency of miRNA binding sites (MRE) on all known primary miRNA sequences. The results indicate MREs are prevalent and enrich on specific primary miRNA sequences.

These overall findings suggest that miRNA to miRNA regulation is occurring in cancer cells, and this form of RNA regulation may be more common than first anticipated. We suspect that the mechanism is via the direct binding of the MRE given the enrichment of miRNA sites on primary miRNA sequences.

65 Overlapping transcripts within gene models can influence bioinformatic analysis

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Many bioinformatic pipelines are dependent on existing gene models for quantification and/or annotation. Transcript information of gene models are efficiently compiled within GTF formatted files which enables the reconstruction and interpretation of complex gene structures. In compiling expression patterns of “high confidence heart developmental” genes we came across one gene, GDF1, that had unexpected low signal in many compiled RNA-Seq databases. A closer examination of GDF1 revealed that this gene belongs to a rare class of bicistronic transcripts that are difficult to describe in GTF format as GDF1 exons are shared with the other cistron annotated as CERS1. As GDF1 and CERS1 have near identical exon structure they are interpreted as being overlapping by many bioinformatic pipelines and are therefore often ignored. We therefore re-analysed single cell datasets and have been able to correctly capture the expression patterns of GDF1, which involves a switch from single cistron to bicistronic transcript isoform. For the first time we also identify the key cell types responsible for this expression pattern. Furthermore we compiled a list of other overlapping transcripts that exist within gene models and explore the implications for various bioinformatic pipelines. From this we identified that 10x single cell RNA-Seq bioinformatic pipelines are susceptible for not quantifying reads that align to overlapping transcripts regions and highlight how this could have important implications for downstream analysis.

66 Transcriptome-wide analysis of the closed-loop model of mRNA translation

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A key feature of eukaryotic translation initiation is the 'closed-loop' model, which describes a process by which the ends of an mRNA molecule, namely the 5' cap and the 3' poly(A) tail, are circularised by protein factors eIF4E, eIF4G and PABP in a manner leading to effective translation. Once thought to form in the majority of cases concerning translation of capped, polyadenylated mRNA, the ubiquity and necessity of the closed-loop complex during initiation has more recently been brought into question. One emerging theory is that the closed-loop may represent an additional layer of translation initiation regulation by preferentially forming to varying extents on different mRNAs. As the closed-loop is implicated in a host of processes such as scanning, translational responses to stress and ribosome recycling, a differential propensity for mRNAs to form the closed-loop may subsequently affect their respective translational outcomes.

To investigate whether closed-loop formation may be mRNA-dependent, we have developed an in vivo approach beginning with formaldehyde-crosslinking of Saccharomyces cerevisiae. This is followed by immunoprecipitation of a tagged closed-loop factor (eIF4E, eIF4G or PABP) - and with it the rest of the closed-loop complex and its interacting mRNAs. Coupling this with mild Rnase treatment, we aim to infer the closed-loop status of different mRNAs by the relative association of their 5' and 3' ends to the closed-loop factors. RNA-seq is currently underway to determine the closed-loop formation profiles of different mRNAs across the transcriptome.

By undertaking this transcriptome-wide analysis of closed-loop complex formation, we hope to elucidate whether closed-loop formation is influenced by mRNA-intrinsic features, and whether it represents an additional form of translation initiation control. In performing this study, we anticipate that a better understanding of closed-loop complex formation during translation initiation will aid any future development of translation-targeting therapeutics.

67 Exquisite timing and mechanistic diversity of gene expression changes in response to a prototypical nutritional stress

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The control of mRNA translation into proteins is critical for the adaptation of eukaryotic cells to environmental changes and stress conditions. Early translation-mediated gene expression changes in glucose starvation are critical to trigger the subsequent transcriptional reprogramming, but the mRNAs involved, key mechanisms and mRNA features that dictate their selective regulation remain obscure especially during rapid (20 seconds) and acute (600 seconds) starvation.

We analysed glucose-specific changes to transcriptome composition and mRNA translation during rapid and acute starvation in budding yeast. To this end, we performed conventional RNA-seq using Total cell lysate to assess the level of mRNA turnover and new transcription, Clarified cell lysate evaluating condensation into stress granules and Translating pool for selective translation control. This data presents an in-depth collection of mRNAs involved in responses to glucose starvation and the enrichment of directly relevant gene ontology terms (e.g., glucose transport and synthesis, heat shock or ribosome biogenesis) in co-regulated mRNA groups.

To determine the distribution of diverse ribosomal complexes along mRNA we employed enhanced TCP-seq protocol (Janapala et al. 2021 JoVE) (Horvath, et al. 2022 bioRxiv). We recorded separate footprint data for singular ribosomes, collided di-ribosomes and small ribosomal subunits in different functional states, e.g., those in polysomes or attached as singletons to mRNA, or those associated with a particular translation initiation factor such as eIF4A. TCP-seq data was used to measure the absolute translation output in the form of Stochastic Translation Efficiency (STE) indicating TPI1, PGK1, RPS21A mRNAs as highly translationally up-regulated underpinning translational acceleration alongside re-adjustment of the RNA metabolism to suit new translational demands.

Taken together, this work provides rich new information on the gene regulatory changes and mechanisms at play in response to starvation. Furthermore, it offers an expanded repertoire of TCP-seq approaches useful in investigating gene regulation mechanisms in diverse cellular contexts.

68 RNA base editing using CRISPR-Cas13 for treatment of inherited retinal degenerations

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Gene therapy promises treatment for many diseases currently untreatable. The eye is of particular interest and is at the forefront of gene therapy due to its accessible nature and relative immune privilege. Inherited retinal degenerations (IRDs) are significant contributors to global vision impairment and blindness, with no cure to date. Gene therapy by supplementation is only effective with smaller genes and loss-of-function mutations. Traditional gene editing approaches also target the genome and compromise safety. Alternative therapeutic approaches are therefore required.

CRISPR-Cas base editing allows targeted modification of single bases through deaminases coupled with inactive Cas enzymes. CRISPR-Cas13 allows for safe and reversible RNA-targeted therapies. However, targeting transient RNA requires continuous therapy for clinical benefit. Compact CRISPR-Cas13 enzymes have now been demonstrated to perform efficient and specific RNA base editing. This allows delivery through single-AAV vectors, that are persistent and non-integrating, ensuring continuous therapeutic effect without genomic changes. To date, A→I and C→U edits has been achieved with RNA base editors. The development of single-AAV RNA base editors for safe and long-term treatment of IRDs is thus conceivable.

To validate ocular RNA base editing, we first compared two single-AAV RNA base editors against mCherry to investigate their editing efficiencies. We then introduced our Cas13 base editors into cells expressing a clinically relevant RPE65 mutation to demonstrate RNA base editing in vitro. Finally, to investigate in vivo RNA base editing, we plan to introduce our RNA base editors into rd12 mice, a rapid retinal degeneration model carrying the same mutation in RPE65 to study rescue of phenotype.

Through this study, we aim to establish single-AAV RNA base editing as a robust strategy against IRDs and potentially other genetic diseases. Our successful demonstration of RNA base editing would provide a novel therapeutic approach against genetic disease; one that is efficient, safe, and long-lasting.

69 Short Interfering RNA to treat SARS-CoV-2 Infection

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the causative agent of the COVID-19 pandemic, which has resulted in >481M infections and >6.1M deaths to date. Vaccines targeted to the to immunogenic regions of the virus are highly effective, however several SARS-CoV-2 variants have emerged with increasing vaccine resistance. Additionally, due to limited vaccine uptake/availability and vaccine-non-responders, further treatment options are needed. Several antiviral drugs are now clinically approved, although efficacies vary markedly across variants, with monoclonal antibodies showing dramatically reduced efficacy for newer variants. Short Interfering RNA (siRNA)-based antiviral therapeutics can specifically target multiple regions of the viral genome and provide potential for broad spectrum antiviral drug development. This approach also directly targets the virus and does not require a functional immune response, as well as bypassing immunogenic virus regions that antibody-based approaches are largely limited to. In this study, we describe a panel of antiviral siRNA targeting highly conserved regions of the SARS-CoV-2 genome that showed virus suppression of seven variants (VIC001, Alpha, Beta, Gamma, Delta, Zeta, and Kappa). These siRNA induced significant protection against virus-mediated cell death in vitro, with up to >99% survival (p <0.0001), using a novel cell survival assay. Similarly, some siRNA induced a significant reduction of viral RNA, with up to a 2-4 log reduction, determined via RT-qPCR analysis. Additionally, the lead antiviral siRNA candidates were to antiviral drugs in clinical use (Remdesivir and Sotrovimab) and showed the siRNA induced virus suppression was greater than that provided by the IC50 for both drugs. These results highlight the potential for an antiviral siRNA therapeutic that is broadly effective against multiple SARS-CoV-2 variants, including those which have shown resistance to neutralizing antibodies and antivirals.

70 The RNA editing enzyme ADAR1 in health and disease

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ADAR1 is an enzyme that deaminates adenosine nucleotides to inosine in doublestranded RNA (dsRNA); this, in turn, modifies the sequence, stability, and structure of the RNA. ADAR1 is expressed as two isoforms with distinct functions; a longer ADAR1 p150 (p150) isoform that is mainly expressed in the cytoplasm and inducible in response to stimuli such as viral infection. Also, a shorter isoform, ADAR1 p110 (p110), that is constitutively expressed and restricted to the nucleus where it edits RNA co-transcriptionally, but its unique function remains unknown. ADAR1 has been associated with various pathological states, from severe autoimmune disease to various cancers. Mutations of ADAR1 are one cause of the development of a rare autoimmune disease named Aicardi-Goutières syndrome (AGS). Given that AGS has limited treatments and is incurable, establishing animal models that can mimic this rare inflammatory genetic disorder will advance progress in understanding of the molecular mechanisms of AGS. A well-established function of ADAR1 is to mark endogenous dsRNAs as "self" through A-to-I editing, thus masking them from the innate immune dsRNA sensor melanoma differentiation associated protein 5 (MDA5) and preventing inappropriate immune activation. On the other hand, mouse genetics indicate there is an essential function of the ADAR1 protein, independent of RNA editing, that is important in normal development. For example, my lab (unpublished) has proposed a liver/metabolism-mediated function of the ADAR1 protein in post-natal development that is not dependent on its role in RNA editing. Overall, my PhD project will further assess isoform specific and RNA editing dependent functions of ADAR1. I will also explore a newly identified role for ADAR1 in metabolism that is protein-dependent but RNA editing independent.

71 The RNA modification N6-methyladenosine (m6A) regulates fear extinction by coordinating the activity of a synapse-enriched variant of the lncRNA Malat1

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The localised accumulation of the RNA modification N6-methyladenosine (m6A) is important for the regulation of RNA splicing, stability, transport, and local translation. Recent work suggests that m6A-modified transcripts accumulate in the synaptic compartment, but whether this is directly involved in plasticity underlying learning and memory is not known. We previously found a role for m6A in regulating RNA stability and the formation of fear memory (Widagdo et al, 2016). Here we examined synapse-enriched m6A-modified transcripts in the adult prefrontal cortex during fear extinction learning. We have identified a unique population of m6A-modified RNA at the synapse, including a variant of the long noncoding RNA (lncRNA) Malat1, and discovered that the interaction between Malat1 and RNA binding proteins CRMP2 and CYFIP2 is driven by m6A. In addition, targeted degradation of the m6A-modified Malat1 variant at the synapse led to impaired fear extinction memory. Together, our findings suggest a critical role for localised activity of m6A-modified lncRNA Malat1 in the formation of fear-extinction memory via the functional regulation of key proteins involved in structural plasticity.

72 Pan-cancer analysis of the Breast Cancer 2 protein interactor Partner and Localizer of BRCA2 gene expression for prognostic and immunological applications

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The Partner and Localizer of BRCA2 (PALB2) gene, along with Breast Cancer 1 and 2 (BRCA1 and BRCA2) genes, express proteins forming the BRCA1:PALB2:BRCA2 complex. BRCA1:PALB2:BRCA2 is considered to be indispensable for homologous recombination (HR) and a potent tumour suppressor, largely due to the constituents' role in double-strand DNA break repair. BRCA1:PALB2:BRCA2 mutations cause defects in HR and the checkpoint control of cell cycle, leading to chromosomal abnormalities and malignant potential. Independently on G2/M checkpoint kinase 1 and 2 activity, BRCA1:PALB2 activates the checkpoint, and PALB2:BRCA2 is needed for the checkpoint maintenance. While BRCA1 and BRCA2 are well-studied, PALB2 diagnostic and prognostic features remain under-characterised.Here we performed multi-omic analyses of the PALB2 gene expression and methylation status in carcinogenesis, using publicly available datasets. We observed multiple co-correlations with the expression of other genes in breast, lung, liver and kidney tissues, and indications of abnormal transcriptional PALB2 regulation in malignancy. Based on the data from Human Protein Atlas project, PALB2 protein abundance was higher in all types of cancers except for those of a liver origin. We found the highest PALB2 mRNA abundance in male and Caucasian breast invasive carcinoma of stage 2, lung adenocarcinoma of stages 2, 1 and 3, liver hepatocellular carcinoma of stage 2 and in kidney renal clear cell carcinoma of stage 1. Our results strongly suggest PALB2 expression is higher in stages 2 and 3, but lower in stage 4. We further found that elevated PALB2 expression positively correlates with immune infiltrates, dendritic cells, neutrophils, macrophages, B-cells, CD8+ and CD4+T-cells significantly in multiple cancers including BRCA, KIRC, LUAD, COAD and LIHC. Our findings will assist researchers in understanding the invasive mechanisms and tumour progression in relation to the PALB2 gene function, and will help to utilise its prognostic potential.

73 Evaluating splicing variants without a patient sample: clinical guidelines for in vitro minigene splicing assays.

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Background: Functional testing of RNA is often essential to assess pathogenicity of genetic variants predicted to alter pre-mRNA splicing, though requires a clinical specimen expressing the candidate gene. In cases where an appropriate clinical specimen is unavailable, in vitro splicing studies using gene constructs can enable evaluation of variant pathogenicity. Herein we devise an in vitro minigene construct to evaluate a COL2A1 variant affecting the splice acceptor of intron-52 (NM_001844.5:c.4075-3C>G) associated with Stickler syndrome 1 in three affected individuals, as part of a clinical co-design project to provide recommendations for the design of in vitro splicing reporters for clinical use.

Method: We designed four EGFP-COL2A1 gene fusion expression constructs, comprising EGFP and the final three introns and exons of COL2A1, with different single nucleotide substitutions at c.4075-3. The effect of c.4075-3 substitutions upon pre-mRNA splicing and encoded EGFP-COL2A1 fusion-proteins from transfected Cos7 and HEK cells were characterised by reverse transcriptase PCR (RT-PCR) and western blotting.

Result: Minigene assay demonstrates wild-type c.4075-3C minigene expresses canonically spliced mRNA encompassing EGFP and COL2A1 exons 52-53-54, encoding the full-length EGFP-COL2A1 fusion protein detected by western blot. In contrast, the c.4075-3C>G minigene is predominantly associated with activation of a cryptic acceptor splice-site created by the variant (AC>AG), encoding a premature termination codon. Concordantly, western blotting reveals absence of full-length EGFP-COL2A1 fusion protein in cell lines transfected with the c.4075-3C>G minigene. Clinical utility of these results for classification of the COL2A1 c.4075-3C>G variant was surveyed from SpliceACORD members, and responses informed draft recommendations for technical design of gene constructs for clinical interpretation.

Conclusion: Our in vitro splicing construct is able to evaluate changes in pre-mRNA splicing and protein quantity in the context of a candidate variant and may provide moderate to strong functional evidence toward splicing variant classification in the absence of available clinical specimens.

74 Persistent changes to the transcriptome following abstinence from nicotine or cocaine in a rat model of addiction

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Neural adaptations in the prefrontal cortex of the brain are critical for maintaining cravings across periods of abstinence from drugs of abuse. While drug induced transcriptomic changes have been investigated in response to acute drug administration, it is unclear whether these persist across abstinence. To identify the key genes and pathways that regulate this process, we performed genome wide strand-specific RNA-sequencing to analyse the transcriptional profiles of rats following 1 or 6 days of abstinence from nicotine or cocaine voluntary intravenous self-administration. We found that abstinence from nicotine and cocaine self-administration led to distinct transcriptomic changes in the prefrontal cortex, that were maintained between day 1 and day 6 of abstinence. Further network analysis using WGCNA identified shared and distinct clusters of genes between rats that had self-administered nicotine versus cocaine. Gene ontology analysis of clusters that varied significantly with treatment revealed biological processes important in protein phosphorylation and regulation of MAPK pathways were regulated in extinction of nicotine and cocaine self-administration, and these pathways continued to be regulated in day 6 of abstinence. Further module preservation analysis revealed unique clusters associated with cocaine and nicotine self-administration, abstinence of nicotine self-administration being uniquely associated with ribosomal regulation. In conclusion, abstinence of nicotine and cocaine self-administration lead to long lasting transcriptomic changes, that are maintained across time and therefore may play a role in lasting vulnerability to relapse.

75 The tripartite synapse: non-coding RNAs as major players in memory and the immune system

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The cellular processes underlying the formation of memory share many similarities with the immune system, as both are intricate networks that affect, and are affected, by the body via multiple 'modes' of operation. Indeed, the two systems are functionally integrated, with the brain's immune system helping to regulate synaptic plasticity and memory. At the molecular level, memory and the immune system are functionally integrated by non-coding RNAs (ncRNAs), which have recently emerged as key regulators of cellular activity throughout the phases of memory and in the adaptive immune system. Given that individual ncRNAs can function either independently or cooperatively between the two systems, these molecules are key targets in understanding the cellular processes underlying memory and the immune system. The Vault and Y ncRNA families are small ncRNAs (sncRNAs) that may
functionally intersect memory and the immune system. We have found that the Vault RNA, Vaultrc5, is heavily upregulated at the synapse, and that when knocked down attenuates fear extinction. Conversely, Y RNA isoforms Rny1 and Rny3 appear to be downregulated during fear extinction. Other studies have shown, however, that both are found in extracellular vesicles , suggesting a role in intercellular communication. Given their key role in the immune system, and our unpublished data, this investigation will provide key insights into how memory and the immune system work together.

76 The role of microRNA in the degenerating retina

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microRNA (miRNA) are small non-coding RNA molecules that regulate gene expression post-transcriptionally, by binding target messenger RNA (mRNA) leading to gene repression or mRNA degradation. They are known to be abundant in the central nervous system (CNS), play a key role in regulating inflammation and their dysregulation are involved in progressive retinal degenerations.
It is hypothesised that homeostatic levels of specific miRNAs are natural indicators of neuronal health. Deviation from natural levels of specific circulating miRNA, including known inflammatory miRNA, have also been reported in the blood of patients with retinal degenerations, including late-stage Age-Related Macular Degeneration (AMD).
While miRNA were only identified in humans as recently as the turn of this century, some miRNA-based agents are already in Phase II clinical trials for treatments and diagnosis of disease. This rapid progression from miRNA discovery to drug and biomarker development reflects the effectiveness of miRNA as strong therapeutic and diagnostic targets.
Developing a deeper understanding of the complex miRNA-mRNA networks that directly underlie the pathogenesis of retinal degenerations, are important in identifying a miRNA signature of retinal diseases, as well as developing novel therapeutic interventions and diagnostics.
In this presentation I will discuss a dynamic role of miRNA in maintaining homeostasis in the degenerating retina through extracellular vesicle (EV) communication and the use of miRNA as early biomarkers and therapeutics for AMD.

77 Fast Tracking mRNA production from lab scale to population scale

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Many mRNA vaccines and therapies have rapidly progressed from pre-clinical leads to population scale vaccines or therapies at rates never seen before. This has created unique challenges for translational researchers, manufacturing process developers, manufactures and regulators. The mRNA manufacturing processes described in this poster have been developed to progress from pre-clinical to commercial scale mRNA within a fast tracked time period and matched with the availability of capital at each stage. We've started with the end in mind - cGMP population scale manufacturing. We've standardised the technology and replicated workflows to enable rapid scale-up/scale-out and technology transfer from pre-clinical, clinical and cGMP population scale manufacturing.

78 METTL3-dependent m6A RNA methylation regulates inflammasome activation

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N6-methyladenosine (m6A) that results from methylation of adenosine residues by the METTL3-METTL14 methyltransferase complex mediates essential processes on RNA including splicing, mRNA decay and translation. In recent work, m6A has emerged as a key regulator of the immune response. However, whether METTL3-mediated RNA m6A methylation is involved in the activation and regulation of the NLRP3 inflammasome remains unknown. The NLRP3 inflammasome is a cytosolic sensor that upon recognition of danger signals fires the innate immune response. NLRP3 mediates caspase-1-dependent maturation and release of the pro-inflammatory cytokines IL-1b and IL-18 and lytic cell death. While a broad range of pathogens can activate the NLRP3 inflammasome, in most cases, it appears to be dispensable to fight infection. However, NLRP3 activation has been identified as a driver of numerous inflammatory disorders. Because of this, considerable efforts have been invested to understand the molecular mechanisms behind the NLRP3 pathway. In this study, we discovered that METTL3 is essential for activation of the NLRP3 inflammasome in vitro and in vivo. METTL3 depletion impaired inflammasome activation in cultured mouse and human primary cells. Furthermore, METTL3 ablation in the myeloid compartment rendered mice resistant to NLRP3-mediated endotoxic shock. Mechanistically, we identified NLRP3 amongst METTL3 targets in macrophages, and found that METTL3 regulates NLRP3 gene expression at two levels: i) co-transcriptionally, promoting H3K9me2 demethylation nearby NLRP3 promoter; and ii) promoting NLRP3 translation via the action of the m6A reader protein, YTHDF1. Our work places m6A-mediated regulation of gene

expression at the centre of immunity and brings forward METTL3 and YTHDF1 as novel potential targets for therapeutic intervention in inflammation-related disorders.

79 Significant and pervasive effects of RNA degradation on Nanopore direct RNA sequencing

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Background:
Oxford Nanopore's direct RNA sequencing (DRS) is a powerful tool capable of sequencing complete RNA molecules and accurately measuring both gene and isoform expression levels. However, as DRS is designed to profile intact native RNA the precision and reliability of gene and isoform quantification may be more heavily dependent upon RNA integrity, (as measured by RIN), than other RNA-seq methodologies. Currently it is unclear what range of RNA integrity DRS can be applied to and if the impact of degradation can be corrected for. RNA from in-vivo or clinical samples have often undergone some degradation, therefore, to allow application of DRS to the widest range of suitable samples it is essential to determine the appropriate range of RIN for DRS.

Results: To determine the impact of RNA degradation on DRS we performed a degradation timecourse, sequencing SHSY5Y neuroblastoma samples where the RNA was allowed to degrade prior to RNA extraction. Our data demonstrate that degradation is a significant and pervasive factor that can bias downstream analysis. This includes a reduction in library complexity resulting in an overrepresentation of short genes and isoforms. Increased gene and isoform length were also closely correlated with rate of degradation, with DNA binding proteins and transcription factors showing the most degradation susceptibility. Degradation also biases differential expression analyses; however, we find explicit correction can almost fully recover meaningful biological signal. Overall, we find samples with RIN greater that 9.6 can be treated as undegraded and samples with RIN greater than 7 can be utilised for DRS with appropriate sample correction.

Conclusions: These results serve to inform the use of DRS by providing a framework for modelling samples impacted by degradation. In so doing DRS can be appropriately applied across a variety of sample RINs whilst limiting the confounding effect of degradation on downstream analysis.

80 Exploring the dynamic transcriptomic m5C modification landscape in post-mitotic neurons and its essential role during neuronal activation

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To date, more than 140 RNA base modifications have been identified although very few have been functionally characterized in the brain. For example, cytosine methylation (m5C) has recently been shown to regulate mRNA translocation, stability, and translation in mammalian cells; however, the function of this novel mRNA modification in the context of neuronal plasticity has yet to be explored. To begin to address this, we performed transcriptome-wide single-nucleotide RNA m5C sequencing on activated primary neurons, in vitro. Overall, we found that m5C-modified mRNA increased in response to neuronal depolarization, with a preference for the accumulation of m5C within the 5'UTR. Moreover, upon closer analysis of the 8310 m5C sites that were unique to neural activation a GO analysis revealed that this RNA modification was associated with genes primarily involved in synaptic activity. Our data imply a functional role for experience-dependent regulation of RNA by m5C, potentially through direct effects on RNA localization.

81 Evolution of circular RNAs in primates

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Circular RNAs (circRNAs) are produce by many primate genes. Nonetheless, their evolutionary conservation it is not know. In this study, we compared tissue-specific transcriptome across eight primates species with a divergence of 70 million years. circular RNAs (circRNAs) are produce by many primate genes. Nonetheless, their evolutionary conservation it is not know. In this study, we compared tissue-specific transcriptome across eight primates species with a divergence of 70 million years.

82 HPV16 E6 and E7 oncogenes can alter the expression of SREBF2 and its intronic miR-33a in Oropharyngeal cancers

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Human papillomavirus (HPV), notably type 16, is a risk factor for up to 90% of oropharyngeal carcinomas (OPC). It has been demonstrated that small non-coding RNAs known as microRNAs play a vital role in the cellular transformation process. The high-risk variant HPV16 encodes for two main oncogenes, E6 and E7, which are consistently expressed in cancer cells and are necessary for induction and maintenance of the transformed state. The aim of this study was to determine the impact of HPV16 E6/E7 on miRNA expression in OPC.

In this study, we used an LNA array to further investigate the impact of HPV16 on the expression of microRNAs in oropharyngeal cancer. From this, we identified miR-33a to be the most upregulated miRNAs in HPV16+ OPC. We also developed an HPV-miRNA interactome to visualise novel mechanistic links between the HPV16 viral oncogenes and the miRNAs. Our interactome analysis revealed miR-33a to be encoded within the intron of the Sterol regulatory binding factor 2 (SREBF2), which is under the regulation of several transcription factor directly target by HPV16 E6/E7. We show that both miR-33a and SREBF2 were significantly upregulated in HPV16+OPC patients and in cell lines and correlation analysis suggested concomitant expression for miR-33a and SREBF2. When E6 and E7 are depleted, SREBF2 and miR-33a were reduced. Interestingly, when SREBF2 is KO by siRNAs, miR-33a is not affected. This may suggest that miR-33a maturation is not linked to SREBF2 turnover.

In this study, we demonstrated constructed a HPV16 viral miRNA interactome to identified possible mechanistic pathways. From this, we showed that E6 and E7, could influence the expression of SREBF2 and miR-33a in OPC, leading to their upregulation. This approach can be used to further our understanding of how viruses can affect the miRNA milieu and how these interactions can drive viral oncogenesis.

83 Decoding the language of cells: Using RBC EV-miRNA supplementation as therapeutic for retinal degenerations

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Cells have a molecular-language that we are only now starting to decipher, and which changes as we age. Cells communicate by transferring molecular cargo, including small gene regulators called microRNA (miRNA), using nanosized delivery vehicles called extracellular vesicles (EV). As we age, there is loss of EV-mediated miRNA communication, leading to the progression of neural degenerations, including Age-related Macular Degeneration (AMD). We hypothesise that supplementation of essential molecular cargo (including miRNA) can restore lost cellular communication and slow the progression of AMD. We propose to use autologous-sourced EV from red blood cells (RBC-EV) as delivery vehicles of these essential retinal EV-miRNA and develop a novel gene therapy for AMD.

RBC-EV (40-200nm) were isolated using differential ultracentrifugation and characterised using nanoparticle tracking analysis (NanoSight NS300), cryogenic electron microscopy and western blotting. RBC-EV were electroporated (GenePulser II) with individual miRNA cargo. Electroporation conditions including voltage, pulse, buffer, and temperature were optimised to achieve efficient loading of miRNA into RBC-EV, which was quantified using Qubit™ microRNA assay. EV loaded with miRNA were transfected into 4 immortalised retinal cell lines and uptake efficiency was analysed using Fluorescence Live cell imaging (Incucyte®) and Flow cytometry.

Electroporation conditions of 300 volts and 2 pulses at 125 µF, in Gene Pulser® Electroporation Buffer with 37° incubation for 1 hr post-electroporation produced optimal loading efficiency, achieving 2 µg miRNA into 1x10^11 RBC-EV, at 70% EV survival rate post-electroporation. Using live-cell imaging and flow cytometry, we observed 661W photoreceptor cell-line to uptake RBC-EV most efficiently, quantified as 88% (4hr post-transfection). In-vivo, we show uptake of RBC-EV by retinal cell types 1-48hr post-intravitreal injection.

We have successfully demonstrated that RBC EV loaded with miRNA can be safely and efficiently transfected into retinal cell types in-vitro and in-vivo. Therefore, we can use RBC-EV for packaging and delivering therapeutic-miRNA during retinal degeneration.

84 Quantification of native nucleic acid integrity from nanopore signals and control of the directional coverage bias

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Long-read sequencing technologies have brought opportunities to observe the near-complete diversity of nucleic acid forms close to their native state. Direct nanopore sequencing further eliminates copying biases and captures in vivo nucleotide chemistry, including modifications. To fully leverage the advantages of direct nanopore sequencing, we must account for any remaining coverage and signal under-representation biases and control the integrity and complexity of the samples. RNA is landmarked by vulnerability to (sequence-specific) chemical and enzymatic attacks, which often complicate or obstruct accurate discovery of functionally and medically relevant RNA features.

Here, we show that coverage bias is typical to DRS experiments. We demonstrate transcript-specific underrepresentation of the functionally important 5' Untranslated Regions (UTRs) and 5'-proximal Open Reading Frame (ORF) regions. We find that native RNA degradation profile, structure and modification-associated stalls, coupled with the 3' to 5' directionality of RNA traversal during nanopore sequencing, underlie DRS coverage bias, limiting the application of DRS to study sequence and RNA processing variations in the upstream parts of transcripts.

To quantify and control the directional coverage effects, we developed nanograd. Nanograd quantifies per-transcript sequence coverage bias and overall library complexity from DRS data, providing a single Direct Integrity Number (DIN). DIN enables seamless, standardised comparison across experiments, libraries and flowcells. Nanograd can be used on any machine with a single command and is applicable in real-time to verify and visualise library quality during sequencing. Nanograd further provides granular data to assess sample complexity and heterogeneity of the RNA degradation transcript-wise, and to segregate true RNA chain termination events from the structure-induced translocation stalls. We discuss DIN and persistent length-dependent biases in the context of sequencing depth of upstream RNA features, highlighting the necessity of DIN coverage correction in identifying key features of RNA such as splice junctions, structural feature marks, and chemical RNA modifications.

85 Investigating how glucose starvation stress condition affects mRNA closed-loop formation transcriptome wide in yeast

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Background: The “closed-loop” mRNA is now a widely accepted model for translation initiation. It is formed through interactions between the 5’ cap, poly(A) tail, PAB (poly(A) binding protein), eIF4E (eukaryotic initiation factor 4E) & eIF4G. In short, the mRNA closed-loop not only stabilises the whole translation initiation complex but also allows ribosome recycling within the same mRNA, which leads to stable and efficient translation. However, there are emerging studies which suggest not all mRNAs form the closed-loop, and the conditions required for the formation of closed-loop structures are also not well- In particular, the role of the closed-loop during global translation inhibition is poorly understood. As glucose starvation in yeast is a well-characterised model of translation inhibition, it will be utilised here to study the role of the closed loop during translation inhibition.

Methods: Here, we will use protein A-tagged yeast strains to immunoprecipitate closed-loop core components (PAB, eIF4E or eIF4G) and use western blotting to investigate whether these components still bind to each other under glucose starvation. For the mRNAs that were pulled down with the closed-loop factors, we will apply limited RNase treatment followed by RT-qPCR to quantify the abundance of 5' and 3' ends of those mRNAs attached to the closed-loop core components, in order to study the extent of closed-loop mRNA under glucose starvation. RNA-seq will then expand this study transcriptome-wide.

Expected outcome: We hypothesize that yeast cells can still produce closed-loop mRNAs under glucose starvation conditions, with the possibility for the production of these closed-loops to also be mRNA specific. Also, RNA-seq will be able to demonstrate the formation and specificity of the closed-loop on a transcriptome-wide range. Combined with further experiments and analysis of databases, we hope to elucidate the specific role of closed-loop mRNAs under glucose starvation conditions.

86 Nuclear Export of Circular RNA

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Circular RNAs (circRNAs), which are increasingly being implicated in a variety of functions in normal and cancerous cells, are formed by back-splicing of precursor mRNAs in the nucleus. circRNAs are predominantly localized in the cytoplasm, indicating that they must be exported from the nucleus. Here, we uncover a pathway specific for nuclear export of circular RNA. This pathway requires Ran-GTP, Exportin-2 and IGF2BP1. Enhancing the nuclear Ran-GTP gradient by depletion or chemical inhibition of the major protein exporter, CRM1, selectively increases nuclear export of circRNAs, while reducing the nuclear Ran-GTP gradient selectively blocks circRNA export. Depletion or knockout of Exportin-2 specifically inhibits nuclear export of circRNA. Analysis of nuclear circRNA binding proteins reveals that interaction of IGF2BP1 with circRNA is enhanced by Ran-GTP. Formation of circRNA export complexes in the nucleus is promoted by Ran-GTP through its interactions with IGF2BP1, Exportin-2 and circRNA. Our findings demonstrate that adaptors such as IGF2BP1 that bind directly to circular RNAs recruit Ran-GTP and Exportin-2 to export circRNAs in a mechanism analogous to protein export, rather than mRNA export.

87 The novel long noncoding RNA lincNeur promotes neuroblastoma by up-regulating E2F1 and Aurora A mRNA as well as Myc protein expression

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Background: Neuroblastoma is the most common solid tumor in early childhood, and accounts for approximately 15% of childhood cancer death. We have recently analyzed RNA sequencing data from 493 human neuroblastoma tissues, and identified the long noncoding RNA lincNeur as one of the transcripts, high expression of which most considerably correlated with poor prognosis in both MYCN oncogene-amplified and MYCN non-amplified neuroblastoma patients.

Aims: To determine the tumorigenic role of lincNeur in neuroblastoma, to identify the mechanism of action through which lincNeur exerts oncogenic function, and to screen for small molecule compound inhibitors of lincNeur.

Methods and results: We performed RNA-binding protein pull-down assay and RNA-immunoprecipitation assay. eIF4B was identified and validated as one of the binding proteins of lincNeur. We also identified down-stream targets of lincNeur via genome-wide differential gene expression analysis. E2F1 and Aurora A were identified as among the transcripts significantly down-regulated after lincNeur knockdown. RT-PCR and western blot analysis showed that lincNeur knockdown reduced E2F1 and Aurora A mRNA and protein expression, and reduced N-Myc and c-Myc protein but not mRNA expression. In addition, lincNeur knockdown reduced, while over-expression of lincNeur promoted, neuroblastoma cell proliferation. eIF4B knockdown also reduced cell proliferation, E2F1 and Aurora A mRNA and protein expression. Mouse xenograft model experiment showed that lincNeur knockdown suppressed neuroblastoma tumor progression in vivo. Importantly, we are screening small molecule compound libraries for inhibitors of lincNeur and eIF4B interaction for neuroblastoma therapy.

Conclusions: The novel long noncoding RNA lincNeur promotes neuroblastoma by interacting with eIF4B to up-regulate E2F1 and Aurora A mRNA and protein, as well as N-Myc and c-Myc protein expression. Small molecule compound inhibitors of lincNeur and eIF4B interaction is a promising therapeutic approach.

88 Inhibiting circRNA CDR1as expression in the ILPFC of adult male C57BL/6J mice impairs fear extinction memory

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Circular RNAs (circRNAs) comprise a novel class of regulatory RNAs that are abundant in the brain, particularly within synapses. They are highly stable, dynamically regulated, and display a range of functional roles, including as decoys for miRNAs and proteins and, in some cases, translation. Early work in animal models revealed an association between circRNAs and neurodegenerative and neuropsychiatric disorders; however, no studies have shown a direct link between circRNA function and memory to date. To address this knowledge gap, we sequenced circRNAs in the synaptosome compartment of the medial prefrontal cortex of fear extinction-trained male C57BL/6J mice, and found 12838 circRNAs enriched at the synapse, including CDR1as. Targeted knockdown of CDR1as in neural processes of the infralimbic prefrontal cortex of male C57BL/6J mice led to impaired fear extinction memory. Altogether, our findings highlight the importance of localised circRNA activity at the synapse for memory formation and suggest that circRNAs may have a more widespread effect on brain function than previously thought.

89 Enhanced RNAi stability through imperfect inverted repeats: nucleotide mismatches prevent intrinsic self-silencing of hairpin RNA transgenes in plants

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Hairpin RNA (hpRNA) transgenes, with a perfect inverted-repeat (IR) DNA, have been the most successful RNA interference (RNAi) method in plants. We found that hpRNA transgenes were invariably methylated in the IR DNA and the adjacent promoter, causing transcriptional self-silencing and preventing the full potential of RNAi. We demonstrated that nucleotide substitutions in the sense sequence, which disrupts the perfect IR DNA structure, were sufficient to prevent the intrinsic DNA methylation resulting in more uniform and persistent RNAi. Substituting all cytosine (C) with thymine (T) nucleotides, in a G:U hpRNA design, prevents DNA methylation and self-silencing but still allows for the formation of perfect hpRNA due to G:U wobble base-pairing. The G:U design induces effective RNAi in 90-96% of transgenic lines, compared to 57-65% for the traditional hpRNA design. Furthermore, while a traditional hpRNA transgene shows increasing DNA methylation and self-silencing from cotyledons to true leaves, the G:U transgenes avoids this developmental progression of self-silencing and induced RNAi throughout plant growth. The G:U and traditional hpRNAs are differently processed, generating siRNAs and dsRNA intermediates with different characteristics, which appear to function through different pathways to induce RNA-directed DNA methylation.

90 Separation-of-function mutations reveal that the RNA-binding surface of PRC2 is required for H3K27me3 deposition to chromatin independently of RNA

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RNA binds to a number of chromatin modifiers, such as the histone methyltransferase polycomb repressive complex 2 (PRC2). There is still no consensus on how RNA regulates the canonical functions of PRC2, including the introduction of the repressive H3K27me3 chromatin mark and the maintenance of transcription programs in lineage-committed cells.

To investigate how RNA regulates PRC2, we assayed two separation-of-function PRC2 mutants, both defective in binding to RNA but retaining methyltransferase activity. Our in vitro enzymatic assays confirmed that the two RNA-binding defective PRC2 mutants indeed retain methyltransferase activity against naked isolated H3 histone substrates, in agreement with previous studies by us [1] and others [2]. However, we found that only one of the mutants is defective in the histone-methylation of naïve chromatin in vitro. Knockout with rescue experiments in lineage-committed human cells, combined with CUT&Tag and RNA-seq, indicates that the mutants in cells mimic their in vitro activities: the PRC2 mutant that was defective in both RNA binding and chromatin modification in vitro phenocopied a catalytically dead PRC2, whereas the true separation of function PRC2 mutant—defective in RNA binding while active in chromatin modification in vitro—resembles the wildtype PRC2 in lineage-committed cells.

Mechanistically, part of the RNA-binding surface of PRC2—rather than the RNA-binding activity per se—is required for the histone methylation of chromatin in vitro and in cells. While previous studies showed that RNA antagonises the interactions between PRC2 to nucleosomes [3], our data provides a mechanistic explanation for this process: during chromatin modification, nucleosomal DNA interacts with a surface of PRC2 that is otherwise available to bind RNA.

91 Structural and functional studies of lncRNA THOR (Testis-associated Highly conserved Oncogenic long non-coding RNA)

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LncRNA THOR is a recently discovered lncRNA that displays strong evolutionary conversation among human, mouse and zebrafish, and is exclusively expressed in testis in healthy human individuals [1]. In contrast, overexpression of THOR is commonly observed in a broad range of carcinomas, including liver, ovarian, lung and colorectal cancer cells. THOR is an oncogenic lncRNA and it promotes cell proliferation, possibly through mediating IL-6/STAT3 signalling pathway [2]. However, a low number of studies on THOR's structure, function and interactome within cells limited further understanding of this lncRNA. We aim to approach this novel lncRNA THOR through both structural and functional perspectives. Here, we present the structure of THOR determined through SHAPE-MaP method, and found that this lncRNA interacts with IGF2BPs, which stabilises mRNAs of other proliferating factors, suggesting a potential oncogenic mechanism of THOR in cancer progression.

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