16TH ASIAN CONFERENCE ON TRANSCRIPTION

2019

1-4 December

St David Lecture Theatre, Dunedin, New Zealand

CONFERENCE HANDBOOK 2019

Welcome

In Dunedin 2019, the Asian Conference on Transcription will include cutting-edge research in genetic and epigenetic mechanisms of gene transcription in prokaryotes and eukaryotes, chromatin structure, cancer biology, antimicrobial resistance, developmental biology, and genomics.

We can't wait to welcome you to beautiful Dunedin, New Zealand, in December 2019 for an exciting meeting on all things transcriptional!

- Julia Horsfield and Justin O'Sullivan
Convenors, 16th Asian Conference on Transcription 2019

Local Organising Committee

Julia Horsfield, University of Otago, Dunedin NZ

Justin O'Sullivan, University of Auckland, NZ

Rebecca Oliver, University of Otago, Dunedin NZ

Sally Boult, Events 4 You Limited (Secretariat)

International Organising Committee (IOC)

Australia	Joel MACKAY
China	WANG Gang
India	Tapas KUNDU
Japan	NAKASONE Kaoru
Korea	CHOY Hyon El
Malaysia	Kalai MATHEE
New Zealand	Julia HORSFIELD
Singapore	Reshma TANEJA
Taiwan	James C. K. SHEN
Thailand	Mayuree FUANGTHONG

International Scientific Committee (ISC)

Australia	WONG Lee
China	SONG Xiaoyuan
India	Jayanta MUKHOPADHYAY
Japan	TSUGE Kenji
Korea	CHO Eun Jung
Malaysia	Nethia M.-KUMARAN
New Zealand	Justin O'SULLIVAN
Singapore	YAN Jie
Taiwan	YEN Betty Linju
Thailand	Nisanart (Puk CHAROENLAP

Contents

Wifi / Internet

Wireless internet: UO_Guest

Follow the instructions

(You will need to create a user name and password)

Message from the SOC Chair

Welcome to Dunedin for ACTXiV. It has been a long road to walk in a very short time to organize this meeting in the deep south of New Zealand. I have to thank my co-organizer Julia Horsfield, Rebecca Oliver, Amarni Thomas and Sally Boult – who really were the local organizing committee - for all their tireless work in making this conference a reality. There were times when it seemed we wouldn't get to the finish line but we made it and what a line-up we have secured.

We have more than 80 delegates attending from around the world – all of them leaders in their fields, here to share their latest results, get constructive feedback, see old friends, make new friends and develop new collaborations.

Thanks to the international and scientific committees for your comments and suggestions. Thanks to the sponsors – your support is invaluable in an endeavour like this - without it our science would be so much more limited. Thanks to you the participants, this conference reflects you and your interests.

I hope that you take full advantage of the beautiful environment that the St David Lecture Theatre complex, nestled by Dunedin's Water of Leith, as you reflect on and co-develop new scientific endeavours. Let's begin – enjoy the meeting!

Sincerely

Justin M. O'Sullivan

Sponsors

Silver Sponsors

GENESEARCH
eFREEZER

LAB SUPPLY

NEW ENGLAND BioLabs®

Humanizing Genomics
macrogen
OCEANIA

Supporting Sponsors

genetics otago

MAURICE & PHYLLIS PAYKEL TRUST

MAURICE WILKINS CENTRE
FOR MOLECULAR BIODISCOVERY

Programme

Preliminary Programme

(as at 4 Nov 2019 - may be subject to change)

Date	Session	Location
Sunday 1 December		**Main Conference Room** (unless otherwise stated)
1.00 pm	**Registration Opens**	
2.00 pm – 5.00 pm	**Dunedin Walking Tour** (optional)	Starts at Dunedin i-SITE Visitor Information Centre, 50 The Octagon
5.00 pm – 5.30 pm	**Official Welcome /Mihi whakatau/ Kapa Haka**	
5.30 pm – 6.15 pm	**Keynote: Leonie Quinn** The John Curtin School of Medical Research, The Australian National University, Canberra *To bind RNA or DNA? The multifaceted functions of DNA/RNA binding proteins in stem cell fate, development and disease* **Chair:**	Sponsored by: NEW ENGLAND BioLabs GENESEARCH e*FREEZER
6.15 pm – 7.15 pm	**Welcome Function:** Drinks and Canapes	Trade Exhibition Area

Date	Session	Location
Monday 2 December		
8.00 am – 8.30 am	**Registration**	
8.30 am – 8.45 am	**Welcome/Housekeeping**	
8.45 am – 9.30 am	**Keynote: Susan Clark** Garvan Institute of Medical Research, Sydney, Australia *Reprogramming of the cancer genome and epigenome in 3D* **Chair:**	
	DNA methylation	**Chair:**
9.35 am – 9.50 am	**Aniruddha Chatterjee,** University of Otago, Dunedin, NZ *Methylome and transcriptome programme segregates PD-L1 expressing and non-expressing melanoma* **1129**	
9.50 am – 10.05 am	**Amy Osborne,** University of Canterbury, NZ *Genome-wide DNA methylation analysis in the Christchurch Health and Development Study reveals potential for epigenetic effects of cannabis use on pathways involved* **1145**	
10.05 am – 10.20 am	**Tim Hore,** University of Otago, Dunedin, NZ *Understanding epigenetic memory in the germline and during sex determination* **1102**	
10.30 am – 11.00 am	**Morning Tea**	Trade Exhibition Area
	Chromatin control of transcription I **Chair**	**Main Conference Room** (unless otherwise stated)
11.00 am – 11.15 am	**Ping Gao,** University of Science & Technology of China *cMyc-mediated epigenetic regulation of cancer metabolic reprogramming* **1143**	
11.15 am – 11.30 am	**Tracy Hale,** Massey University, NZ *Heterochromatin Protein 1Î± interacts with parallel RNA and DNA G-quadruplexes* **1093**	
11.30 am – 11.45 am	**JHA Sudhakar,** Cancer Science Institute of Singapore *TIP60 keeps the endogenous oncogenic demons repressed* **930**	
11.45 am – 12.00 pm	**Helen Fitzsimons,** Massey University, NZ *Investigating nuclear and cytoplasmic roles of HDAC4 in the brain* **1094**	

Date	Session	Location
Monday 2 December *continued*		
12.00 pm – 12.15 pm	**Lynette Brownfield,** University of Otago, Dunedin, NZ *Male germline specification in flowering plants* **1105**	
12.15 pm – 12.30 pm	**Sheri Johnson,** University of Otago, Dunedin, NZ *Paternal hypoxia exposure primes offspring for increased hypoxia resistance* **1131**	
12.30 pm – 12.45 pm	**Melanie Laird,** University of Otago, Dunedin, NZ *Epigenetic reprogramming of the brushtail possum (Trichosurus vulpecula) germline* **1109**	
12.45 pm – 1.00 pm	**Megan Wilson,** University of Otago, Dunedin, NZ *Epigenetic regulation of Botrylloides leachii whole body regeneration* **1092**	
1.00 pm – 2.00 pm	**Lunch**	Trade Exhibition Area
	RNA and transcription	**Chair:**
2.00 pm – 2.15 pm	**Paul Gardner,** University of Otago, Dunedin, NZ *Protein expression is controlled by the accessibility of translation initiation sites* **1146**	
2.15 pm – 2.30 pm	**Sarah Diermeier,** University of Otago, NZ *Long non-coding RNAs as regulators of gene expression in cancer* 1100	
2.30 pm – 2.45 pm	**Xiaoyuan Song,** University of Science & Technology of China *The function of lncRNAs in brain aging* **1166**	
2.45 pm – 3.00 pm	**Robert Weatheritt,** Garvan Institute of Medical Research, Sydney, Australia *The transcriptional and co-transcriptional mechanisms that regulate novel exon acquisition* **1157**	
3.00 pm – 3.15 pm	X**iangting Wang,** USTC, China *LncRNA 887L regulates tumor progression via transcriptional activationÂ of CA9* **1150**	
3.15 pm – 3.30 pm	**Chun Shen Lim,** University of Otago, Dunedin, NZ *Improving the expression and solubility of recombinant proteins by smart sequence design algorithms* **1152**	
3.30pm – 4.00 pm	**Afternoon Tea**	Trade Exhibition Area

Date	Session	Location
Monday 2 December ***continued***	**Student/Early Career Researcher Awards Session** Sponsored by:  	**Main Conference Room** (unless otherwise stated)
	3 minute Quick Fire Poster Presentations	**Chair:**
4.00 pm – 4.05 pm	**Sophie Farrow**, Liggins Institute, NZ *A Novel Insight into the Regulation of GBA in Parkinson's Disease* **1138**	
4.05 pm – 4.10 pm	**Sreemol Gokuladhas,** The University of Auckland *Shared regulatory pathways reveal novel genetic correlations between grip strength and neuromuscular disorders* **1139**	
4.10 pm – 4.15 pm	**Sarah Inwood**, University of Otago, NZ *Dual-species transcriptomics to investigate parasitism resistance in a classical biological control system* **1098**	
4.15 pm – 4.20 pm	**Chanshin Kang,** Seoul National University, South Korea *CHD Family have Distinct Translocation Dynamics on Nucleosome Complex* **1159**	
4.20 pm – 4.25 pm	**Damien Muckle,** Australian National University *FUBP1/Psi functions in the niche to non-autonomously control neural stem cell fate* **1164**	
4.25 pm – 4.30 pm	**Stephanie Workman,** The University of Otago, NZ *Haploinsufficiency of LIM Homeobox 9 (Lhx9) during genital ridge development impacts ovarian function and infertility* **1097**	
4.30 pm – 4.35 pm	**Olga Zaytseva,** John Curtin School of Medical Research, Australia *Psi, the Drosophila ortholog of the FUBP1 single stranded DNA binding protein, fine-tunes transcription of developmental patterning genes* **1126**	
4.35 pm – 4.40 pm	**Eunho Song,** Seoul National University, South Korea *RNAP-bound factor Rho finely regulates premature transcription termination in prokaryote* **1122**	

Date	Session	Location
Monday 2 December *continued*		
4.40 pm – 4.45 pm	**Gunhyoung Lim,** Seoul National University, South Korea *Single molecule studies on R-loop formed during transcription* **1113**	
4.45 pm – 4.50 pm	**Jurairat Chittrakanwong,** Chulabhorn Royal Academy, Thailand *Characterization of TrmA on oxidative stress response in Pseudomonas aeruginosa* **1133**	
4.50 pm – 4.55 pm	**Rebecca Clarke,** University of Otago, NZ *Why heal when you can regenerate? Whole body regeneration in Botrylloides leachii* **1107**	
4.55 pm – 5.00 pm	**Sulagna Banerjee,** University of Otago, NZ *Using Xenous laevis tadpole as a model for testing anti-inflammatory drugs as treatment for intractable epilepsy* **1095**	
5.00 pm – 5.05 pm	**Sarada Ketharnathan,** University of Otago, NZ *Subfunctionalization of cohesin STAG1/2 in zebrafish development and disease* **1169**	
5.05 pm – 5.10 pm	**Lana Ly,** University of New South Wales, Australia *The role of ZBTB7A in foetal globin repression* **1172**	
5.10 pm – 5.15 pm	**Manan Shah,** University of New South Wales, Australia *WDR5 is a novel partner of KLF3 and is important in KLF3 genomic localisation and gene regulation* **1173**	
5.15 pm – 7.15 pm	**Poster Session:** Drinks and Nibbles	Trade Exhibition/ Poster Area

Poster Display (in #ID order)		
ID	**Title**	**Presenting Author**
1095	*Using Xenous laevis tadpole as a model for testing anti-inflammatory drugs as treatment for intractable epilepsy*	**Sulagna Banerjee**
1097	*Haploinsufficiency of LIM Homeobox 9 (Lhx9) during genital ridge development impacts ovarian function and infertility*	**Stephanie Workman**
1098	*Dual-species transcriptomics to investigate parasitism resistance in a classical biological control system*	**Sarah Inwood**
1107	*Why heal when you can regenerate? Whole body regeneration in Botrylloides leachii*	**Rebecca Clarke**
1113	*Single molecule studies on R-loop formed during transcription*	**Gunhyoung Lim**
1115	*Structural study on prokaryotic transcription - how RNA polymerases pause and go*	**Jin Young Kang**
1122	*RNAP-bound factor Rho finely regulates premature transcription termination in prokaryote*	**Eunho Song**
1126	*Psi, the Drosophila ortholog of the FUBP1 single stranded DNA binding protein, fine-tunes transcription of developmental patterning genes*	**Olga Zaytseva**
1127	*High-energy initiation complex retaining o70identified as complex hypersensitive to pyrophosphate*	**Nobuo Shimamoto**
1132	*Genome-wide DNA methylation and gene expression analysis of non-invasive and invasive melanoma cell lines*	**Michael Eccles**
1133	*Characterization of TrmA on oxidative stress response in Pseudomonas aeruginosa*	**Jurairat Chittrakanwong**
1138	*A Novel Insight into the Regulation of GBA in Parkinson's Disease*	**Sophie Farrow**
1139	*Shared regulatory pathways reveal novel genetic correlations between grip strength and neuromuscular disorders*	**Sreemol Gokuladhas**
1144	*RNA polymerase can reinitiate at opposite direction promoter by hopping after intrinsic termination*	**Wooyoung Kang**
1154	*SoDoPE: Soluble Domain for Protein Expression*	**Bikash Bhandari**
1155	*E2A-PBX1 functions as a coactivator for RUNX1 in acute lymphoblastic leukemia*	**Wen-Chieh Pi**
1159	*CHD Family have Distinct Translocation Dynamics on Nucleosome Complex*	**Chanshin Kang**
1160	*Drosophila models reveal FUBP1 and CIC, predicted oligodendroglioma driver mutations, inhibit expansion of the neural stem cell lineage in vivo*	**Nan-Hee Kim**
1161	*Highly accessible translation initiation sites are predictive of successful heterologous protein expression*	**Chun Shen Lim**

Poster Display (in #ID order)		
ID	**Title**	**Presenting Author**
1164	*FUBP1/Psi functions in the niche to non-autonomously control neural stem cell fate*	**Damien Muckle**
1168	*Characterisation of zebrafish meiotic cohesin subunits and their roles in gametogenesis and fertility*	**Stephanie Lee**
1169	*Subfunctionalization of cohesin STAG1/2 in zebrafish development and disease*	**Sarada Ketharnathan**
1170	*Dysregulated transcriptional response to differentiation signals in cohesin-mutant leukemia cells*	**Jisha Antony**
1172	*The role of ZBTB7A in foetal globin repression*	**Lana Ly**
1173	*WDR5 is a novel partner of KLF3 and is important in KLF3 genomic localisation and gene regulation*	**Manan Shah**
1175	*A synthetic lethal drug screen identifies exploitable vulnerabilities in cohesin-deficient cells*	**Chue Vin Chin**
1178	*Decoding the multimorbidities among psychiatric disorders and cognitive*	**Evgeniia Golovina**
1180	*Pericentromeric heterochromatin clustering is impaired in muscle atrophy*	**Hongmin Lee**
1182	*Good genes gone bad: are placental genes hijacked by cancer cells to facilitate invasion?*	**Chi Lynch-Sutherland**
1183	*Analysis of Menin in breast cancers reveals its role in cancer epigenome regulation*	**Taewan Kim**

Date	Session	Location
Tuesday 3 December		**Main Conference Room** (unless otherwise stated)
8.30 am – 9.15 am	**Keynote:** David Levens Center for Cancer Research, National Cancer Research Institute, Maryland, USA *MYC and the principles and consequences of global transcription amplification* **Chair**:	Sponsored by: 
	Transcription and cell homeostasis	**Chair:**
9.15 am – 9.30 am	**Kate Hannan,** The Australian National University, Australia *Targeting RNA polymerase I transcription for cancer therapy* **1149**	
9.30 am – 9.45 am	**Kate Quinlan,** University of New South Wales, Sydney, Australia *Transcriptional regulation of adipose tissue energy expenditure* **1135**	
9:45 am -10:00 am	**Jerry Workman,** Stowers Institute for Research, Missouri, USA *Phosphorylation of histone H3 threonine 11 by the Tda1 kinase under nutritional stress requires the AMPK and CK2 kinases* **1119**	
10.00 am – 10.15 am	**Inkyung Jung,** Korea Advanced Institute of Science and Technology, Daejeon, South Korea *3D genome organization in colon cancer* **1176**	
10.15 am – 10.30 am	**Andrew Keniry,** Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia *Unexpected contributors to the establishment of X Inactivation* **1111**	
10.30 am – 10.45 am	**William Schierding,** University of Auckland, NZ *Common SNPs and Cohesin Genes: Long Distance Compensatory Regulation Reveals Gene Hubs in Cohesinopathies* **1141**	
10.45 am – 11.15 am	**Morning Tea**	Trade Exhibition Area
	Transcription and human disease	**Chair:**
11.15 am – 11.30 am	**Wei-Yi Chen,** National Yang-Ming University, Taiwan *Mechanism of E2A-PBX1-mediated transcription in acute lymphoblastic leukemia* **1167**	

Date	Session	Location
Tuesday 3 December *continued*		**Main Conference Room** (unless otherwise stated)
	Transcription and human disease (continued)	**Chair:**
11.30 am – 11.45 am	**Edwin Cheung,** University of Macau, Macao *Regulation of androgen receptor-mediated transcription by TRIM33* **1140**	
11.45 am – 12.00 pm	**Lee Wong,** Monash University, Australia *Chromatin abnormalities and genome instability in pediatric brain cancers* **1136**	
12.00 pm – 12.15 pm	**Nor Effa Zulkafli,** Advanced medical And Dental Institute, Universiti Sains Malaysia *Crosstalk between PPARÎ³ ligands and Foxp3 expression in natural T-regulatory cells from Type 1 Diabetes Mouse Model (NOD) and control strain Non-Obese Resistant Mouse Model (NOR)* **904**	
12.15 pm – 12.30 pm	**Gang Wang,** Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, China *Transcriptional Mediator subunit MED23 regulates inflammatory responses and liver fibrosis* **933**	
12.30 pm – 12.45 pm	**Vincent Harley,** Hudson Institute of Medical Research, Melbourne Australia *Sex-specific neuroprotection by inhibition of the Y-chromosome gene, SRY, in experimental Parkinson's disease* **1091**	
12.45 pm – 1.00 pm	**Denis Nyaga,** University of Auckland, NZ *Regulatory mechanisms and underlying genetic variation in the development of Type 1 diabetes* **110**	
1.00 pm – 2.00 pm	**Lunch**	Trade Exhibition Area
1.00 pm – 2.00 pm	SOC/IOC Meeting: 2021 Conference	Room 1
	Transcription and human disease (continued)	**Chair:**
2.00 pm – 2.15 pm	**Kalai Mathee,** International Forensic Research Institute, Florida, USA *Temporal interactions of genes, taxa, and metabolites of the microbiota in patients with inflammatory bowel disease* **1174**	

Date	Session	Location
Tuesday 3 December *continued*		**Main Conference Room** (unless otherwise stated)
	Transcription and human disease (continued)	**Chair:**
2.15 pm – 2.30 pm	**Ping Hu,** Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, China *Vgll4 functions as transcription co-repressor or co-activator at different stage of myogenesis* **1179**	
	Features of prokaryotic transcription	**Chair:**
2.30 pm – 2.45 pm	**Ranjan Sen,** Centre for DNA Fingerprinting and Diagnostics, India *Design of bacterial transcription terminator-inhibitory peptides by directed evolution* **1147**	
2.45 pm – 3.00 pm	**Mayuree Fuangthong,** Chulabhorn Research Institute, Bangkok, Thailand *tRNA modification and oxidative stress response in Pseudomonas aeruginosa* **1116**	
3.00 pm – 3.15pm	**Valakunja Nagaraja,** Indian Institute of Science, India *Regulation of transcription- topology coupling: genome-wide interplay probed by topology perturbation* **1104**	
3.15 pm – 3.30 pm	**Changwon Kang,** Korea Advanced Institute of Science and Technology *Transcription reinitiation by E. coli RNA polymerase recycling and diffusing on DNA after intrinsic termination* **1096**	
3.30 pm – 4.00 pm	**Afternoon Tea**	Trade Exhibition Area
	Features of prokaryotic transcription (continued)	**Chair:**
4.00 pm – 4.15 pm	**Nisanart Charoenlap,** Chulabhorn Research Institute, Bangkok, Thailand *Modulation of antibiotic resistances by oxidative stress responsive transcriptional factor in Stenotrophomonas maltophilia* **1117**	
4.15 pm – 4.30 pm	**Iain Lamont,** University of Otago, Dunedin, NZ *Using gene expression to understand bacterial physiology during infection in humans* **1153**	

Date	Session	Location
Tuesday 3 December *continued*		**Main Conference Room** (unless otherwise stated)
	Features of prokaryotic transcription (continued)	**Chair:**
4.30 pm – 4.45pm	**Sungchul Hohng,** Seoul National University, South Korea *Single-molecule studies on rho-dependent termination mechanisms of E. coli transcription* **1110**	
4.45 pm – 5.00 pm	**Dipankar Chatterji,** Indian Institute of Science, India *Structure - Function Relationship of omega-subunit of RNA polymerase: Through the lens of silent mutations* **997**	
5.00 pm – 5.15pm	**Chris Brown,** Department of Biochemistry, Univeristy of Otago, Dunedin *Development of new bioinformatic tools for identification and characterisation of CRISPR ncRNA and protein coding operons*	
7.00 pm - late	**Conference Dinner**	Toitu Early Settlers Museum

Date	Session	Location
Wednesday 4 December		**Main Conference Room** (unless otherwise stated)
9.30 am – 10.15 am	**Keynote: Ed Seto,** The George Washington Cancer Center, Washington, USA *Mechanisms and functions of HATs and HDACs: transcription and beyond* **Chair:**	
10.15 am – 10.45 am	**Morning Tea**	
	Chromatin control of transcription II	**Chair:**
10.45 am – 11.00 am	**Huafeng Zhang,** University of Science & Technology of China *Tumor microenvironment and cancer cell metabolism* **1148**	
11.00 am – 11.15 am	**Reshman Taneja,** National University of Singapore *G9a epigenetically controls canonical Wnt signalling in embryonal rhabdomyosarcoma* **1089**	
11.15 am – 11.30 am	**Cheng-Wen Wu,** Academia Sinica, Taiwan *OCT4B-ORF1, a novel protein derived from unspliced OCT4B RNA in LSCC, plays a key role in cancer stemness and cell survival* **1101**	
11.30 am – 11.45 am	**Alyson Ashe,** The University of Sydney, Australia *Transgenerational Epigenetic Inheritance: initiation, establishment and maintenance have differing genetic requirements* **1177**	

Date	Session	Location
Wednesday 4 December *continued*		**Main Conference Room** (unless otherwise stated)
	Chromatin control of transcription II (continued)	**Chair:**
11.45 am – 12.00 pm	**Chen Davidovich,** Monash University, Melbourne, Australia *Context-dependent master-regulator: molecular basis for the regulation of the histone methyltransferase PRC2* **910**	
12.00 pm – 12.15 pm	**Yichen Zhong,** University of Sydney, Australia *CHD4 Slides nucleosomes by decoupling entry – and exit – side DNA translocation* **1171**	
12.15 pm – 12.30 pm	**Jaehoon Kim,** Korea Advanced Institute of Science and Technology, Korea *Regulation of Heat Shock-Responsive Gene Transcription by RNF20/40-Mediated eEF1BdL Monoubiquitylation* **902**	
12.30 pm – 1.00 pm	**Awards / Farewell**	
1.00 pm – 2.00 pm	**Lunch**	Trade Exhibition Area
2.00 pm onwards	**Optional Tours:** Monarch Peninsula Tour Taieri Gorge Railway Olveston Anatomy Museum	

Keynote Speakers

David L. Levens, M.D., Ph.D.

Center for Cancer Research, National Cancer Institute, Maryland, USA

Dr Levens studies fundamental mechanisms of gene regulation, emphasizing the control of MYC function and expression. He and his team showed that MYC is a univesal amplifier of gene expression. This explains much of MYC physiology and pathology, but also defines the needs to control MYC levels precisely in real time. The Levens Lab has shown that torque generated during transcription of MYC modifies DNA structure dynamically at the FUSE element, that together with FUSE Binding Protein and FBP Interacting Repressor is molecular cruise control for MYC. Most recently he is exploring the genome-wide utilization of supercoiled driven changes in DNA to regulate genes in health and disease.

Professor Susan Clark, FAA

Garvan Institute of Medical Research NSW Australia

Professor Susan Clark has a highly acclaimed international reputation for her work in cancer epigenetics. Susan is a NHMRC Senior Principal Research Fellow and Director of the Genome and Epigenetics Division at the Garvan Institute of Medical Research in Sydney, Australia. She graduated in 1982 with a PhD in Biochemistry, University of Adelaide. Her molecular studies over her career have addressed profound questions about the importance of epigenetics in early development and in disease, especially in cancer. The techniques she pioneered in the early 1990s, including bisulphite methylation sequencing, helped to revolutionise epigenomic research. Susan was a founding member of IHEC (International Human Epigenome Consortium) and led the formation of the AEpiA (Australian Epigenetics Alliance).

Dr Leonie Quinn

John Curtin School of Medical Research, The Australian National University, ACT, Austraila

After completing her PhD studies in Adelaide (1999) and conducting postdoctoral research at the Peter MacCallum Cancer Centre (2000-2007), Dr Quinn established her laboratory at the University of Melbourne in 2007. In 2016 Dr Quinn relocated to The John Curtin School of Medical Research (ANU, Canberra) to establish the Quinn Group - Cancer Models in the ACRF Department of Cancer Biology and Therapeutics.

Professor Ed Seto

George Washington Cancer Center, Washington, USA

Professor Ed Seto is the Associate Center Director for Basic Sciences in the GW Cancer Center and a Professor in the Department of Biochemistry at the George Washington University Medical School. Dr. Seto's research interest focuses on understanding gene regulation with a particular emphasis on studying the functions, mechanisms of action, and regulation of histone deacetylases (HDACs). The ultimate goal of his lab is to obtain a thorough understanding of HDACs in order to provide not only tremendous insights into transcription, epigenetics, and gene regulation, but also potential diagnostic and therapeutic approaches for the treatment of diseases such as cancer.

Exhibitor List

1 **Lab Supply**
labsupply.co.nz

2 **Custom Science**
customscience.co.nz

3 **Macrogen**
macrogen.com.au

4 **Genesearch**
genesearch.co.nz

The Trade Exhibition Floorplan can be viewed on the back cover

General Information

Website

Otago.ac.nz/actvi2019

Registration and Information Desk

The registration desk is situated near the road side of the St David Lecture Theatre Complex. We welcome your enquires on any conference detail including local information. The desk will be open at the following times:

Sunday	1.00 pm – 7.30 pm
Monday	8.00 am- 7.30 pm
Tuesday	8.00 am – 5.00 pm
Wednesday	8.30 am – 2.00 pm

Contact Phone Numbers

Registration Desk Staff: 027 562 5949

Dunedin Taxis: 03 477 7777

Super Shuttles www.supershuttle.co.nz

Police/Ambulance/Fire 111

Urgent Doctor 00 000 0000

Abstracts

Abstracts for the presentations and posters are available electronically on the conference website:

Attendee List

There is a list of conference attendees available at the registration desk. Please note this only includes delegates who have consented to having their information included.

Certificate of Attendance & Evaluation

A certificate of attendance will be emailed directly to delegates following the conclusion of the conference along with a link to an evaluation form for your valuable feedback.

Internet Access

Wireless internet: UO_Guest

Follow the instructions (You will need to create a user name and password)

Mobile Phones/Devices

Mobile phones are allowed in the conference rooms, however please turn all devices to silent mode.

Name Badges

All conference attendees and industry representatives area requested to wear their name badges at all times during the conference and social functions. It is your official entrance pass to the sessions and conference catering.

We invite you to return your name badge to the registration desk at the end of the conference for recycling.

Parking

There is plenty of street parking in the surrounding streets. Please note that most parking is pay and display (up to 4 hours) with free parking on Sunday.

Smoking

The University of Otago is completely smoke-free and this includes the conference venue and surrounding property.

Special Diets

Vegetarian options are included in all refreshment breaks. If you have advised any special dietary requirements on your registration these would have been notified to the caterers. All lamb, beef, chicken served at the main conference venue is certified Halal. There will be a pre-registered special diets table located in the exhibition area, with all meals labeled by name. Please make yourself known to the catering staff if you require help finding your meal.

Suggestions for Dining Near to Venue:

Buddha Stix: 678 George Street

Ombrellos 10 Clarendon Street

Eureka 116 Albany Street

Poppas Pizza Albany Street

More dining options are available on the Dunedin Tourism Website:

www.dunedinnz.com

Presenter Information

Oral Presenters

Powerpoint presentations are to be loaded at the audio visual desk located next to the registration desk. Please take your presentation to the audio visual technician on your arrival at the conference and ensure this done the day prior to your presentation. It would be helpful if you would name your file with your surname and the day you are presenting (eg: SmithMonday). Due to time contraints, only Keynote Presenters will be able to use their own laptop in the Main Conference Room.

Please note that the total time scheduled includes questions and answers.

Poster Presenters

Poster presenters are asked to have their posters up in the Foyer area by Monday morning ready for the first morning tea break. Poster tubes can be stored at the Registration Desk – authors must take their own posters down and remove before 3 pm on Wednesday.

Please refer to the poster list on Page which lists posters in poster id order. The Poster Boards will be labeled with the poster id number and surname.

Social Events

Dunedin City walking tour

Venue Meet outside the iSite Visitor Information Centre (Octagon)

Date Sunday 1 December

Time 2.00 – 4.30 pm

Price No charge

This guided tour will start at the iSite Visitor Information Centre in the Octagon will include key Dunedin city landmarks as well as a number of the street art murals painted around the city centre finishing at the conference venue. There will be a stop for refreshments at a local café (at your own cost). This tour is approximately 6.5km of walking over a 2.5 hour period – it will be on footpaths and paved areas at all times and there are only short bursts of uphill with the majority of the tour being flat.

Welcome function

Venue Foyer Area, St David Lecture Theatre Complex

Date Sunday 1 December

Time 5.00 – 7.15 pm

Price Included in your registration

Delegates will be officially welcomed followed by an opening lecture. This will be followed by a chance to catch up with colleagues with drinks and canapés in the St David Foyer.

Poster session

Venue St David Lecture Theatre Complex

Date Monday 2nd December

Time 5:00 – 7:00pm

Price Included in your registration

Delegates are invited to hear short presentations by poster authors in the poster display area. Refreshments will be provided (first drink is complimentary, followed by a cash bar). Posters will be on display for the duration of the conference.

Conference dinner

Venue Toitu Early Settlers Museum

Date Tuesday 3 December

Time 7.00 pm – late

Price $95

Join fellow delegates for the official conference dinner at the Toitu Early Settlers Museum, located next to the Dunedin Railway Station. Conference dinner tickets can be purchased via online registration and includes dinner and drinks.

Post-Conference Tours

Tours listed below were available to pre-book via online registration. If you have not booked and would like to participate in one of the activities, please see the Registration Desk.

W D Trotter Anatomy Museum Tour

Venue Lindo Ferguson Building, 270 Great King Street

Date Wednesday 4 December

Time 2.00 – 3.30 pm

Price No charge

The Anatomy Museum at the University of Otago was established in 1874, and holds a large collection of anatomical specimens and models, many of which are unique in Oceania. Some models date back to the late 1800s. The Museum Curator will provide a guided tour of the museum.

Olveston Historical Home Guided Tour

Venue Olveston, 42 Royal Terrace

Date Wednesday 4 December

Time 2.45 – 4.00 pm

Opened as a historic house museum in 1967, Olveston is a time capsule as little has changed inside the house since it was occupied as a family home between 1906 to 1966. Olveston is an authentic and original historic home depicting the life of a wealthy merchant family in the early part of the twentieth century. Tour runs for one hour, allow some extra time to view the garden.

Taieri Gorge Railway – Train Trip

Venue Departs from Dunedin Railway Station

Date Wednesday 4 December

Time 3.00 – 7.30 pm

Departing from the historic Dunedin Railway station travel to Pukerangi return – seeing stunning scenery including Wingatui Viaduct.

Monarch Otago Peninsula Wildlife Tour (T2)

Venue Departs from Fryatt Street

Date Wednesday 4 December

Time 3.30 – 7.00 pm

Travel by bus to Wellers Rock then cruise the length of the harbour and Tairoa Head in the Monarch boat taking in the beauty of the harbour and local sea wildlife. Bus out, boat back.

Abstracts: Keynote

In Alphabetical Order
(presenting author surname)

id #XXXX

PLACE HOLDER FOR KEYNOTE Transgenerational Epigenetic Inheritance: initiation, establishment and maintenance have differing genetic

Alyson Ashe[1]

1. University of Sydney, Darlington, NSW, Australia

Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea. His et dolore integre definitionem. Populo patrioque et duo. Rebum alterum voluptatum ea vel, ei elit everti propriae vim, ea apeirian praesent nam. Nec vocibus eligendi in, mutat eleifend comprehensam ea est.Qui no errem solet vitae, constituto argumentum an duo. Porro alterum laoreet cu usu, assum corpora fastidii no qui. Ei est diam conclusionemque, vim et clita labores conclusionemque. Sumo feugiat vel ut, sit eu summo commune detracto, duis ullum homero pri ei.

Ea tota mundi corpora vis. Vidit animal appetere quo ea, in postea admodum fastidii duo. Quo odio cibo everti in, mel amet dolores facilisis te. Ne sea nullam epicuri, eum accusam elaboraret te. Usu ut dolore putant, facer philosophia vim ex, duo choro dissentiet ne. No accusamus gubergren mei, ne qui ponderum consequuntur, clita mucius qui cu.

Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea. His et dolore integre definitionem. Populo patrioque et duo. Rebum alterum voluptatum ea vel, ei elit everti propriae vim, ea apeirian praesent nam. Nec vocibus eligendi in, mutat eleifend comprehensam ea est.

Ea tota mundi corpora vis. Vidit animal appetere quo ea, in postea admodum fastidii duo. Quo odio cibo everti in, mel amet dolores facilisis te. Ne sea nullam epicuri, eum accusam elaboraret te. Usu ut dolore putant, facer philosophia vim ex, duo choro dissentiet ne. No accusamus gubergren mei, ne qui ponderum consequuntur, clita mucius qui cu. Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea.

id #XXXX

PLACE HOLDER FOR KEYNOTE Transgenerational Epigenetic Inheritance: initiation, establishment and maintenance have differing genetic

Alyson Ashe[1]

1. University of Sydney, Darlington, NSW, Australia

Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea. His et dolore integre definitionem. Populo patrioque et duo. Rebum alterum voluptatum ea vel, ei elit everti propriae vim, ea apeirian praesent nam. Nec vocibus eligendi in, mutat eleifend comprehensam ea est.Qui no errem solet vitae, constituto argumentum an duo. Porro alterum laoreet cu usu, assum corpora fastidii no qui. Ei est diam conclusionemque, vim et clita labores conclusionemque. Sumo feugiat vel ut, sit eu summo commune detracto, duis ullum homero pri ei.

Ea tota mundi corpora vis. Vidit animal appetere quo ea, in postea admodum fastidii duo. Quo odio cibo everti in, mel amet dolores facilisis te. Ne sea nullam epicuri, eum accusam elaboraret te. Usu ut dolore putant, facer philosophia vim ex, duo choro dissentiet ne. No accusamus gubergren mei, ne qui ponderum consequuntur, clita mucius qui cu.

Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea. His et dolore integre definitionem. Populo patrioque et duo. Rebum alterum voluptatum ea vel, ei elit everti propriae vim, ea apeirian praesent nam. Nec vocibus eligendi in, mutat eleifend comprehensam ea est.

Ea tota mundi corpora vis. Vidit animal appetere quo ea, in postea admodum fastidii duo. Quo odio cibo everti in, mel amet dolores facilisis te. Ne sea nullam epicuri, eum accusam elaboraret te. Usu ut dolore putant, facer philosophia vim ex, duo choro dissentiet ne. No accusamus gubergren mei, ne qui ponderum consequuntur, clita mucius qui cu. Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea.

id #XXXX

PLACE HOLDER FOR KEYNOTE Transgenerational Epigenetic Inheritance: initiation, establishment and maintenance have differing genetic

Alyson Ashe[1]

1. University of Sydney, Darlington, NSW, Australia

Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea. His et dolore integre definitionem. Populo patrioque et duo. Rebum alterum voluptatum ea vel, ei elit everti propriae vim, ea apeirian praesent nam. Nec vocibus eligendi in, mutat eleifend comprehensam ea est.Qui no errem solet vitae, constituto argumentum an duo. Porro alterum laoreet cu usu, assum corpora fastidii no qui. Ei est diam conclusionemque, vim et clita labores conclusionemque. Sumo feugiat vel ut, sit eu summo commune detracto, duis ullum homero pri ei.

Ea tota mundi corpora vis. Vidit animal appetere quo ea, in postea admodum fastidii duo. Quo odio cibo everti in, mel amet dolores facilisis te. Ne sea nullam epicuri, eum accusam elaboraret te. Usu ut dolore putant, facer philosophia vim ex, duo choro dissentiet ne. No accusamus gubergren mei, ne qui ponderum consequuntur, clita mucius qui cu.

Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea. His et dolore integre definitionem. Populo patrioque et duo. Rebum alterum voluptatum ea vel, ei elit everti propriae vim, ea apeirian praesent nam. Nec vocibus eligendi in, mutat eleifend comprehensam ea est.

Ea tota mundi corpora vis. Vidit animal appetere quo ea, in postea admodum fastidii duo. Quo odio cibo everti in, mel amet dolores facilisis te. Ne sea nullam epicuri, eum accusam elaboraret te. Usu ut dolore putant, facer philosophia vim ex, duo choro dissentiet ne. No accusamus gubergren mei, ne qui ponderum consequuntur, clita mucius qui cu. Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea.

id #XXXX

PLACE HOLDER FOR KEYNOTE Transgenerational Epigenetic Inheritance: initiation, establishment and maintenance have differing genetic

Alyson Ashe[1]

1. University of Sydney, Darlington, NSW, Australia

Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea. His et dolore integre definitionem. Populo patrioque et duo. Rebum alterum voluptatum ea vel, ei elit everti propriae vim, ea apeirian praesent nam. Nec vocibus eligendi in, mutat eleifend comprehensam ea est.Qui no errem solet vitae, constituto argumentum an duo. Porro alterum laoreet cu usu, assum corpora fastidii no qui. Ei est diam conclusionemque, vim et clita labores conclusionemque. Sumo feugiat vel ut, sit eu summo commune detracto, duis ullum homero pri ei.

Ea tota mundi corpora vis. Vidit animal appetere quo ea, in postea admodum fastidii duo. Quo odio cibo everti in, mel amet dolores facilisis te. Ne sea nullam epicuri, eum accusam elaboraret te. Usu ut dolore putant, facer philosophia vim ex, duo choro dissentiet ne. No accusamus gubergren mei, ne qui ponderum consequuntur, clita mucius qui cu.

Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea. His et dolore integre definitionem. Populo patrioque et duo. Rebum alterum voluptatum ea vel, ei elit everti propriae vim, ea apeirian praesent nam. Nec vocibus eligendi in, mutat eleifend comprehensam ea est.

Ea tota mundi corpora vis. Vidit animal appetere quo ea, in postea admodum fastidii duo. Quo odio cibo everti in, mel amet dolores facilisis te. Ne sea nullam epicuri, eum accusam elaboraret te. Usu ut dolore putant, facer philosophia vim ex, duo choro dissentiet ne. No accusamus gubergren mei, ne qui ponderum consequuntur, clita mucius qui cu. Lorem ipsum dolor sit amet, cu ridens noster deseruisse duo, viderer inimicus ea mea.

Abstracts: Oral

In Alphabetical Order
(presenting author surname)

id #1177

Transgenerational Epigenetic Inheritance: initiation, establishment and maintenance have differing genetic requirements

Alyson Ashe[1]

1. University of Sydney, Darlington, NSW, Australia

It has recently become clear that in some circumstances Lamarck may have been right. There are a growing number of examples where a clear case can be made for the inheritance from parent to offspring of environmentally acquired gene expression changes. We have developed a sensor in C. elegans in which RNAi-induced silencing of a GFP transgene is robustly inherited for multiple generations in the absence of the initial RNAi trigger. The visible nature of this phenotype provides an exquisitely sensitive system, whereby we can separate individual animals according to their silencing status and measure effects in these distinct groups. Using this approach, we show that two putative histone methyltransferases are required in the RNAi-exposed generation for effective transmission of transgene silencing, but are dispensable for silencing inheritance in subsequent generations. This shows that these proteins are required for the establishment of a transgenerational silencing signal but not for long-term maintenance of this signal, suggesting that transgenerational epigenetic inheritance is a multi-step process with distinct genetic requirements for establishment and maintenance of heritable silencing.

Previous studies have implicated secondary siRNAs as the effector molecules of heritable silencing. We have found that the abundance of secondary siRNAs does not correlate well with silencing phenotypes in the sequenced generation or in the next generation, suggesting that the current mechanistic models of epigenetic inheritance are incomplete.

id #1105

Male germline specification in flowering plants

Benjamin Peters[1], Shiny Varghese[1], Olivia Kelly[1], Shereen Asha Murugayah[1], Stuart Zohrab[1], David Twell[2], Lynette Brownfield[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

2. Genetics, University of Leicester, Leicester, Leicestershire, UK

Development of complex multicellular organisms depends upon the generation of diverse cell types. Asymmetric division, where a mother cell produces daughter cells that are immediately distinct, is a fundamental mechanism for generating cellular diversity. In flowering plants, a critical asymmetric division that leads to distinct daughter cell fate occurs during pollen development. This asymmetric nature of this division is essential for the specification of the male germline. However, little is known about the nature of male germline fate determinants, and how they are unequally distributed into the daughter cells.

While the identity of the germline fate determinants are unknown, they likely lead to the expression of the transcription factor DUO POLLEN1 (DUO1) in the smaller daughter cell soon after the asymmetric division. DUO1 then promotes expression of a diverse array of male germline genes required for sperm cell formation and fertilization. The male germline-specific expression of DUO1 appears to be controlled largely at the transcriptional level. By identifying factors that activate *DUO1* expression, we aim to identify the unequally segregated male germline fate determinants.

We have identified a conserved 85 bp region of the *DUO1* promoter, called *Regulatory Region of DUO1* (*ROD1*), that replicates the *DUO1* expression pattern in pollen. *ROD1* contains three *cis*-regulatory elements that play different roles; one is essential for transcription, one enhances transcription and the other represses transcription. Work is now focused on identifying the transcription factors that bind to these *cis*-regulatory elements and several candidate transcription factors have been identified.

id #1117

Modulation of antibiotic resistances by oxidative stress responsive transcriptional factor in *Stenotrophomonas maltophilia*

Nisanart Charoenlap[1], Jurairat Chittrakanwong[2], Sorayut Chattrakarn[2], Paiboon Vattanaviboon[1], Skorn Mongkolsuk[1]

1. Laboratory of Biotechnology, Chulabhorn Research Institute, Bangkok, Thailand

2. Program in Applied Biological Science: Environmental Health, Chulabhorn Graduate Institute, Bangkok, Thailand

Stenotrophomonas maltophilia is a cause of nosocomial infections especially in the immunocompromised patients. It inherits several antibiotic resistance mechanisms. Understanding the antibiotic resistance mechanisms is crucial for development of alternative treatments. Transcriptional factor SoxR is known as superoxide sensing regulator, which controls several oxidative stress response genes, including superoxide dismutase gene. Our finding revealed that SoxR in *S. maltophilia* regulates not only *sod* genes, but also the major facilitator superfamily, *mfsA,* gene. MfsA functions as an efflux pump, which is responsible for resistance to several compounds. When *S. maltophilia* contains plasmid-mediated overexpression of *mfsA*, the bacteria showed an increased resistance not only to paraquat but also to fluoroquinolone antibiotics. Moreover, heterologous expression of *mfsA* in other Gram-negative pathogens conferred resistance to paraquat as well as to fluoroquinolones. In *S. maltophilia*, oxidized SoxR, a consequence of exposure to superoxide stress, could increase *mfsA* expression, leading to multidrug resistance. We hypothesized that mutation of SoxR to the form that is easily oxidized would up-regulate genes contributing to both oxidative stress protection and multiple antibiotic resistance, thereby enhancing bacterial virulence and its ability to survive in hostile conditions. By using random mutagenesis methods, we got 5 groups of SoxR mutations that lead to antibiotic resistances. Some of these mutations were selected and confirmed by site-directed mutagenesis. The structural analysis of the mutated SoxR is required for better understanding of the mechanism at molecular level.

id #1129

Methylome and transcriptome programme segregates PD-L1 expressing and non-expressing melanoma

Aniruddha Chatterjee[1], Euan J Rodger[1], Antonio Ahn[1], Peter A Stockwell[1], Matthew Parry[1], Stuart Gallagher[2], Jessamy Tiffen[2], Peter Hersey[2], Michael Eccles[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

2. Centenary Institute, University of Sydney, Sydney, NSW, Australia

The programmed death-ligand 1 (PD-L1) receptor is an important immune checkpoint and is often upregulated in cancer cells to allow immune evasion. In melanoma, the patients with PD-L1 expression and absence of tumour infiltrating lymphocytes (TILs) (i.e. "constitutive PD-L1 or PD-$L1_{CON}$") show worse response rates and prognosis than patients with PD-L1 expression and the presence of TILs (i.e. "inducible PD-L1 or PD-$L1_{IND}$"). However, how PD-L1 expression is regulated in melanoma cells remains elusive. We hypothesised that epigenetic state regulates constitutive and inducible PD-L1 expression in melanoma.

We have generated whole-genome scale DNA methylomes (using reduced representation bisulfite sequencing) and transcriptomes (RNA-Seq) for patient derived melanoma cell lines (in PD-$L1_{IND}$ and PD-$L1_{CON}$ groups). We discovered extensive global hypomethylation in the constitutive lines, particularly pronounced in intergenic repeat regions and gene bodies. RNA-Sequencing data indicated that the hypomethylated state of the PD-$L1_{CON}$ cells was correlated with higher upregulation of the differentially expressed genes at a global-scale and the upregulated genes were associated with cancer hallmark properties. Transcriptome signature revealed viral mimicry, oxidative phosphorylation and differentiation as the major altered pathway between PD-$L1_{IND}$ and PD-$L1_{CON}$ melanoma groups and this signature was strongly validated in melanoma patient expression signatures (cancer genome atlas data). We believe these results are the first to show that DNA methylation mediated transcriptome regulation play a role in controlling PD-L1 expression in melanoma and suggest they may have important implications for combined treatments targeting methylation (DNMTi) and PD1/PD-L1 (anti-PD1 antibodies).

id #977

Structure - Function Relationship of omega- subunit of RNA polymerase: Through the lens of silent mutations

dipankar chatterji[1]

1. Indian Institute of Science, Bangalore, KARNATAKA, India

RNA polymerase is composed of a total of 6 subunits. Information is available on the contribution of all the subunits in the primary functioning of bacterial RNA polymerase except the smallest subunit omega. A toxic mutant screen was used to discover the role of omega in RNAP holoenzyme. This led to the serendipitous discovery of a silent mutant which was lethal. The primary focus of our study is to decode the mechanism behind this lethality. The native omega is intrinsically disordered but the silent mutant was having a predominantly helical structure. So, we hypothesized that this transition from intrinsically disordered to ordered structure is responsible for the lethal phenotype and the disordered nature of omega is prerequisite for maintaining the plasticity of the active site. We generated several silent mutants of omega to investigate the role of codon bias and effect of rare codons with respect to its position in the protein sequence. Not all silent mutations affect the structure equally. RNA polymerase reconstituted with these omega silent mutants (structured in comparison to wild type) were found to be inactive. Concomitant changes in structure and transcriptional profile led to understand that proteins with same sequence can have different fold and function. Codon plus strain was used to see the rescue of phenotype in lethal silent mutants. We performed ribosome profiling experiments with the wild type and mutant strains of ω and observed that they are translated at a differential rate. ppGpp interaction studies also showed difference.

id #1167

Mechanism of E2A-PBX1-mediated transcription in acute lymphoblastic leukemia

Wei-Yi Chen[1]

1. National Yang-Ming University, Taipei, TAIWAN, Taiwan

The dimeric E2A, which acts in concert with other lineage-specific factors, plays an important role in the development of B- and T- lymphocytes, and is also of significance for certain pediatric leukemias. In this regard, genetic lesions generate E2A-fusion proteins (E2A-PBX1 and E2A-HLF) with novel biochemical properties, including binding to distinct DNA sequences, and functional activities that contribute to tumor formation. Numerous studies have established the oncogenic functions of these two fusion proteins in cell and animal models, as well as biochemical properties in vitro. However, the E2A-fusion-enforced cistrome and related mechanisms underlying leukemogenesis in human acute lymphoblastic leukemia cells remain far from clear.

In current study, we determined the unbiased genome-wide binding and transcription signatures of the oncogenic E2A-PBX1 in human pre-B ALL-derived cell lines. Our results demonstrate that E2A-PBX1 preferentially binds to a subset of RUNX1-occupied enhancers through a direct interaction with DNA-bound RUNX1 – and not through the PBX1 DNA-binding homeodomain as had been proposed. In addition, we also show that enrichments of p300, MED1/Mediator and K27 acetylated histone H3 activation signals are a feature of E2A-PBX1-targeted RUNX1 sites that could promote the enhanced expression of associated genes. Importantly, we find that E2A-PBX1 not only binds to RUNX1-controlled genes, but also targets the positive feedback loop of the RUNX1 locus. Hence, the positive impact of E2A-PBX1 on activation of the RUNX1 locus and downstream RUNX1 targets may contribute to a sustained RUNX1 transcriptome in pre-B cells that leads to leukemogenesis.

id #1140

Regulation of androgen receptor-mediated transcription by TRIM33

Edwin Cheung[1]

1. University of Macau, Taipa, MACAU, Macao

Prostate cancer is one of the most common cancer in men worldwide. Androgen receptor (AR) is a master transcription factor in driving the development and progression of the disease. Alteration in the expression or activity of AR co-factors can influence the outcome of the disease, including therapy resistance. Using a proteomics approach called rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME), we identified Tripartite Motif Containing 33 (TRIM33, aka TIFly) as a novel protein interactor of AR. We show TRIM33 co-localizes with AR globally on chromatin to directly regulate genes relevant to prostate cancer progression. We demonstrate TRIM33 achieves this by stabilizing AR from ubiquitin-mediated proteasomal degradation. We also show TRIM33 is essential for prostate cancer cell growth. Finally, we found TRIM33 is upregulated in prostate cancer patients and identified a TRIM33 gene regulatory signature that can predict disease-free survival. Overall, our study has identified TRIM33 as a novel AR co-activator that enhances oncogenic AR signaling pathways in prostate cancer. These findings suggest TRIM33 may be a potential therapeutic target for prostate cancer treatment.

id #910

Context-dependent master-regulator: molecular basis for the regulation of the histone methyltransferase PRC2

Chen Davidovich[1, 2]

1. Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton, VIC 3800, Australia

2. EMBL-Australia and the ARC Centre of Excellence in Advanced Molecular Imaging, Clayton, VIC, Australia

The polycomb repressive complex 2 (PRC2) is a histone methyltransferase that maintains cell identity during development in all multicellular organisms. At the molecular level, PRC2 mark repressed genes and chromatin domains with the H3K27me3 repressive epigenetic mark. The interactions between PRC2 to some of its accessory subunits increase its enzymatic activity, while interactions with RNA inhibits it. PRC2 binds to RNA with high affinity and low sequence specificity, with preference to G-tracts and G-quadruplex-forming sequences [1]. Observations of high-affinity and inhibitory interactions between PRC2 to RNA opened a mechanistic conundrum: how can PRC2 methylate histones within the RNA-rich environment of the nucleus?

We recently mapped the RNA-binding surfaces of PRC2 and identified an RNA-binding patch within its allosteric regulatory site, adjacent to the methyltransferase centre [2]. Accordingly, RNA-mediated inhibition of PRC2 is alleviated by allosteric activation using H3K27me3 histone-tail peptides. Most common types of holo-PRC2 complexes bind RNA, providing a unified model to explain how RNA and allosteric stimuli antagonistically regulate the enzymatic activity of PRC2. I will present results from our ongoing work, revealing how positive and negative effectors regulates PRC2 at the molecular level, within the context of transcriptional regulation.

References

[1] X. Wang, et al., T.R. Cech & C. Davidovich, *Mol Cell*. **(2017)**; "Targeting of Polycomb Repressive Complex 2 to RNA by Short Repeats of Consecutive Guanines."

[2] Q. Zhang, et al. R. Bonasio & C. Davidovich. *Nat Struct Mol Biol*. **(2019)**; "RNA exploits an exposed regulatory site to inhibit the enzymatic activity of PRC2."

id #1100

Long non-coding RNAs as regulators of gene expression in cancer

Sarah D Diermeier[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

Recent genome-wide studies revealed that 1-2% of the human genome encodes for proteins, while as much as 50% of the genome can be transcribed. Of these "non-coding" transcripts, long non-coding RNAs (lncRNAs) represent the largest and most diverse class. LncRNAs can be spliced and polyadenylated, lack a significant open reading frame, and are expressed in a tissue-specific manner. They have been implicated as regulatory molecules in a variety of cellular functions, including epigenetic gene regulation, splicing, mRNA stability and translation. However, a detailed molecular mechanism is lacking for most lncRNAs.

We previously identified 30 potentially oncogenic lncRNAs in breast cancer, termed *Mammary Tumour Associated RNAs*(*MaTAR*s). To functionally validate the role of *MaTAR*s, we performed

knockdown experiments and observed reduced cell proliferation, invasion and/or organoid branching in a cancer-specific context. One of the identified lncRNAs, *hMaTAR17*, is over-expressed in several different types of cancer compared to normal tissue. Notably, injection of antisense oligonucleotides targeting *MaTAR17*into a transgenic mouse model of breast cancer resulted in a significant decrease of tumour size, and increased tumour differentiation. We generated loss-of-function cell lines using CRISPR/Cas9 genome editing, and were able to reproduce the reduced proliferative potential both *in vitro* and *in vivo*.

Ongoing studies to investigate the molecular mechanism by which *hMaTAR17*acts include RNA-seq, single molecule RNA-FISH and Chromatin Isolation by RNA Purification in breast and colorectal cancer cells. Our results suggest that this lncRNA is likely an important driver of mammary tumour progression, and represents a promising new therapeutic target in cancer.

id #1094

Investigating nuclear and cytoplasmic roles of HDAC4 in the brain

Helen L Fitzsimons[1], Patrick J Main[1]

1. School of Fundamental Sciences, Massey University, Palmerston North, MANAWATU, New Zealand

Both neuronal development and memory formation involve epigenetic mechanisms that coordinate changes in gene expression that lead to synaptic rearrangement and growth. Haploinsufficiency of the histone deacetylase HDAC4 is associated with intellectual disability and developmental delay in humans, indicating that it plays an essential role in normal neuronal function. However HDAC4 is increased in abundance in brains of individuals with Alzheimer's disease and we have shown in our *Drosophila* model that overexpression results in severe developmental malformation of the brain and also prevents long-term memory formation in the adult fly. Unlike most other HDACs, HDAC4 undergoes nucleocytoplasmic shuttling and is largely non-nuclear in neurons, therefore to investigate whether these phenotypes were due to increased abundance of nuclear and/or cytoplasmic HDAC4, we expressed HDAC4 mutants that were restricted to either the nucleus (3SA) or cytoplasm (L175A) of neurons and examined the impact on development and long-term memory. Severe developmental defects and memory deficits were observed on expression of 3SA whereas L175A had no impact. RNA-seq surprisingly revealed that expression of only 29 genes was significantly altered by 3SA, suggesting that HDAC4 may be acting through largely non-transcriptional mechanisms. HDAC4 aggregates into punctate foci in nuclei through tetramerisation of its glutamine-rich N-terminus. An increasing number of studies have identified HDAC4 to be present in intranuclear and cytoplasmic inclusion associated with several neurodegenerative disorders, therefore further investigation is warranted into whether the impairments in neuronal function are caused by intranuclear aggregation of HDAC4.

id #1116

tRNA modifications and oxidative stress response in *Pseudomonas aeruginosa*

Narumon Thongdee[1], Juthamas Jaroensuk[1], Sopapan Atichartpongkul[2], Jurairat Chittrakanwong[1], Paiboon Vattanaviboon[2], Skorn Mongkolsuk[2], Mayuree Fuangthong[2]

1. Applied Biological Sciences Program, Chulabhorn Graduate Institute, Lak Si, Bangkok, Thailand

2. Laboratory of Biotechnology, Chulabhorn Research Institute, Lak Si, Bangkok, Thailand

tRNA modifications have been identified in a wide range of organisms, where they play a large variety of functions in different biological processes and cellular stress responses. A link between tRNA modifications and the oxidative stress response in *Pseudomonas aeruginosa*, an opportunistic human pathogen, was recently established in our previous work. We found that tRNA modifications located either inside or outside the anticodon loop contributed to H_2O_2 resistance in *P. aeruginosa*. The loss of tRNA modifying genes, such as *trmJ, trmB* or *trmA*, altered the expression pattern of H_2O_2-responsive genes, resulting in H_2O_2 hypersensitivity. Moreover, the level of certain tRNA modifications was shown to be reprogrammed as a part of the cellular response to H_2O_2 exposure. We identified their enzymatic reactions, ribonucleoside modifications, tRNA substrates, and the locations of tRNA modifications. Characterization of the physiological role of tRNA modifications that confer H_2O_2 sensitivity would provide insight into the regulatory networks of the oxidative stress response, which is crucial for the pathogenicity of *P. aeruginosa.*

id #1143

cMyc-mediated epigenetic regulation of cancer metabolic reprogramming

Ping Gao[1]

1. School of Medicine and Institutes for Life Sciences, South China University of Technology, Guangzhou, China

It is known that cancer cells generally adapt a specific metabolic phenotype that is characterized by the switch to aerobic glycolysis, or Warburg Effect. While this metabolic phenotype is largely believed to be responsible for the growth advantage of various cancers, the underlying mechanisms are not very clear. This talk will be focused on our current progress related to cMyc-mediated epigenetic regulation of cancer metabolic reprogramming.

id #1146

Protein expression is controlled by the accessibility of translation initiation sites

Bikash K Bhandari[1], Chun Shen Lim[1], Paul Gardner[1]

1. University of Otago, Dunedin, TE WAIPOUNAMU, New Zealand

Recombinant protein production in microbial systems is a widely used technique, yet up to half of these experiments fail at the expression phase. A number of contributing factors to failures have been proposed, e.g. codon-bias, mRNA folding, mRNA:ncRNA avoidance, tRNA abundance and G+C content. Determining which, if any, of these features explains experiment failures is an active area of research. We have discovered that an ensemble energy model of RNA folding that captures the accessibility of translation initiation sites greatly outperforms other features in predicting the outcomes of 11,430 recombinant protein expression experiments in *Escherichia coli*. We have developed a new computational tool called Tisigner, that optimises the first nine codons of an mRNA to improve (or impair) accessibility. Our evaluations have shown that this approach is generally sufficient to elevate the chances of a successful experiment, with the advantage that straightforward PCR cloning methods can be used to integrate optimised sequences.

id #1093

Heterochromatin Protein 1α interacts with parallel RNA and DNA G-quadruplexes

Tracy Hale[1], Ruby Roach[1], Vyacheslav Filichev[1], Geoffery Jameson[1], Carlos Gonzalez[2]

1. Massey University, Palmerston North, MANAWATU, New Zealand

2. Instituto de Química Física 'Rocasolano', CSIC, Madrid, Spain

The maintenance of telomeric heterochromatin is essential to genome integrity as it protects the ends of chromosomes from degradation and the DNA damage response machinery. In mammals, telomeres comprise of highly conserved tandem repeats of $(TTAGGG)_n$ terminating in a 3› overhang. Transcription from these repeats results in TERRA transcripts of heterogeneous lengths with the majority consisting of UUAGGG tracks that are shorter than 400 bases. These guanine-rich transcripts form G-quadruplex (G4) structures by the association of four guanines bound through Hoogsteen hydrogen bonding. While it has been known for some time that DNA and RNA can form non-B structures *in vitro*, only recently has it been demonstrated that G4 and intercalated C-rich motif (i-motif) structures exist *in vivo* and contribute to genome function. The presence of TERRA within the telomere nucleoprotein complex is proposed to act as a docking site for proteins involved in telomere function including Heterochromatin Protein 1α (HP1α).

To identify if the interaction of HP1α with RNA is structure dependent, we investigated whether HP1α recognises the G4 structures formed by TERRA. We found that the hinge region of HP1α binds with high affinity not only the TERRA G4 structures of parallel topology but also DNA G4 structures of the same topology. This suggests not only a mechanism by which TERRA expression can promote heterochromatin formation at telomeres, but the formation of G4s within the genome could influence HP1α function in gene silencing, RNA processing or DNA repair.

id #1149

Targeting RNA polymerase I transcription for cancer therapy.

Kate Hannan[1], Nadine Hein[1], Rita Ferreira[1], Yulia Manenkova[2], Mustapha Haddach[2], Denis Drygin[2], Ross Hannan[1]

1. ACRF Dept Cancer Biology and Therapeutics, John Curtin School of Medical Research, ANU, Acton, ACT, Australia

2. Pimera, San Fransico, California, USA

Despite the overwhelming evidence of dysregulated RNA Polymerase I (Pol I) transcription of the ribosomal RNA (rRNA) genes (rDNA) in cancer, only one selective Pol I transcription inhibitor, CX-5461, has entered clinical trials. Studies using *in vivo* models for various blood and solid cancers have demonstrated efficacy and support a broad use for CX-5461 as a cancer therapy. A Phase I trial with patients with advanced hematological cancer reported that CX-5461 is tolerated with minimal side effects and a second trial has commenced in breast cancer.

While pioneering and promising, CX-5461 does have additional activities (e.g., Top2a inhibition and DNA damage), which possibly contributes to its efficacy, toxicity profiles and acquired resistance mechanisms.

We have developed a series of 2nd generation Pol I transcription inhibitors with improved toxicology, tissue distribution (penetrates the blood brain barrier), reduced plasma protein binding and higher efficacy compared to CX-5461. Our lead compound, PMR-116, has a high orally bioavailability, is well tolerated and improves survival in murine models of acute myeloid leukemia, B-cell lymphoma and prostate cancer.

Preliminary studies suggest PMR-116 impairs Pol I recruitment to the rDNA repeat in a similar fashion to CX-5461. Most importantly, however, under equivalent tIC50 (50% inhibition of rDNA transcription) doses PMR-116 is effective irrespective of p53 status and does not activate the DNA damage response (DRR) as observed for CX-5461. Thus, unlike CX-5461, PMR-116 may not interfere with Top2a or initiate DDR, a significant distinguishing characteristic between these two compounds. PMR-116 is poised to enter clinical trials.

id #1091

Sex-specific neuroprotection by inhibition of the Y-chromosome gene, *SRY*, in experimental Parkinson's disease

Joohyung Lee[1, 2], Paulo Pinares-Garcia[1, 2], Hannah Loke[1], Seungmin Ham[1], Eric Vilain[3], Vincent Harley[1, 2]

1. Hudson Institute of Medical Research, Clayton, VIC, Australia

2. Monash University, Clayton, VIC, Australia

3. University of California, Los Angeles, CA, USA

Parkinson's disease (PD) is a debilitating neurodegenerative disorder caused by the loss of midbrain dopamine (DA) neurons. While the cause of DA cell loss in PD is unknown, male sex is a strong risk factor. Aside from the protective actions of sex hormones in females, emerging evidence suggests that sex-chromosome genes contribute to the male bias in PD. We previously showed that the Y-chromosome gene, *SRY*, directly regulates adult brain function in males independent of gonadal hormone influence. *SRY* protein colocalizes with DA neurons in the male substantia nigra, where it regulates DA biosynthesis and voluntary movement. Here we demonstrate that nigral *SRY* expression is highly and persistently up-regulated in animal and human cell culture models of PD. Remarkably, lowering nigral *SRY* expression with antisense oligonucleotides in male rats diminished motor deficits and nigral DA cell loss in 6-hydroxydopamine (6-OHDA)-induced and rotenone-induced rat models of PD. The protective effect of the *SRY* antisense oligonucleotides was associated with male-specific attenuation of DNA damage, mitochondrial degradation, and neuroinflammation in the toxin-induced rat models of PD. Moreover, reducing nigral *SRY* expression diminished or removed the male bias in nigrostriatal degeneration, mitochondrial degradation, DNA damage, and neuroinflammation in the 6-OHDA rat model of PD, suggesting that *SRY* directly contributes to the sex differences in PD. These findings demonstrate that *SRY* directs a previously unrecognized male-specific mechanism of DA cell death and suggests that suppressing nigral Sry synthesis represents a sex-specific strategy to slow or prevent DA cell loss in PD.

id #988

Fertilizer produced from neem (*azadirachta indica*) seeds

Yusuf Haruna[1], Muhammad Aliyu[1]

1. Kebbi State University of Science and Technology Aliero, Aliero, KEBBI STATE, Nigeria

Organic fertilizers are derived from animal matter, human excreta, and vegetable matter (manure). Naturally occurring *organic* fertilizers include animal wastes from meat processing, *manure*, slurry etc. This research was carried out to study the production, bio-safety and the chemistry of organic fertilizer from neem seeds, rice husk, blood meal, bone meal, and calcium carbonate; proximate analysis was carried out using standard procedures to determine the plant's nutritive value of the formulated organic fertilizer for the presence of nitrogen by Khadjel distillation method, phosphorus by Bray No.1 method, Elements like Potassium and Nitrogen were confirmed Organic Carbon by Wakley- Black dichromate method and other micronutrients. Phosphorus is primarily used for the growth and repair of body cells and tissues. Potassium is required by plants to maintain the turgor pressure of the cell by keeping the cell strong. The result from the analysis indicated following based on the N.P.K content in each. Formulation 1 (N=4810mg/kg, P=4.76mg/kg, K=1166.7mg/kg), Formulation 2 (N= 7140mg/kg, P=19.83mg/kg, K = 1250mg/kg, Formulation 3 (N= 4060mg/kg, P=19.39mg/kg, K=600mg/kg), Formulation 4 (N=7700mg/kg,P=19.28mg/kg, K = 650mg/kg) and Formulation 5 (N= 148400mg/kg,P= 20.10mg/kg, K=600mg/kg). The result shows that formulation 5 had the highest nitrogen content (N=148400mg/kg) while formulation 3 had the lowest nitrogen content (N=40600mg/kg) which is due to the increase in proportion of blood meal, poultry litters and neem seeds. This suggests that organic fertilizer could be a better substitute to conventional inorganic fertilizer as a soil conditioner and good plant manure when applied based on the soil nutrient requirements.

id #1110

Single-molecule studies on rho-dependent termination mechanisms of *E. coli* transcription

Sungchul Hohng[1], Eunho Song[1], Heesoo Uhm[1], Palinda Munasingha[2], Yeon-Soo Seo[2], Kuk Sun Ha[2], Jin Young Kang[3], Changwon Kang[2]

1. Seoul National University, Seoul, 1 GWANAK-RO, GWANAK-GU, South Korea

2. Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, South Korea

3. Department of Chemistry, Korea Advanced Institute of Science and Technology, Daejeon, South Korea

Rho is a hexameric helicase that induces bacterial transcription termination, but there has been a controversy about the mechanism of rho-dependent termination. In a conventional model called 'RNA-dependent pathway', rho first binds *rut* (rho-utilizing) site of RNA transcript, then chases RNA polymerase (RNAP) waiting on a pausing site, and finally disassembles the transcription complex. In a competing model called 'RNAP-dependent pathway', rho makes a stable complex with RNAP, and upon binding the *rut* site, induces termination through an allosteric mechanism. To clearly elucidate the mechanism of rho-dependent termination, we developed single-molecule fluorescence assays that can monitor the processes of rho-dependent termination of *E. coli* transcription. We found that whereas both RNA- and RNAP-dependent pathways operate for all tested rho-dependent termination sites with varying proportions, the RNAP-dependent pathway becomes more dominant as the pausing time at

the termination site increases. This property of RNAP-dependent termination pathway makes it ideal for riboswitch-based regulation of rho-dependent termination as observed in the leader region of *mgtA* gene.

id #1102

Understanding epigenetic memory in the germline and during sex determination

Oscar J Ortega[1], Tim Hore[1]

1. Department of Anatomy, University of Otago, Dunedin, OTAGO, New Zealand

The vast majority of inherited biological information is hard-coded in DNA sequence; however, epigenetic modification provides an additional conduit for intergenerational transmission of molecular memory. In mammals, inheritance of epigenetic memory in the form of DNA methylation is largely prevented by extensive erasure and reprogramming early in germline formation. We recently discovered that, unlike mammals, the germline of zebrafish does not undergo genome-wide erasure of DNA methylation during development. The significance of epigenetic memory preservation and its consequences for basic biological processes such as sex determination, is discussed.

id #1179

Vgll4 functions as transcription co-repressor or co-activator at different stage of myogenesis

Xue Feng[1], Fei Wang[1], Lei Zhang[1], Ping Hu[1]

1. Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, SHANGHAI, China

VGLL4 is originally identified as a negative regulator of YAP-dependent gene transcription. Here we show that VGLL4 regulates muscle regeneration in both YAP-dependent and YAP-independent manners at different stages. Vgll4 also serves as either a co-repressor or co-activator depending on whether YAP is involved. We find that knockout of VGLL4 leads to smaller myofiber size and defective muscle contraction force. What's more, our studies reveal that knockout of VGLL4 results in increased muscle satellite cell proliferation and impaired myoblast differentiation, ultimately leading to delayed muscle regeneration. Mechanistically, our results show that VGLL4 works as a conventional YAP inhibitor at the proliferation stage of muscle regeneration. At the differentiation stage, VGLL4 acts as a co-activator of TEAD4 to promote MyoG transactivation and facilitate the initiation of differentiation in a YAP-independent manner. Moreover, VGLL4 stabilizes the protein-protein interaction between MyoD and TEAD4 to achieve efficient MyoG transactivation. Our findings define the dual roles of VGLL4 in regulating muscle regeneration at different stages, and may open novel therapeutic perspectives for muscle regeneration.

id #1181

Role of p300-mediated modification of Hypoxia-inducible Factor (HIF)-1α in the regulation of HIF-1α stability and function

Kwanyoung Jeong[1]

1. SUNGKYUNKWAN University, Suwon-si, GYEONGGI-DO, South Korea

Cells operate two major protein degradation pathways, the ubiquitination-proteasome system (UPS) pathway and the autophagy-lysosomal system (ALS) pathway, to control the stability and turnover of proteins. In UPS, proteins are selectively ubiquitinated and subsequently targeted for degradation by the 26S proteasome. Whereas, in ALS, proteins and various cytoplasmic constituents are delivered to lysosomes for catabolic turnover. One of the best target of UPS-mediated regulation of protein stability is Hypoxia-inducible factor 1 alpha (HIF-1α), which is an essential component in the transcriptional response of tumours under hypoxia. HIF-1α controls the transcription of over 60 genes involved in many aspects of cancer biology including angiogenesis, metastasis, invasion, and proliferation. HIF-1α is also degraded by ALS, although the detailed mechanism or its role in hypoxia and tumorigenesis is not yet elucidated. In this study, we investigated p300-dependent post-translational modification of HIF-1α and present a potential role of this modification in the HIF-1α regulation.

id #930

TIP60 keeps the endogenous oncogenic demons repressed

Sudhakar Jha[1]

1. Cancer Science Institute of Singapore, Singapore, NA

TIP60 is a lysine acetyltransferase which belongs to the MYST family of acetyltransferases and is known to be a haploinsufficient tumor suppressor. TIP60 downregulation is an early event in tumorigenesis which has been observed in several cancer types including breast and colorectal cancers. Colorectal cancers are characterized by inflammation wherein inflammatory bowel disease greatly increases the risk of colorectal cancer. In this symposium, I will discuss the role of TIP60 in the silencing of endogenous retroviral elements (ERVs). We have identified a unique mechanism of ERV regulation in cancer cells mediated by TIP60 and BRD4 through regulation of Histone H3K9 trimethylation. I will also discuss our efforts to exploit this pathway to sensitize colorectal cancer to reverse transcriptase inhibitors.

id #1131

Paternal hypoxia exposure primes offspring for increased hypoxia resistance

Alexandria Ragsdale[1], **Ludovic Dutoit**[1], **Anne Besson**[1], **Tania King**[1], **Neil Gemmell**[1], **Tim Hore**[1], **Sheri Johnson**[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

Increasingly, studies are revealing that the environmental challenges experienced by an organism can not only have multiple effects on an individual level, but that these challenges may also impact unexposed offspring. Hypoxia is a physiological challenge that many aquatic

organisms encounter in their environment, resulting in numerous physiological, phenotypic, and epigenetic changes. In this study, we use zebrafish (*Danio rerio*) as a model to investigate how paternal hypoxia experience impacts subsequent progeny. Males were exposed to moderate hypoxia (11-13 kPA) for 2 weeks, crossed to unexposed females to create an F1 generation, and progeny underwent an acute hypoxia (0-1 kPA) tolerance assay. Using time to loss of equilibrium as a measure of hypoxia resistance, we show that paternal exposure to hypoxia endow offspring with a greater tolerance to acute hypoxia, compared to offspring of unexposed males. In addition to phenotypic alternations, we also investigated changes in gene expression in offspring. We conducted RNA-Seq on whole F1 fry and detected 89 differentially expressed genes, including two hemoglobin genes and a selenoprotein that are upregulated more than 4-fold in offspring from male parents exposed to hypoxia. Paternal exposures to physiological challenges are thus able to impact the phenotype and gene expression of their unexposed progeny. We are now investigating whether changes in DNA methylation underpin the observed changes in phenotype and gene expression.

id #1176

3D genome organization in colon cancer

Inkyung Jung[1]

1. Department of Biological Sciences, Korea Advanced Institute of Science & Technology, Daejeon, South Korea

Chromosomal rearrangements are a hallmark of various cancer types, but delineating their effect in gene regulation has been challenging because many of them are occurred at noncoding sequences. To elucidate the effect of noncoding associated chromosomal rearrangements in oncogenesis we employ high-throughput chromatin conformation capture method to generate genome-wide chromatin contact maps for colorectal cancer patients' tumor samples and systematically identify disorganized 3D genome structure. The alteration of 3D genome structure frequently rewires or disrupts the promoter-*c*RE (*cis*-regulatory element) relationships, correlated with either activation of proto-oncogenes or inhibition of tumor-suppressor genes. Unexpectedly, we reveal chromosome-wide disorganized 3D genome structure, associating with widespread abnormal gene expression changes. This study will provide a new insight to decipher the effect of noncoding associated chromosomal rearrangement in the context of 3D genome structure in cancer biology.

id #1096

Transcription reinitiation by *E. coli* RNA polymerase recycling and diffusing on DNA after intrinsic termination

Wooyoung Kang[1], Kook Sun Ha[2], Heesoo Uhm[1], Kyuhyong Park[1], Ja Yil Lee[3], Sungchul Hohng[1], Changwon Kang[2]

1. Department of Physics and Astronomy, Seoul National University, Seoul, Republic of Korea

2. Korea Advanced Institute of Science and Technology, Deajeon, Republic of Korea

3. School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan, Republic of Korea

It is unknown how transcription termination complexes are disassembled, especially in what order the essential components dissociate from transcription complexes. We primarily used single-molecule fluorescence measurements to examine the dissociations of fluorescently labeled Cy3-RNA transcript and Cy5-DNA template from unlabeled RNA polymerase (RNAP) in *E. coli* intrinsic termination, and their post-terminational fates. RNA product release precedes RNAP dissociation from immobilized DNA template much more often than their concurrent dissociations in intrinsic termination. As termination is defined by the release of product RNA from transcription complex, the subsequent retention of RNAP on DNA template constitutes a previously unidentified stage, termed as ‹recycling.› In experiments with Cy5 placed on RpoD (σ^{70}) factor instead of DNA, Cy5-σ never dissociates before Cy3-RNA. Post-terminational retention of RNAP on DNA is little affected by the presence of transcription factor σ^{70}, NusA or NusG. RNAP's post-terminational possession of σ and diffusion on DNA during the recycling stage allow for transcription 'reinitiation,' which occurs not only on a downstream promoter oriented in the sense direction but also on the original promoter. Thus, RNAP can diffuse downward and upward on DNA template for reinitiation on any promoter that can be reached within a diffusion lifetime. With flipping of RNAP on DNA, antisense reinitiation at an oppositely oriented promoter could be possible. Furthermore, σ supplement increases the reinitiation efficiency, suggesting that even core enzyme can become reinitiation-competent during the recycling stage. In summary, after releasing RNA product at intrinsic termination, recycling RNAP diffuses on DNA template for reinitiation most times.

id #1111

Unexpected contributors to the establishment of X inactivation

Andrew Keniry[1], Natasha Jansz[1], Linden J Gearing[1], Iromi Wanigasuriya[1], Quentin Gouill[1], Matthew Ritchie[1], Douglas Hilton[1], Marnie E Blewitt[1]

1. Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia

Major complications with *in vitro* culture of female embryonic stem cells (mESC) have impeded study of sex-specific pluripotency; however, from the published work female pluripotency significantly differs to male. We report a replenishable female mESC system that has enabled us to produce an optimised protocol for preserving the XX karyotype; a protocol that also improves male mESC fitness. To demonstrate the utility of the system, we screened for regulators of the female-specific process of X chromosome inactivation. This is the first screen that has studied the establishment of X inactivation in its native context, again based on the challenges of female mESC culture. We reveal a new role for chromatin remodellers *Smarcc1* and *Smarca4* in establishment of X inactivation. The remodellers create a nucleosome depleted region at gene promotors on the inactive X during exit from pluripotency, without which gene silencing fails. To the best of our knowledge this hasn't been reported previously. Our female mESC system provides a tractable model for XX mESC culture that will expedite study of female pluripotency and has enabled us to discover new features of the female-specific process of X inactivation.

id #902

Regulation of Heat Shock-Responsive Gene Transcription by RNF20/40-Mediated eEF1BδL Monoubiquitylation

Jaehoon Kim[1]

1. Korea Advanced Institute of Science and Technology, Daejeon, South Korea

RNF20/40 E3 ubiquitin ligase-mediated histone H2B monoubiquitylation plays important roles in many cellular processes, including transcriptional regulation. However, the multiple defects observed in RNF20-depleted cells suggest additional ubiquitylation targets of RNF20/40 beyond histone H2B. Here, using biochemically defined assays employing purified factors and cell-based analyses, we demonstrate that RNF20/40, in conjunction with its cognate E2 ubiquitin-conjugating enzyme RAD6, monoubiquitylates lysine 381 of eEF1BδL, a heat shock transcription factor. Notably, monoubiquitylation of eEF1BδL increases eEF1BδL accumulation and potentiates recruitment of p-TEFb to the promoter regions of heat shock-responsive genes, leading to enhanced transcription of these genes. We further demonstrate that cooperative physical interactions among eEF1BδL, RNF20/40, and HSF1 synergistically promote expression of heat shock-responsive genes. In addition to identifying eEF1BδL as a novel ubiquitylation target of RNF20/40 and elucidating its function, we provide a molecular mechanism for the cooperative function of distinct transcription factors in heat shock-responsive gene transcription.

id #1109

Epigenetic reprogramming of the brushtail possum (*Trichosurus vulpecula*) germline

Melanie K Laird[1], **Tim A Hore**[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

Epigenetic reprogramming of mammalian primordial germ cells (PGCs)- the precursors to the sperm and egg cells of adults- occurs in the early embryo as PGCs undergo active genome-wide demethylation following migration to the developing gonad. Demethylation, and subsequent re-methylation, resets parentally-inherited imprints to reflect the sex of the developing embryo.

Our understanding of both the mechanisms and evolution of this phenomenon is in its infancy. Marsupials are fascinating models of germline reprogramming as PGC migration and development occurs largely after birth- in the gonads of developing pouch young. Marsupial PGCs also appear to be reprogrammed post-natally, based on semi-quantitative global studies and single loci analysis of male wallaby PGCs. To further investigate the dynamics of epigenetic memory in marsupials, we isolated germline cells (PGCs) from male and female brushtail possums (*Trichosurus vulpecula*) throughout gonadal differentiation. We used post-bisulfite adaptor tagging (PBAT) to assess methylation of PGCs in a fully-quantitative manner, assigning reads against the recently-assembled possum genome. Our findings indicate that the mechanisms of germline reprogramming are conserved between marsupials and eutherian mammals, but also divergent. We discuss the potential implications of this work for the generation of transgenic marsupials using a PGC transplantation approach, and in particular, its application for possum biocontrol in New Zealand.

id #1153

Using gene expression to understand bacterial physiology during infection in humans

Iain Lamont[1], Lois Martin[1], Ben Brockway[1], Andrew Gray[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

The bacterial pathogen *Pseudomonas aeruginosa*infects the lungs of patients with cystic fibrosis, causing a decline in lung function and a reduction in life expectancy. The physiology of *P. aeruginosa*has been well studied in laboratory culture but not during infection in patients, where it may exist under anoxic conditions. Gene expression in bacteria is responsive to environmental conditions. We have taken advantage of regulated changes to gene expression to investigate the conditions experienced by the bacteria during infection. We isolated*P. aeruginosa*from sputum from the lungs of 20 patients with cystic fibrosis and used RT-qPCR to measure expression of three genes *narI*, *nirS*and *ccoN*that encode proteins required for growth of *P. aeruginosa*when oxygen tension is low. Expression of all three genes was significantly increased (median increases of 6-fold, 14-fold and 200-fold for *ccoN*, *narI* and *nirS*) when the bacteria were grown in the absence of oxygen. In parallel RNA was extracted directly from sputum from the same patients, with no bacterial subculture, and used RT-qPCR to obtain a snapshot of bacterial gene during infection. Expression of the target genes was at levels similar to those of bacteria grown in the absence of oxygen in laboratory culture. Our findings show that during chronic lung infection *P. aeruginosa*exists under conditions of oxygen deprivation. This finding has implications for the effectiveness of antibiotics, which we have shown have altered activity against bacteria grown in the absence of oxygen.

id #1152

Improving the expression and solubility of recombinant proteins by smart sequence design algorithms

Chun Shen Lim[1], Bikash K. Bhandari[1], Paul P. Gardner[1]

1. Department of Biochemistry, University of Otago, Dunedin, New Zealand

Recombinant protein production is a widely used technique, yet half of these experiments fail at the expression phase and only a quarter of target proteins are successfully purified. Failures are largely due to toxicity, low protein expression, and poor protein solubility. We have discovered that the energetics of RNA structure ensembles, that model the 'accessibility' of translation initiation sites, accurately predicts the expression outcomes of 11,430 recombinant protein production experiments in *Escherichia coli*. We have further discovered that normalised B-factors, that model the 'flexibility' of amino acid residues, accurately predicts the solubility of 12,158 recombinant proteins expressed in *Escherichia coli*. One important implication of our findings is that the chances of successful experiments can be increased by improving both 'accessibility' and 'flexibility'. We have developed TIsigner (Translation Initiation coding region designer) and SoDoPE (Soluble Domain for Protein Expression) that allows users to choose a protein region of interest for optimising expression and solubility, respectively. The final results will suggest synonymous codon changes within the first few codons of the DNA fragments of interest, meaning that gene optimisation can be done using standard PCR cloning.

id #1174

Temporal interactions of genes, taxa, and metabolites of the microbiota in patients with inflammatory bowel disease

Daniel Ruiz-Perez[1], Jose Lugo-Martinez[2], Natalia Bourguignon[3, 4], Betiana Lerner[3], Shekhar Bhansali[4], Ziv Bar-Joseph[2], Giri Narasimhan[1], Kalai Mathee[1]

1. Florida International University, Miami, FL, United States

2. Carnegie Mellon School of Computer Science, Pittsburgh, Pennsylvania, USA

3. Universidad Technologica Nacional (UTN), Buenos Aires, Argentina

4. College of Engineering and Computing, FIU, Florida, USA

Microbiomes are dynamic, forming a "social network" interacting with each other and their host through different metabolites. The host physiology, age, or gender can confound the biological processes and the microbiota. Thus, longitudinal data are necessary to understand the complex interaction. Metatranscriptomics, metagenomics, and metabolomics data generated by the Integrative Human Microbiome Project that followed 132 individuals with inflammatory bowel disease over one year were used in our analyses. We compensated for the variable biological process, non-uniform sampling, noisy and missing data by using Dynamic Bayesian Networks (DBNs). The DBNs are ideally suited to model heterogeneous dynamic systems and infer temporal interactions between their constituents. Using DBN, a framework was developed to identify relationships between genes, taxa, and metabolites. MIMOSA validated a significant (based on a Poisson-Binomial distribution) number of the edges with the highest bootstrap confidence predicted by the DBN. In-depth knowledge of this network would improve our understanding of the disease, metabolic potential of each taxon, and potentially critical metabolites that may lead to better therapeutics. The work represents novel and valuable research on an integrated analysis of multiomic longitudinal data.

id #1162

Dual inhibition of anti-apoptotic proteins BCL-XL and MCL-1 enhance cytotoxicity of Nasopharyngeal carcinoma cell lines

Siti Fairus Abdul Rahman[1], Kalaivani Muniandy[1], Azali Azlan[1], Mohd Ghows Mohd Azzam[1], Nethia Mohana Kumaran[1]

1. Universiti Sains Malaysia, Georgetown, PENANG, Malaysia

The BCL-2 family proteins are critical regulators of the intrinsic apoptosis pathway. They are up-regulated in many cancers and have become attractive therapeutic targets. Given that different cell population rely on different anti-apoptotic proteins for survival, it is crucial to establish which of these proteins are important for Nasopharyngeal carcinoma (NPC) cell survival. A human apoptosis RT^2 **Profiler PCR Array was first employed to profile the anti-apoptotic gene expressions in NPC cell lines. The NPC HK1 cells expressed all the anti-apoptotic genes (***MCL-1, BFL-1, BCL-2, BCL-XL, and BCL-w*). On the other hand, NPC C666-1 cells expressed all except for *BFL-1*. Given that there are no specific BFL-1 inhibitors, the role of BFL-1 in NPC cell survival was determined by deleting the gene using the CRISPR/Cas9 technique. The *BFL-1* **single guide RNAs were cloned into the PX459 plasmid (**pSpCas9(BB)-2A-Puro) and were transfected into the cells. To this end we have generated bulk cells of the *BFL-1* **knockout NPC cells. Parallel to this experiment, NPC cell lines were tested with BH3 mimetics ABT-199, A1331852 and** S63845 which inhibits BCL-2, BCL-XL and MCL-1,

respectively, alone and in combination. The cells were resistant to single agent treatment of these drugs implying that the cells do not solely rely on BCL-2, BCL-XL or MCL-1 for survival. Co-inhibition of MCL-1 and BCL-XL more profoundly inhibited NPC cell proliferation compared to co-inhibition of MCL-1 and BCL-2. Collectively, our data show that combination of BH3 mimetics targeting specific anti-apoptotic proteins could be potential treatment strategies for NPC.

id #1104

Regulation of transcription- topology coupling: genome-wide interplay probed by topology perturbation

Valakunja Nagaraja[1]

1. Indian Institute of Science, Bangalore, KARNATAKA, India

In bacteria, transcription generated positive and negative supercoils are removed by DNA gyrase and topoisomerase I acting ahead and behind the transcribing RNA polymerase (RNAP) to overcome the topological barriers. We have investigated *in vivo* recruitment and interaction of both the topoisomerases with mycobacterial genome. We have mapped the genome-wide footprints of topoisomeraseI and DNA gyrase along with RNAP in the deadly pathogen, *Mycobacterium tuberculosis(Mtb),* taking advantage of minimal topoisomerase representation in the organism. We show that *in vivo* distribution of topoisomerases is guided by active transcription and both the enzymes co-occupy active transcription units (TUs). The recruitment was higher at the genomic loci with higher transcriptional activity and/or at regions under high torsional stress compared to silent genomic loci. We establish their interaction with the regions of genome having propensity to accumulate negative and positive supercoiled domains, validating their role in managing the twin supercoiled domains. Next, we have probed genome wide action by trapping enzyme- DNA complexes - either by using chemical inhibitors or poisonous mutants. Comparison of these genome wide action sites with binding sites determined by Chip Seq provide insights into topoisomerase I function in transcription elongation and chromosome segregation.

id #1106

Regulatory mechanisms and underlying genetic variation in the development of Type 1 diabetes

Denis M Nyaga[1], **Mark H Vickers**[1], **Craig Jefferies**[1, 2], **Jo K Perry**[1], **Justin M O'Sullivan**[1]

1. University of Auckland, Grafton, AUCKLAND, New Zealand

2. Starship Children's Health, Auckland, New Zealand

Background

Type 1 diabetes (T1D) is a chronic metabolic disorder characterised by the autoimmune destruction of insulin-producing pancreatic beta cells in genetically predisposed individuals. Genome-wide association studies (GWAS) have identified over 60 risk loci across the human genome, marked by single nucleotide polymorphisms (SNPs), which confer genetic susceptibility to T1D. Evidence indicates that disease-associated SNPs can alter gene expression through spatial interactions that involve distal loci, in a tissue- and developmental-specific manner.

Objectives
We sought to elucidate the mechanisms through which population-based genetic variants (SNPs) identified from GWAS and prospective studies on T1D contribute to the disease development by identifying the genes regulated by these variants.

Methods
We utilised data derived from three-dimensional (3D) genome mapping to identify genes that physically co-localize with DNA regions that are marked by T1D-associated SNPs. Analysis of these SNP-gene pairs using the Genotype-Tissue Expression (GTEx) database identified a subset of SNPs that significantly affected levels of gene expression in human tissues.

Results
We observed that T1D-associated SNPs are associated with the differential expression of genes including *HLA-DQB2, TAP2, BTN3A2, CTLA4, RPS26, NOTCH4*, *IGF2-AS*, and long non-coding RNA *RP11-973H7.1*, which exhibit age- and tissue-specific effects in tissues including the spleen and pancreas. Furthermore, the spatially regulated genes are enriched for immune regulatory pathways that involve antigen presentation, immune cell activation, and cytokine signalling.

Discussion
Our results demonstrate that T1D-associated genetic variants contribute to adaptive immune regulatory pathways and emphasize the importance of early life events in new-borns at genetic risk of developing autoimmunity and T1D.

id #1145

Genome-wide DNA methylation analysis in the Christchurch Health and Development Study reveals potential for epigenetic effects of cannabis use on pathways involved neurodevelopment and neuronal signalling

Amy Osborne[1], John Pearson[2], Alexandra Noble[1], Neil Gemmell[3], John Horwood[2], Joseph Boden[2], Tim Hore[3], Miles Benton[4], Donia Macartney-Coxson[4], Martin Kennedy[2]

1. University of Canterbury, Christchurch, CANTERBURY, New Zealand

2. University of Otago Christchurch, Christchurch City, New Zealand

3. University of Otago, Dunedin

4. Environmental Science and Research, Porirua

Cannabis use is associated with an increased risk of adverse psychosocial outcomes, depression and schizophrenia, and has been shown to alter human DNA methylation. Examining cannabis-only and cannabis + tobacco users from The Christchurch Health and Development Study, we found the most significantly differentially methylated sites in cannabis + tobacco users were in the AHRR and F2RL3 genes, replicating previous studies on the effects of tobacco. Cannabis-only users had no evidence of differential methylation in these genes (P=0.97), or at any other loci at the epigenome-wide significance level ($P<10^{-8}$). However, there were 521 sites differentially methylated at P<0.001. The top cannabis-only loci are in, near or interact with genes whose function is consistent with the psychosocial outcomes associated with cannabis use. We conclude that the effects of cannabis use on the mature human methylome differ from the effects of tobacco use, with further research required to further identify specific loci.

id #1135

Transcriptional Regulation of Adipose Tissue Energy Expenditure

Alexander J Knights[1], Emily J Vohralik[1], Kyle L Hoehn[1], Merlin Crossley[1], Kate GR Quinlan[1]

1. University of NSW, Sydney, NSW, Australia

Beige adipocytes residing within white adipose tissue burn fuels to generate heat and therefore may reduce obesity by burning rather than storing excess fuels. Cells of the immune system – including macrophages, innate lymphoid cells and eosinophils – appear to be essential in the beiging of white adipocytes.

Knockout mice for the transcriptional repressor Kruppel-like Factor 3 (KLF3) are lean and are protected from diet-induced obesity. Interestingly, these mice show evidence of an increased capacity for thermogenesis. We performed a bone marrow transplantation study and were able to confer the lean beige phenotype on wild type mice. This suggested that KLF3 deficiency in cells of the haematopoietic lineage may drive leanness in this mouse model. We interrogated different types of adipose-resident immune cells and discovered that there are three times as many eosinophils in KLF3-deficient adipose tissue.

We performed genome-wide expression analyses on eosinophils isolated from white adipose tissue and uncovered widespread gene expression differences in the absence of KLF3. Interestingly, we saw expression of a number of genes that encode secreted proteins known for their role in beiging. The eosinophils from KLF3 knockout mice, where we see enhanced beiging, expressed higher levels of these secreted proteins.

Our data suggest that KLF3 is a master regulator of gene expression programs in adipose tissue-resident eosinophils. We are now testing whether novel secreted proteins we have identified are able to induce beiging and energy expenditure and may present druggable targets for obesity.

id #1141

Common SNPs and Cohesin Genes: Long Distance Compensatory Regulation Reveals Gene Hubs in Cohesinopathies

William Schierding[1, 2], Julia Horsfield[3, 2], Justin O'Sullivan[1, 2]

1. Liggins Institute, University of Auckland, Auckland, New Zealand

2. Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Auckland, New Zealand

3. Department of Pathology, Dunedin School of Medicine, The University of Otago, Dunedin, New Zealand

Intergenic and even intronic variants mark DNA elements that regulate distant genes. These spatial-regulatory connections are largely set during human development, when the cohesin complex plays a key role in genome organisation. Variation in cohesin subunits or regulators of cohesin (loading and unloading from chromosomes) has been associated with an overlapping spectrum of human syndromes known as cohesinopathies. Here we used a common SNP approach across each cohesin-associated gene locus: all SNPs with cis-eQTLs (nearby regulation) in the GTEx portal to one of the genes comprising the cohesin ring subunits (SMC1A, SMC1B, SMC3, STAG1, STAG2, STAG3, RAD21, REC8, and RAD21L1) or the cohesin-ring regulators (CTCF, WAPL, NIPBL, MAU2, PDS5A, PDS5B). For each SNP, distant, allele-specific regulatory

connections were determined by Hi-C spatial connection and verified by significant regulatory evidence through GTEx eQTL (FDR < 0.05). This analysis revealed common themes of cohesin-relevant pathways (genome organization, cell cycle, and DNA damage/repair), highlighting hubs of genes co-regulated in cohesinopathies. For example, SNPs in the RAD21L1 locus connect to RFC3 and KIF18A, which regulate cell cycle (GO:0007049). Long distance eQTLs originating from the MAU2 locus target ERCC1, ZBTB46, and ZNF790. Collectively, these results demonstrate the impacts of genetic variation on the coordinated expression of cohesin and functionally related genes. This highlights co-expressed genes that represent additional targets that may help improve our understanding of the pleiotropic impacts associated with cohesinopathies.

id #1147

Design of bacterial transcription terminator-inhibitory peptides by directed evolution.

Ranjan Sen[1]

1. Center For DNA Fingerprinting and Diagnostics, Hyderabad, TELANGANA, India

In bacteria, Rho-dependent transcription termination plays a major role in the regulation of gene expression. It regulates many physiological processes directly or indirectly making the conserved Rho protein a very attractive drug target. The Psu protein is a capsid protein in bacteriophage P4 that moonlights as a specific inhibitor of Rho. Expression of Psu induces lethality to *Escherichia coli* as well as many other pathogens. Here, we report the generation of peptide inhibitors against Rho from the Psu-CTD through a directed evolution method. We screened peptides capable of inhibiting *in vivo* Rho-dependent termination as well as inducing lethality in a similar way as Psu. *In vitro*, the peptides inhibited the RNA binding, RNA release and ATPase activity by Rho. Direct interaction of the peptides with Rho was evident from pull down and ITC assays. Deletion of 8 amino acids from the C-terminal of the peptide did not affect its function. The N-terminal Histidine tag was important for their solubility *in vitro* and presumably *in vivo*. The ITC assays indicated that the peptides occupy the N-terminal domain of four of the subunits of Rho. Thus, the mode of interaction of the peptides is different from that of Psu. These peptides are also lethal against the *Mycobacterium smegmatis and Mycobacterium bovis. In vitro,* the peptides could inhibit the ATPase activities of *Mycobacterium tuberculosis* and the plant pathogen*, Xanthomonas orizae* Rho proteins. Our results establish an alternative way to design new bactericidal molecules using the bacteriophage proteins as platforms.

id #1166

The function of lncRNAs in brain aging

Fei Wang[1], Amr R Ghanam[1], Baowei Liu[1], Shengwei Ke[1], Ke Zhang[1], Shuya Zhang[1], Sisi Ma[2], Juan Shi[1], Xiaoyuan Song[1]

1. School of Life Sciences, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, 230026, China

2. National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, CAMS and PUMC, Beijing , 100005, China

Both categories and quantities of lncRNAs are high in mammalian brain, which is one of the organs that is highly susceptible to aging. However, many lncRNAs remain uncharacterized and the roles of most lncRNAs have not been studied in brain

aging. Through microarray and RNA-Seq screening with young and old mouse brains, we have identified several candidate lncRNAs that play roles in brain aging. Using multiple methods, including FISH, ChIRP-MS, RIP combining with knockdown and knockout models, we are dissecting the function and underlying mechanism of these lncRNAs in brain aging.

id #1089

G9a epigenetically controls canonical Wnt signalling in embryonal rhabdomyosarcoma

Reshma Taneja[1]

1. National University of Singapore, Singapore, SINGAPORE, New Zealand

The Wnt signaling pathway is down-regulated in embryonal rhabdomyosarcoma (ERMS) and contributes to the block of myogenic differentiation. Epigenetic mechanisms leading to its suppression are unknown and could pave the way towards novel therapeutic modalities. In this study, we demonstrate that the lysine methyltransferase G9a suppresses canonical Wnt signalling by directly activating expression of DKK1, a Wnt antagonist. Inhibition of G9a expression or its pharmacological activity resulted in reduced DKK1 expression and consequently elevated b-catenin levels that impaired growth and induced differentiation of ERMS cells in vitro and in vivo. Loss of DKK1 mimicked G9a depletion phenotypes. Conversely, the impact of G9a deficiency on tumor growth was reversed by recombinant DKK1, or LGK974, which also inhibits Wnt signaling. Consistently, among thirteen drugs targeting chromatin modifiers, G9a inhibitors were highly effective in reducing ERMS cell viability. Together, our demonstrate that ERMS cells are vulnerable to G9a inhibitors and suggest that targeting the G9a-DKK1-b-catenin node holds promise for differentiation therapy.

id #933

Transcriptional Mediator subunit MED23 regulates inflammatory responses and liver fibrosis

Gang Wang[1]

1. Fudan University, Shanghai, SHANGHAI, China

Liver fibrosis, often associated with cirrhosis and hepatocellular carcinomas, is characterized by hepatic damage, an inflammatory response, and hepatic stellate cell (HSC) activation, although the underlying mechanisms are largely unknown. Here, we show that the MED23 subunit of the transcriptional Mediator complex participates in the development of experimental liver fibrosis. Compared with their control littermates, mice with hepatic *Med23* deletion exhibited aggravated CCl_4-induced liver fibrosis, with enhanced chemokine production and inflammatory infiltration as well as increased hepatocyte regeneration. Mechanistically, the orphan nuclear receptor RORα activates the expression of the liver fibrosis-related chemokines CCL5 and CXCL10, which is suppressed by the Mediator subunit MED23. We further found that the inhibition of *Ccl5* and *Cxcl10* expression by MED23 likely occurs due to G9a-mediated H3K9 dimethylation of the target promoters. Collectively, these findings reveal hepatic MED23 as a key modulator of chemokine production and inflammatory responses and define the MED23-CCL5/CXCL10 axis as a potential target for clinical intervention in liver fibrosis.

id #1150

LncRNA *887L* regulates tumor progression *via* transcriptional activation of CA9

Tao Shen[1, 2], Wangxiao Xia[3], Sainan Min[4], Zixuan Yang[1, 2], Wei Wang[1, 2], Huafeng Zhang[2, 5], Liling Wu[6], Guangyan Yu[4], Qingpeng Kong[3], Xiangting Wang[1, 2]

1. Department of Geriatrics, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, China

2. Hefei National Laboratory for Physical Sciences at the Microscale, Hefei, Anhui, China

3. State Key Laboratory of Genetic Resources and Evolution/Key Laboratory of Healthy Aging Research of Yunnan Province, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, Yunnan, China

4. Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology, Beijing, China

5. Key Laboratory of Innate Immunity and Chronic Disease, the Chinese Academy of Sciences, Hefei, Anhui, China

6. Department of Physiology and Pathophysiology, Peking University Health Science Center, Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, and Beijing Key Laboratory of Cardiovascular Receptors Research, Beijing, China

Publish consent withheld

id #1157

The transcriptional and co-transcriptional mechanisms that regulate novel exon acquisition.

Robert Weatheritt[1]

1. Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

The long introns of mammals are pools of evolutionary potential due to the multiplicity of sequences that permit the acquisition of novel exons. However, the permissibility of genes to this type of acquisition and its influence on the evolution of cell regulation is poorly understood. Here, we observe that human genes are highly permissive to the inclusion of novel exonic regions permitting the emergence of novel regulatory features. Our analysis reveals the potential for novel exon acquisition to occur in over 30% of evaluated human genes. Regulatory processes including the rate of splicing efficiency and RNA polymerase II (RNAPII) elongation control this process by modulating the “window of opportunity” for spliceosomal recognition. Our work demonstrates that the inclusion of repeat-associated novel intronic regions is a tightly controlled process capable of expanding the regulatory capacity of cells.

id #1092

Epigenetic regulation of *Botrylloides leachii* whole body regeneration

Megan J Wilson[1], Lisa Zondag[1], Rebecca Clarke[1]

1. Anatomy, University of Otago, Dunedin, OTAGO, New Zealand

The colonial tunicate *Botrylloides leachii* is exceptional at regenerating from a piece of vascular tunic after loss of all adults from the colony. Previous transcriptome analyses indicate a brief period of healing before regeneration of a new adult (zooid) in as little as 8-10 days. However, there is little understanding of how the resulting changes to gene expression, required to drive regeneration, are initiated and how the overall process is regulated. Rapid changes to transcription often occur in response to chromatin changes, mediated by histone modifications such as histone acetylation. Here, we investigated a group of key epigenetic modifiers, histone deacetylases (HDAC) that are known to play an important role in many biological processes such as development, healing and regeneration.

Through our transcriptome data, we identified and quantified the expression levels of HDAC and histone acetyltransferase (HAT) enzymes during whole body regeneration (WBR). To determine if HDAC activity is required for WBR, we inhibited its action using valproic acid (VPA) and Trichostatin A (TSA). HDAC inhibition prevented the final morphological changes normally associated with WBR and resulted in aberrant gene expression. *B. leachii* genes including *Slit2*, *TGF-β*, *Piwi* and *Fzd4* all showed altered mRNA levels upon HDAC inhibition in comparison to the control samples. Additionally, atypical expression of *Bl_Piwi* was found in immunocytes upon HDAC inhibition.

Together, these results show that HDAC function, specifically HDAC I/IIa class enzymes, are vital for *B. leachii* to undergo WBR successfully.

id #1136

Chromatin abnormalities and genome instability in pediatric brain cancers

Maheshi Udugama[1], Linda Hii[1], Andrew Garvie[1], Jeffrey Mann[1], Hsiao Voon[1], Lee Wong[1]

1. Monash University, Clayton, VIC, Australia

One striking finding in the cancer epigenetics was the identification of mutated histone genes (oncohistones) in paediatric glioblastomas (pGBMs). Two H3.3 mutations are found. The first mutation replaces lysine 27 with a methionine. The second one replaces glycine 34 by an arginine (G34R). H3.3G34R overlaps with ATRX and p53 mutations, and these pGBMs are activated in the Alternative Lengthening of Telomeres (ALT) telomere maintenance pathway, suggesting that H3.3G34R/ATRX/p53 mutations cooperate to drive ALT and pGBM development.

We have created cell models carrying H3.3G34R/ATRX/p53 mutations to recapitulate the initial driver epigenetic events that promote ALT. KDM4 proteins are demethylases or epigenetic erasers that remove the methyl group from trimethylated H3K9 and H3K36. We find that the H3.3G34R inhibits KDM4 catalytic function of and drives its aberrant distribution. Thus, it induces aberrant histone methylation pattern and affects telomere chromatin maintenance. Our success in inducing ALT in H3.3G34R/ATRX/TP53/TERT cell model verifies the roles of H3.3 and ATRX in ALT activation and ALT is a multifactorial process. We propose KDM4 chromatin network as a major driver that promotes ALT and the oncogenic process.

In the H3.3G34R/ATRX mutants, we detect DNA copy loss at ATRX-bound ribosomal DNA (rDNA) repeats, accompanied with severely reduced rRNA synthesis. ALT positive human sarcoma tumours are substantially reduced in rDNA copy. Moreover, ALT cancer cells show increased sensitivity to RNA Polymerase I transcription inhibitor, suggesting the therapeutic potential of targeting Pol I transcription in ALT cancers. Our study provides insights into chromatin defects associated with ATRX/H3.3 mutations and ALT.

id #1119

Phosphorylation of histone H3 threonine 11 by the Tda1 kinase under nutritional stress requires the AMPK and CK2 kinases.

Seunghee Oh[1], Jerry Workman[1]

1. Stowers Institute for Medical Research, Kansas City, MO, United States

Nutritional stress signaling in budding yeast includes phosphorylation of histone H3T11 (H3pT11).

To understand upsteam signaling of H3pT11, we dissected kinases involved in this process. We found that H3pT11 requires both AMPK and CK2 kinases. However, these kinase do not directly phosphorylate H3T11 but instead directly phosphorylate the Tda1 kinase. Tda1 is a yeast AMPK interacting kinase that phosphorylates H3T11 in vitro and in vivo. Phosphorylation of Tda1 is required for its proper histone kinase activity and its protein stability. The Tda1 kinase thus integrates signals of multiple upstream kinases to regulate H3T11 phosphorylation upon nutrient stress.

id #1101

OCT4B-ORF1, a novel protein derived from unspliced OCT4B RNA in LSCC, plays a key role in cancer stemness and cell survival.

Erh-Hsuan LIN[1], CHENG-WEN WU[1]

1. Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan

OCT4 is an embryonic stemness factor that plays key roles to maintain the self-renewal and pluripotency of embryonic stem cells, and is also associated with tumorigenesis. Our study showed that in lung cancer, lung adenocarcinoma (LAC) and squamous-cell carcinoma (LSCC) express distinct isoforms of OCT4. An unspliced OCT4B RNA was found in LSCC (but not LAC), which played an important role in cell survival. Knockdown the unspliced OCT4B with shRNAs targeting intron regions led to LSCC cell death, but showed no effect in LAC cells. An open-reading-frame flanking exon 2ab and Intron 2 on the unspliced OCT4B RNA was identified, which encodes a functional protein (indicated as “ORF1”) with a molecular weight ~14 kD. Customized antibody confirmed the expression of endogenous ORF1 protein in LSCC cell lines and patient samples.

Bioinformatic analysis showed that ORF1 could be involved in cancer stemness regulation and EMT and invasion phenotypes. We also found that ORF1 can positively regulate SOX2, a key stemness factor and amplified lineage-survival oncogene in LSCC. Specifically, ORF1 does not regulate SOX2 promoter activity, but likely modulates SOX2 mRNA stability. Overexpression of SOX2 through an exogenous promoter cannot recover the cell death mediated by knockdown of ORF1, emphasizing the key role of ORF1 in LSCC survival.

In summary, ORF1 is a novel protein derived from unspliced OCT4B RNA, which plays a key role in LSCC cell survival. The mechanisms that promote the generation of ORF1 in LSCC, and its clinical significance will be further elucidated.

id #904

Crosstalk between PPARγ ligands and Foxp3 expression in natural T-regulatory cells from Type 1 Diabetes Mouse Model (NOD) and control strain Non-Obese Resistant Mouse Model (NOR)

Nor Effa S. Z.[1], Nik S Yaacob[2], Norazmi MN[3]

1. Regenerative Medicine Cluster, Advanced medical And Dental Institute, Universiti Sains Malaysia, Bertam, Penang, Malaysia

2. Pathology Department, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

3. School of Health Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

Immunosuppressive natural T-regulatory (nTreg) cell is critical in mediating peripheral self-tolerance. Concordantly, the anti-inflammatory properties of nuclear receptor peroxisome proliferator-activated receptor-g (PPARg) have been intensely studied as adjuvant to immunosuppressant. Current study examined the possible crosstalk between PPARg and Foxp3 expression in nTreg cells from NOD and NOR mice. Splenic nTreg cells from mice were cultured with CD3/CD28 stimulant, IL-2, treated or untreated with the PPARg ligands, ciglitazone or 15d-prostaglandin-J_2 with or without the GW9662 as inhibitor. Gene expression was measured by qPCR and PCR Array. PPARg-PPRE binding was measured by ligand-binding assay. Phosphorylation levels of signaling molecules were analysed by flow cytometry. Data showed that Foxp3 mRNA expression in nTreg cells from both mice strains were down-regulated via PPAR-independant mechanism following treatments and synergized by GW9662. Binding activity between PPARg-PPRE was not significant in treated and untreated nTreg cells. PCR array data showed that genes involved in inflammatory-related pathways were differentially regulated in PPARγ ligand treated-nTreg cells from NOR and NOD. Ciglitazone induced expression of NF-κβ-related genes while suppressed MAPK, TGF-β, NFAT and PKC-related genes in NOR. Prostaglandin-J_2 induced *odc1* in treated-nTreg cells while down-regulated *fasl*, *il2* and *nab2* genes associated with NFAT and MAPK pathways. While both ligands suppressed gene expressions in MAPK, TGF-β, NKFB, NFAT and PKC pathways but not *myc* and *odc1* in NOD. Our findings suggest the negative regulation between PPARγ and Foxp3 expression in nTreg cells during normal and autoimmune T1D. The underlying mechanism of suppression is differentially regulated in healthy and T1D models.

id #1148

Tumor microenvironment and cancer cell metabolism

Huafeng Zhang[1]

1. School of Basic Medical Sciences, University of Science and Technology of China, Hefei, China

Cancer cells rewire metabolic pathways to obtain sufficient energy or building blocks to support rapid cell growth and proliferation. Hypoxia and nutrition deprivation are central characterictics of tumor microenvironment. Our recent studies revealing the interplay between cancer cell metabolism and tumor microenvironment will be presented.

id #1171

CHD4 slides nucleosomes by decoupling entry – exit-side DNA translocation

Yichen Zhong[1], **Bishnu Prasad Paudel**[2, 3], **Daniel P Ryan**[1], **Jason K K Low**[1], **Charlotte Franck**[1], **Karishma Patel**[1], **Max J Bedward**[1], **Richard J Payne**[3], **Antoine M van Oijen**[2, 3], **Joel P Mackay**[1]

1. University of Sydney, Darlington, NSW, Australia

2. University of Wollongong, NSW, Australia

3. Illawarra Health and Medical Research Institute, Wollongong, Australia

Chromatin remodellers convert the chemical energy of ATP hydrolysis to the mechanical movement of nucleosomal DNA relative to a histone octamer. The molecular basis for this activity is only partially resolved, and it is not clear if the mechanism is conserved among the four classes of remodelling enzyme. Of these enzymes, the least well understood family are the chromo-helicase domain (CHD) enzymes exemplified by CHD4, the remodelling component of the Nucleosome Remodelling and Deacetylase (NuRD) complex. Here, we use single-molecule assays to demonstrate that the flanking DNA enters and exits the nucleosome through two decoupled translocation processes, and the formation of a CHD4-nucleosome complex – even in the absence of nucleotide – is sufficient to prime the system. In combination with recent analyses of yeast remodelling enzymes, our data lead to a model for the mechanism of nucleosome sliding.

Abstracts: Posters

In Alphabetical Order
(presenting author surname)

id #1170

Dysregulated transcriptional response to differentiation signals in cohesin-mutant leukemia cells

Jisha Antony[1, 2], Gregory Gimenez[1], Terry Taylor[2], Umaima Khatoon[1], Robert Day[1], Ian M Morison[1], Julia A Horsfield[1]

1. The University of Otago, Dunedin, NZ, New Zealand

2. Southern Community Laboratories, Dunedin, New Zealand

Mutations in the subunits of the cohesin complex, particularly in the STAG2 subunit, have been identified in a range of myeloid malignancies, but it is unclear how these mutations progress leukaemia. Here, we created isogenic K562 erythromyeloid leukaemia cells with and without the known leukemic STAG2 null mutation, R614*. STAG2 null cells acquired stem cell and extracellular matrix gene expression signatures that accompanied an adherent phenotype. Chromatin accessibility was dramatically altered in STAG2 null K562, consistent with gene expression changes. Enhanced chromatin accessibility was observed at genes encoding hematopoietic transcription factors, ERG and RUNX1. Upon stimulation of megakaryocyte differentiation using phorbol 12-myristate 13-acetate (PMA), STAG2 null cells showed a precocious spike in *RUNX1* transcription that was associated with enhanced transcription from its proximal *P2* promoter. A similar precocious spike was observed in transcription of *ERG*. Interestingly, unrestrained transcription of these genes was confined to early time points of differentiation. Treatment of STAG2 null cells with enhancer-blocking BET inhibitor, JQ1, dampened precocious *RUNX1 P2* expression and blocked of *RUNX1 P1* and *ERG* transcription during PMA stimulation in both parental and STAG2 null cells These results suggest that precocious *RUNX1* and *ERG* transcription in STAG2 null cells is enhancer-driven. Furthermore, JQ1 treatment reduced stem cell-associated KIT expression in STAG2 null mutants. We conclude that STAG2 depletion in leukemic cells amplifies an enhancer-driven transcriptional response to differentiation signals, and that this characteristic is dampened by BET inhibition. The results are relevant for development of therapeutics in cohesin mutant myeloid leukaemia.

id #1095

Using *Xenous laevis* tadpole as a model for testing anti-inflammatory drugs as treatment for intractable epilepsy.

Sulagna Banerjee[1], Erin Damsteegt[1], Paul Szyszka[1], Caroline Beck[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

Epileptic encephalopathies are a group of catastrophic seizure syndromes often caused by *de novo* genetic mutations. Seizures typically manifest before 1 year of age, and patients often develop developmental and intellectual disabilities. They are at risk of sudden unexpected death and about 25% of the children die before the age of 20 years. One third of patients also develop drug resistance, where the patient's seizures cannot be managed through conventional anti-epileptic drugs, and only a few can be treated by brain surgery. In search of alternative therapy targets, current epilepsy research has put the spotlight on neuroinflammation. There is a correlation between brain inflammation and epileptogenesis, as well as worsening of the epileptic conditions. Anti-inflammatory drugs (AIDs) have shown disease modifying effects in some rat models. Here we test the hypothesis that inhibiting pro-inflammatory pathways reduces epileptic seizures in *Xenopus laevis* tadpoles – an epilepsy model which is more easily accessible to genetic manipulation than rats. We use behavioural and electrophysiological

assays to quantify epileptic seizures in this model. We first established the effects of AIDs like Losartan in the Pentylenetetrazol induced status epilepticus model of epilepsy. We are now developing *Xenopus laevis* epileptic encephalopathy models using CRISPR/Cas9 to induce gene alterations mimicking those found in human patients. We hope that with our model of intractable epilepsy, we can have a better understanding of how targeting neuroinflammation may help in reducing the severity of the seizures in these chronic conditions.

id #1154

SoDoPE: Soluble Domain for Protein Expression

Bikash Bhandari[1], Chun Shen Lim[1], Paul Gardner[1, 2]

1. University of Otago, Dunedin, OTAGO, New Zealand

2. Biomolecular Interaction Centre, University of Canterbury, Christchurch, Canterbury, New Zealand

Expression of soluble recombinant proteins is the first aim of functional and structural studies. Biotechnology and pharmaceutical applications often require soluble proteins at high concentrations. We have found that normalised temperature factors (B factors), can be used to accurately predict the solubility of recombinant proteins expressed in *Escherichia coli.* Based on this finding, we developed SoDoPE (Soluble Domain for Protein Expression). The tool first identifies functional protein domains and predicts their solubility using normalised B factors. Options will be given to users to maximise the solubility of a target protein region, i.e., extended regions with higher probabilities of solubility. After finalising the target protein region, protein expression level can then be optimised at the nucleotide sequence level using our recently developed tool TIsigner.

id #1175

A synthetic lethal drug screen identifies exploitable vulnerabilities in cohesin-deficient cells

Chue Vin Chin[1], Jisha Antony[1], Jinshu He[2], Kate M Parsons[2], Amee J George[2], Ross D Hannan[2], Julia Horsfield[1]

1. University of Otago, Dunedin, New Zealand

2. John Curtin School of Medical Research, Australian National University, Acton, Australia

The cohesin complex comprises four core subunits: RAD21, SMC3, SMC1A, and STAG2. Cohesin is required for many biological processes, including sister chromatid cohesion, DNA damage response, and ribosomal biogenesis. Somatic mutations in cohesin subunits are found in a wide range of human cancers, including acute myeloid leukemia (AML) (12%), Ewing sarcoma (15%) and glioblastoma (19%). Currently, there is no targeted therapeutic strategy for cancer patients with cohesin mutations. Synthetic lethality has emerged as a promising approach for targeted therapy. To identify synthetic lethal drug activity with cohesin mutations, we generated three isogenic cohesin-deficient MCF10A cell lines (RAD21+/-, SMC3+/-, and STAG2-/-) using CRISPR/Cas9. Cohesin-deficient MCF10A cell lines exhibit alterations in nucleolar morphology and increased γH2AX and p53 (except in STAG2-/-). RNA-sequencing analysis reveals downregulation of genes involved in regulation of RNA synthesis in these cell lines. Furthermore, ribosomal stress mediated by Actinomycin D led to severe nucleolar fragmentation in cohesin-deficient

MCF10A, but not MCF10A parental, suggesting that cohesin mutation might confer vulnerability to perturbation of ribosome biogenesis. Using cohesin-deficient MCF10A cell lines, we performed a high-throughput drug screen against 3,009 compounds of FDA-approved drugs, kinase, and epigenetic inhibitors. Several classes of inhibitors targeting mTOR signaling, Wnt signaling and chromatin modification were found to selectively inhibit the growth of cohesin-deficient MCF10A cells by 30% or more. In summary, we have identified potential synthetic lethal compounds that may provide the basis for development of targeted therapies of cancers with cohesin deficiency or mutation.

id #1133

Characterization of TrmA on oxidative stress response in *Pseudomonas aeruginosa*

Jurairat Chittrakanwong[1], Narumon Thongdee[1], Sopapan Atichartpongkul[2], Thanyaporn Srimahaeak[1], Skorn Mongkolsuk[1, 2], Mayuree Fuangthong[1, 2]

1. Applied Biological Sciences: Environmental Health Program, Chulabhorn Royal Academy, Chulabhorn Graduate Institute, Lak si, Bangkok, Thailand

2. Laboratory of Biotechnology, Chulabhorn Research Institute, Lak si, Bangkok, Thailand

Pseudomonas aeruginosa is a gram negative bacterium, which can cause nosocomial infection, particularly in immunocompromised patient. Regulation of gene expression at post-transcriptional level has been shown to be crucial for oxidative stress adaptation. *P. aeruginosa* TrmA shows about 50% identity to m^5U methyltransferase enzyme or TrmA in *E. coli*. Recombinant *P. aeruginosa* TrmA methylated tRNAs extracted from *P. aeruginosa* using *in vitro* methyltransferase assay, in which SAM was used as a methyl donor. Using disk diffusion and killing assay, *trmA* mutant was more sensitive to hydrogen peroxide (H_2O_2) than *P. aeruginosa* wild-type. The H_2O_2 sensitive phenotype could be complemented by introducing the *trmA* full length gene into the *trmA* mutant strain. Catalase activity was determined, since this is a main enzyme detoxifying H_2O_2. Interestingly, the *trmA* mutant had lower catalase activity than that of the wild-type and *trmA* complemented strains, supporting the H_2O_2 sensitive phenotype of the the *trmA* mutant. The increased TrmA protein expression concurs with the increased m^5U modification, when exposed to H_2O_2, demonstrating a possible function of TrmA-catalyzed m^5U formation in response to oxidative stress. Altogether, TrmA possesses methyltransferase activity for the formation of m^5U, and the TrmA-mediated m^5U may play a role in oxidative stress response.

id #1107

Why heal when you can regenerate? Whole body regeneration in *Botrylloides leachii*

Rebecca M Clarke[1], Michael Meier[1], Miles Lamare[2], Megan J Wilson[1]

1. Developmental Biology and Genomics Lab, Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand

2. Department of Marine Science, University of Otago, Dunedin, Otago, New Zealand

Whole body regeneration (WBR) is the ability to regrow an entire body or adult from a small collection of cells. Wound healing is the process that occurs after injury, and which utilises

components of the immune system, blood coagulation cascade and the inflammatory pathways to repair the wound. We study these processes using the colonial ascidian, *Botrylloides leachii*. *B. leachii* are chordates, they live in colonies that consist of adults, referred to as zooids, that share a common gelatinous matrix or tunic which is embedded with a vascular system. *B. leachii* have the ability to regenerate from only a few hundred cells, to form a fully functioning zooid within 8 days. Regeneration arises from a small section of blood vessels but it must not contain any adult zooids for the process to occur. Wound healing occurs in *B. leachii* when injury results in at least one zooid being left on the colony. It is currently unknown, besides the presence or absence of zooids, what triggers regeneration verses wound healing.

We are using RNA-sequencing at 1, 3, 5, and 10 hour times points to determine the genes and pathways unique to wound healing and regeneration. Preliminary data indicates that there are 121 up-regulated genes in 1h WBR in comparison to 1h WH and 228 with increased expression during 1h WH.

Understanding what triggers regeneration and wound healing may give insight as to why certain species have lost the ability to regenerate, along with the triggers of *B. leachii* WBR.

id #1132

Genome-wide DNA methylation and gene expression analysis of non-invasive and invasive melanoma cell lines

Jyoti Motwani[1], Euan J Rodger[1], Peter A Stockwell[1], Michael R Eccles[1]

1. University of Otago, Dunedin, NZ, New Zealand

Melanoma is a serious form of skin cancer: New Zealand has among the highest rates in the world (50 per 100,000). Although survival has improved due to advances in therapies, metastasis is still a major cause of mortality in melanoma patients. Metastasis is very likely to be related to tumour microenvironment, e.g. hypoxia, but a better understanding of the underlying mechanisms is required. Previously, we, and others, demonstrated two main subgroups of gene expression, which correlated with non-invasive and invasive behavioural characteristics of melanoma cell lines. In the present study we hypothesized that differential DNA methylation could be associated with invasive and non-invasive melanomas. We carried out genome-wide DNA methylation analysis using reduced representation bisulphite sequencing (RRBS). A total of 39 differentially methylated fragments (DMFs) were identified in invasive versus non-invasive cell lines, with 20 DMFs being hypermethylated and 19 DMFs hypomethylated. Integrated analysis of DNA methylation and RNA sequencing (RNA-Seq) was carried out on these cell lines to identify possible relationships between DNA methylation and gene expression, resulting in a total of 17 common differentially expressed genes associated with differential DNA methylation. Further analyses of these data are on-going, including RNA-Seq analysis of 70+ melanoma cell lines, including 8/9 of the cell lines used. In summary, integrated analysis of DNA methylation and gene expression between invasive and non-invasive melanoma cell lines has revealed a list of differentially methylated and differentially expressed genes that are potentially involved in the mechanisms associated with the invasive phenotype exhibited in melanoma cell lines.

id #1138

A Novel Insight into the Regulation of GBA in Parkinson's Disease

Sophie L Farrow[1], William Schierding[1], Sara Qubisi[2], Alan Davidson[2], Jo Perry[1], Antony Cooper[3, 4], Justin O'Sullivan[1]

1. Liggins Institute , University of Auckland, Auckland, New Zealand

2. Molecular Medicine and Pathology , University of Auckland, Auckland, New Zealand

3. Division of Neuroscience, Garvan Institute of Medical Research, Sydney, New South Wales, Australia

4. St Vincent's Clinical School, UNSW Sydney, Sydney, New South Wales, Australia

Parkinson's disease (PD) is a complex neurodegenerative illness affecting more than 7 million individuals worldwide. The majority of cases are idiopathic, yet evidence increasingly points to a strong genetic component. Mutations within the GBA gene, which impact GCase activity, are the greatest genetic risk factor for familial PD. However, reduced GCase activity has been identified in idiopathic PD patients lacking GBA mutations, consistent with alternative disease mechanisms. We hypothesised that enhancers located at the GBA locus and throughout the 3D genome regulate GBA and modifier genes that impact on alternative mechanisms of PD development. We utilised an algorithm that uses information on the 3D organization of the genome to screen variants within the GBA locus for regulatory potential. Seventy-three variants were identified that collectively regulate 143 genes. We subsequently performed a genome-wide search of 31,471,836 SNPs for regulatory activity on GBA in PD relevant tissues and identified a *trans*-acting regulatory region in ELFN2 (chr.22) that downregulates GBA expression in the substantia nigra (SN). We contend that this regulatory region contributes to the observed selective reduction of GBA/GCase activity in the SN of idiopathic PD patients lacking GBA missense mutations. Our results illustrate that GBA is a hub of regulatory activity, fundamental to PD pathology. Expanding these efforts to functional validation of the regulatory regions will quantify the effects and their potential for disease stratification.

id #1139

Shared regulatory pathways reveal novel genetic correlations between grip strength and neuromuscular disorders

Sreemol Gokuladhas[1], William Schierding[1], David Cameron-Smith[2], Melissa Wake[3], Emma L Scotter[5, 4], Justin O'Sullivan[1]

1. Liggins Institute, The University of Auckland, Auckland, New Zealand

2. Singapore Institute for Clinical Sciences, Agency for Science, Technology and Research, Singapore

3. Murdoch Children's Research Institute, The University of Melbourne, Victoria, Australia

4. Department of Pharmacology and Clinical Pharmacology, The University of Auckland, Auckland, New Zealand

5. Centre for Brain Research, The University of Auckland, Auckland, New Zealand

Muscle weakness and muscle wasting can be a consequence of aging (sarcopenia) and neuromuscular disorders (NMD). Genome-wide association studies have identified genetic variants associated with grip strength (GS; an inverse measure of muscle weakness) and NMD (multiple sclerosis (MS), myasthenia gravis (MG) and amyotrophic lateral sclerosis (ALS)).

However, how these variants contribute to the muscle weakness caused by aging or NMD remains obscure. We have integrated GS and NMD associated SNPs in a multimorbid analysis that leverages high-throughput chromatin interaction (Hi-C) data and expression quantitative trait loci (eQTL) data to identify allele-specific gene regulation (*i.e.* eGenes). Pathways and shared drug targets that are enriched by colocalised eGenes were then identified using pathway and drug enrichment analysis. We identified gene regulatory mechanisms (eQTL-eGene effects) associated with GS, MG, MS, and ALS. The eQTLs associated with GS regulate a subset of eGenes that are also regulated by the eQTLs of MS, MG, and ALS. Yet, we did not find any eGenes commonly regulated by all four phenotype-associated eQTLs. By contrast, we identified three pathways (mTOR signaling, axon guidance, and alcoholism) that are commonly affected by the gene regulatory mechanisms associated with all four phenotypes. Furthermore, 13% of the eGenes we identified were known drug targets, and GS shares at least one druggable eGene and pathway with each of the NMD phenotypes. Collectively, these findings identify significant biological overlaps between GS and NMD, demonstrating the potential for spatial genetic analysis to identify mechanisms underlying muscle weakness caused by aging and NMD.

id #1178

Decoding the multimorbidities among psychiatric disorders and cognitive functioning

Evgeniia Golovina[1, 2], M H Vickers[1], C D Erb[3], J M O'Sullivan[1, 2]

1. Liggins Institute, The University of Auckland, Auckland, New Zealand

2. A Better Start National Science Challenge, Auckland, New Zealand

3. School of Psychology, The University of Auckland, Auckland, New Zealand

Epidemiological studies have identified multimorbidities for psychiatric disorders and cognitive functioning and suggest that common biological mechanisms may underlie these phenotypes. Single nucleotide polymorphisms (SNPs) represent common predisposing genetic factors. These can occur at regulatory sites and contribute to multimorbidities via their impact on gene expression and biological pathways. Genome-wide association studies (GWAS) have identified thousands of SNPs associated with psychiatric disorders and cognition. However, the regulatory contributions that these SNPs make to multimorbidity are largely unknown.

Here, we integrate 3D genome organization and expression quantitative trait (eQTL) analyses to identify genes and pathways that are functionally impacted by 2,893 GWAS SNPs ($p < 1{\times}10^{-6}$) associated with cognition and five psychiatric disorders (i.e. ADHD, anxiety, bipolar disorder, unipolar depression and schizophrenia). The analysis revealed 33 genes and 62 biological pathways that were commonly affected by the gene regulatory interactions associated with all six phenotypes despite there being no common SNPs and eQTLs. 16% of the ADHD-risk, 16.7% of the anxiety-risk, 20.5% of the BD-risk, 15.4% of the UD-risk, 17.1% of the SCZ-risk and 15.8% of the cognition-associated genes we identified represent known drug targets. Four druggable genes (i.e. *AS3MT*, *FLOT1*, *HLA-A* and *PBRM1*) were affected by eQTLs from all six phenotypes.

Collectively, our analyses inform the extent of genetic impacts on regulatory mechanisms and pathways that contribute to multimorbidities among psychiatric and cognitive phenotypes. Our results represent the foundation for a shift from a gene-targeted towards a pathway-based approach to the treatment and enhancement of multimorbid psychiatric and cognitive conditions.

id #1098

Dual-species transcriptomics to investigate parasitism resistance in a classical biological control system

Sarah N Inwood[1], Tom W Harrop[1], Peter K Dearden[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

The Argentine stem weevil (*Listronotus bonariensis*) is an economically significant pasture pest in New Zealand, primarily controlled by *Microctonus hyperodae*; a parasitoid wasp biological control agent. *M. hyperodae* parasitises adult weevils and develops within the host, killing it as the parasitoid larvae emerges. Initial parasitism rates were as high as 80% but are now declining significantly, resulting in severe pasture damage totalling an estimated NZ$160M per annum. This is the first reported example of resistance in a biological control system worldwide, and a genetic basis for this parasitism resistance is suspected. Resistance to parasitism is most likely to be either a barrier preventing the parasitism event itself, or a host-mediated immune response towards the oviposited parasitoid egg.

Dual-species RNAseq was used to investigate the possibility of a host immune response toward the parasitoid egg. Results show that while parasitism causes changes in expression in a small subset of genes in the *L. bonariensis*transcriptome; these are involved in metabolism, regulation of fertility, and the innate immune system (against potential bacterial infection). Dual-species transcriptomic techniques also allowed for gene expression analysis of the simultaneously developing parasitoid egg, revealing changes to metabolism, epigenetic regulation and the initiation of nervous system development. The absence of genes involved in the cellular immune system, responsible for immune response and subsequent encapsulation of parasitoid eggs in *Drosophila melanogaster*, and the similarity of parasitism-induced changes with hosts of model-parasitoid *Nasonia vitripennis,* which do not display parasitism resistance, implies resistance is unlikely to be a post-parasitism immune response.

id #1159

CHD Family have Distinct Translocation Dynamics on Nucleosome Complex

Chanshin Kang[1], Eunhye Lee[2], Ji-Joon Song[2], Sungchul Hohng[1]

1. Seoul National University, Gwanak-Gu, SEOUL, South Korea

2. Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea

ATPase remodelers translocate DNA which bound along nucleosome. The remodeling mechanism takes huge parts of a central dogma which might be affect to DNA replication or transcription. Among various ATPase remodelers, Chromodomain-helicase-DNA-binding protein(CHD) family has similar helicase and ATPase domains which relates to SNF2. We used a single-molecule fluorescence approach to observe quantitative kinematics of remodelers on nucleosome complex that 601 DNA is bound along histone octamers. According to Fluorescence Resonance Energy Transfer(FRET) pictures, we got traces of a distance DNA related to histone nucleosome when ATPase remodelers are applied in high time resolution. Focus on CHD7, it relocates DNA in large steps but the step is composed of 1-2 fundamental steps. Compare to CHD1 which is also CHD family, CHD7 has not only similarity in remodeling activity but also noticeable different fundamental step size aspect. Our results distinguish ATPase remodelers: CHD7, CHD1 and ACF/Snf2h.

id #1115

Structural study on prokaryotic transcription - how RNA polymerases pause and go

Jin Young Kang[1]

1. KAIST, Daejon, DAEJON, South Korea

The transcriptional pausing plays pivotal roles in transcription initiation, elongation, termination, RNA folding, and translation. In E.coli, RNA polymerase (RNAP) pauses at every ~100 bps during transcription and hairpin structure of nascent RNA increases pause lifetimes ten-fold or more. Despite its central life-maintaining function, the underlying molecular mechanism of transcriptional pausing has remained elusive. To probe this, we determined a 3.8 Å cryo-electron microscopy (cryo-EM) structure of an E. coli his pause elongation complex (hisPEC). From the structure, we found that i) the RNA hairpin stem forms within the RNA exit channel of RNAP, ii) the DNA:RNA hybrid is trapped in a distinctive half-translocated conformation, and iii) mobile domains of the RNAP swiveled in a concerted manner. To understand how these pauses are regulated, we solved structures of anti-pausing factors, NusG- and RfaH-bound elongation complexes at 3.7 Å and 3.5 Å resolution, respectively. These revealed that NusG and RfaH bind to the β protrusion, β gate loop, and β' clamp helices of RNAP, stabilizing the RNAP in a conformation of active elongation state. While both NusG and RfaH reduce backtrack pausing, only RfaH blocks RNA hairpin-stabilized pausing by binding to the elongation complex tightly enough to resist RNAP swiveling. Based on our results, we propose that the pause RNA hairpin stabilizes global conformational changes in the RNAP that secures the paused state while NusG and RfaH enhance transcription elongation by maintaining the active conformation of the elongation complex.

id #1144

RNA polymerase can reinitiate at opposite direction promoter by hopping after intrinsic termination

Wooyoung Kang[1], **Changwon Kang**[2], **Sungchul Hohng**[1]

1. Department of Physics and Astronomy, Seoul National University, Seoul , Republic of Korea

2. Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon , Republic of Korea

Despite previous studies about intrinsic termination, it is uncovered what is the fate of RNA polymerase (RNAP) after termination. In our previous single molecule fluorescence study, RNAP dissociation is often followed by RNA transcript release in bacterial intrinsic termination. Moreover, remained RNAPs on DNA can diffuse one dimensionally in bi-direction, and these RNAPs can bind to nearby promoter on template DNA and initiate transcription again. In this study, we measure the diffusion coefficient of remained RNAPs under variable salt condition and we unveiled that diffusion of RNAPs after termination can be described as hopping. Furthermore, this hopping mode makes it possible that diffusion of RNAPs recognizes the promoter located on not only template DNA but non-template DNA.

id #1169

Subfunctionalization of cohesin *STAG1/2* in zebrafish development and disease

Sarada Ketharnathan[1], A Labudina[1], S Lee[1], J Antony[1], I Morison[1], J Horsfield[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

Cohesin is a multimeric protein made up of three core subunits in somatic cells: *RAD21*, *SMC3*, *SMC1A* and additionally, either *STAG1* or *STAG2*. Cohesin-STAG1 and cohesin-STAG2 have both redundant and unique functions in key processes such as cell division and transcriptional regulation via genome organization. STAG2 is the most frequently mutated cohesin complex member and is one among only 12 genes that are significantly mutated in at least four cancer types, including acute myeloid leukaemia.

Zebrafish represent a well-established model to study haematopoiesis. Using CRISPR-Cas9 we have generated germline zebrafish mutants for three of four *stag* paralogs, namely *stag1a*, *stag1b* and *stag2b*. We observed disruption of both primitive and definitive embryonic haematopoiesis in our *stag1a* mutants, with primitive myeloid skewing and a decrease in the number of definitive haematopoietic stem cells. Our s*tag1b* and *stag2b* mutants showed a reduction in primitive erythropoiesis and in addition exhibited striking defects in tail development, such as mis-localization of certain cell types and the development of ectopic secondary structures. *stag2a* transcript is maternally inherited and expressed in the ovary, therefore mutants may not have been recovered owing to a critical role for *stag2a* in germ cell development.

Our results raise the possibility of unique cell type-specific requirements for the different *stag* paralogs in zebrafish while confirming their importance in haematopoiesis.

id #1160

Drosophila models reveal FUBP1 and CIC, predicted oligodendroglioma driver mutations, inhibit expansion of the neural stem cell lineage *in vivo*

Nan-Hee Kim[1], Olga Zaytseva[1, 2], Naomi Mitchell[1, 2], Caroline Delandre[3], Mark Gilbert[4], David Levens[4], Owen Marshall[3], Leonie Quinn[1, 2]

1. Department of Cancer Biology and Therapeutics, The Australian National University, Canberra, ACT, Australia

2. The University of Melbourne, Melbourne, VIC, Australia

3. Menzies Institute for Medical Research, Hobart, TAS, Australia

4. Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA

FUBP1, a single stranded DNA/RNA binding protein, was initially characterised as a transcriptional activator of the *MYC* oncogene and thus driver of cell growth and proliferation. Genome sequencing in oligodendroglioma, however, identified putative loss-of-function FUBP1mutations as tumour drivers, suggesting that FUBP1 behaves as a tumour suppressor in the context of the brain. Exciting preliminary data demonstrate FUBP1/*Psi* knockdown specifically in the neural stem cells drives lineage expansion, consistent with tumour suppressor function in oligodendroglioma. Not only were genes controlling neural stem cell lineage maintenance and

proliferation identified as direct FUBP1/Psi targets using Targeted DamID (TaDa), but analysis of TCGA data revealed 72% of the human orthologs of the *Drosophila* targets were dysregulated in low grade glioma. As FUBP1 mutations significantly co-occur with loss-of-function mutations in the CIC transcriptional repressor of receptor tyrosine kinase signalling, we are testing whether expansion of the neural stem cell lineage driven by FUBP1/*Psi* knockdown is modified by concurrent CIC/*cic* knockdown. We will further investigate genome wide CIC binding in the stem cell lineage, correlate with active and inactive chromatin state, and determine whether CIC binding is modified by Psi abundance (and vice versa). Thus we will determine how FUBP1/Psi and CIC interact to control neural stem cell fate in *Drosophila*, providing *in vivo* insight into how loss-of-function for the FUBP1 and CIC transcriptional regulators drives oligodendroglioma.

id #1183

Analysis of Menin in breast cancers reveals its role in cancer epigenome regulation

Taewan Kim[1], Eun-Jung Cho[1]

1. Sungkyunkwan university, Suwon, GYEONGGI-DO, South Korea

Menin, encoded by the Multiple Endocrine Neoplasia Type 1 (MEN1), functions as a scaffold protein interacting with a variety of transcription factors to control active or repressive expression of target genes involved in myriad cellular processes. While menin has been originally reported as a tumor suppressor in pancreatic tumors, it is also associated with tumorigenesis by interaction with MLL to promote tumors in the blood and other solid tissues, including luminal breast cancer, prostate cancer, and liver cancer. These studies place firmly menin as a therapeutic target for the development of small molecules that inhibit Menin-MLL interaction. More recently, DAXX (Death-domain associated protein) a chaperone for histone H3.3, was identified as an interaction partner, to which Menin competes with H3.3 for binding. Since DAXX is involved in H3.3 loading through PML and global heterochromatin formation, Menin has a potential to affect the DAXX-dependent H3.3 deposition into chromatin via PML. In this study, we found that targeting Menin impairs the growth of breast cancer cells of both luminal and triple negative breast cancer (TNBC) types. Furthermore, we found that cellular localization of DAXX was profoundly affected upon inhibition of menin. The nuclear colocalization of DAXX with PML foci was significantly increased by menin inhibition, indicating the impact of menin on the H3.3 cancer epigenome. We further discuss the implication of Menin-DAXX-H3.3 interaction required for the chromatin dynamics during breast cancer tumorigenesis.

id #1180

Pericentromeric heterochromatin clustering is impaired in muscle atrophy

HongMin Lee[1], Jinyoung Park[1], Eun-Jung Cho[1]

1. Sungkyunkwan University School of Pharmacy, South Korea

During skeletal muscle development and regeneration, myogenic precursors termed satellite cells undergo several cellular and molecular changes while they differentiate to multinucleated myotubes that express a large amount of contractile muscle proteins. Such myogenic differentiation is particularly highlighted by a progressive clustering of heterochromatin that form a large repressive compartment within nucleus, called chromocenters. Meanwhile, muscle

atrophy, initiated by various conditions such as aging or cancer, is characterized by a large decrease in muscle proteins and reduction of fiber diameter. When muscle atrophy undergoes, it is well described that proteolytic systems such as the ubiquitination-proteasome system (UPS) pathway or the autophagy-lysosomal system (ALS) pathway are activated, leading to decrease of contractile muscle proteins. However, it's not known whether the expression of muscle genes or the chromatin condition were altered. Therefore, we have investigated any changes in chromatin signatures in atrophy-induced muscle cells. We found that heterochromatin integrity of myotube nuclei was primarily affected during muscle atrophy. Here we show that the number of chromocenters of atrophy muscle cells were increased with heterochromatin foci being scattered. Also, H4K20me3, a heterochromatin-enriched histone mark and the histone chaperone DAXX tended to be delocalized from the chromocenter foci throughout the nucleus, suggesting that focal localization of chromatin factors and heterochromatin clustering might be affected during muscle atrophy. We further discuss the underlying mechanisms that are required for the maintenance of muscle integrity.

id #1168

Characterisation of zebrafish meiotic cohesin subunits and their roles in gametogenesis and fertility

Stephanie Lee[1], Neil Gemmell[1], Julia Horsfield[1]

1. University of Otago, Dunedin, New Zealand

Aneuploidy due to germ cell chromosome mis-segregation during meiosis is a leading cause of miscarriage, age-related infertility and high Down's syndrome incidence in humans. Sister chromatid cohesion during meiosis facilitates accurate chromosome segregation in germ cells.

The cohesin complex is important for sister chromatid cohesion in mammalian meiosis, particularly in oocytes. Lack of Rec 8-cohesin turnover in oocytes and loss of chromosomal-associated Rec8 contributes towards maternal age related aneuploidies. Premature ovarian insufficiency has been traced to Stag3 truncating variants in a few families.

Fish are a good model for understanding germ cell aneuploidy as they share many aspects of gamete development with mammals. However little is known about the roles of genes involved in meiosis in fish. In the present study, we characterise the zebrafish homologues of the meiotic cohesin subunits *rec8* and *stag3* with the aim of understanding how they influence gametogenesis and fertility.

id #1161

Highly accessible translation initiation sites are predictive of successful heterologous protein expression

Chun Shen Lim[1], Bikash K. Bhandari[1], Paul P. Gardner[1]

1. Department of Biochemistry, University of Otago, Dunedin, New Zealand

Recombinant protein production in microbial systems is well-established, yet half of these experiments have failed in the expression phase. Failures are expected for 'difficult-to-express' proteins, but for others, codon bias, mRNA folding, avoidance, and G+C content have been suggested to explain observed levels of protein expression. However, determining which of these is the strongest predictor is still an active area of research. We used an ensemble average

of energy model for RNA to show that the accessibility of translation initiation sites outperforms other features in predicting the outcomes of 11,430 experiments of recombinant protein production in *Escherichia coli*. We developed TIsigner and showed that synonymous codon changes within the first nine codons are sufficient to improve the accessibility of translation initiation sites. Our software produces scores for both input and optimised sequences, so that success/failure can be predicted and prevented by PCR cloning of optimised sequences.

id #1113

Single molecule studies on R-loop formed during transcription

Gunhyoung Lim[1], Sungchul Hohng[1]

1. Seoul National University, Sillim 9-dong, Gwanak-gu, SEOUL, South Korea

R-loop is one of the noncanonical structures of nucleic acid able to be formed during transcription. As a three-stranded nucleic acid structure, it consists of two components: one DNA:RNA hybrid, and a remnant ssDNA. Since the exposed ssDNA is frail, it could be said that there is tentative downsides in respect of genome stability. This R-loop structure, however, is not a merely malign factor which should be eliminated instantly, because it has crucial functions in some biological processes. For instance, recent studies on R-loop shed light on its positive roles like R-loop driven ATR pathway required for proper chromosome segregation. Considering its positive and negative aspects, it is important that this structure should be appropriately regulated. So, for better understanding of these regulation processes, our study has focused on observing the formation and elimination of R-loop using single-molecule FRET technique. As a result, for the first time, we pulled off observing the co-transcriptional formation of R-loop at the single molecule level in real time. And then, we found out the detail features of this structure such as reaction to RNase H known for eliminating R-loop, relation with G-quadruplex, and formation efficiency in different sequences including trinucleotide repeat expansion sequence causing neurodegenerative disorders like Huntington's disease.

id #1172

The role of ZBTB7A in foetal *globin* repression

Lana Ly[1], Kate G.R Quinlan[1], Merlin Crossley[1]

1. University of New South Wales, Kensington, NSW, Australia

The *globin* loci encode subunits of haemoglobin, an oxygen-carrying protein that is abundant in red blood cells. Beta-haemoglobinopathies, such as sickle cell anaemia and beta-thalassemia, arise from mutations in the adult *beta-globin* gene. There are no fully effective treatments for these conditions and since the number of affected infants born each year is rising as populations increase across areas with high carrier rates, the search for effective long-term treatments is ongoing.

Reactivation of the silenced foetal *gamma-globin* genes can compensate for dysfunctional adult beta-globin and alleviates symptoms. One strategy towards reactivating the foetal *globin* genes is to interfere with the mechanism of repression. We have shown that the Zinc Finger and BTB Domain Containing 7A (ZBTB7A) acts by binding to the -200 proximal promoter site and directly represses foetal globin expression.

Here we investigate ZBTB7A's mechanism of action. ZBTB7A consists of four classical zinc fingers and an N-terminal POZ domain that can mediate self-association and contact to partner proteins. We have investigated the sequence recognition motifs of individual zinc fingers and the requirement for self-association via the POZ domain. We are investigating a model whereby ZBTB7A may bind at multiple sites across the locus to ensure the appropriate 3D topology of the locus, including looping of the power Locus Control Region enhancer to individual *globin* gene promoters.

id #1182

Good genes gone bad: are placental genes hijacked by cancer cells to facilitate invasion?

Chi Lynch-Sutherland[1], P A Stockwell[1], M R Eccles[1, 2], A Chatterjee[1, 2], E C Macaulay[1]

1. Department of Pathology, University of Otago, Dunedin School of Medicine, Dunedin, NZ

2. Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, NZ

The human placenta may offer novel insights into the molecular mechanisms that drive cancer invasion and metastasis. During early pregnancy the placenta invades into the uterus and manipulates the maternal immune response, showing striking similarities to cancer. The placenta and cancer lack DNA methylation at some retrotransposon sequences. Demethylation of retrotransposons in the placenta has given rise to new genes and regulatory elements, some of which are critical for placental development. Surprisingly, these placental genes are also overexpressed in a number of cancers, but their function is not yet known. This project seeks to document the abundance and diversity of functional retrotransposons in the placenta and reveal the functional role of these elements in cancer. We have developed a bioinformatic pipeline to identify functional retrotransposons in the placenta and other early developmental stages. Expression of these elements has also been investigated in melanoma revealing that they are upregulated in comparison to corresponding somatic tissue. Currently we are investigating the mechanism which permits activation of these functional retrotransposons in cancer along with the functional significance. We expect that reactivation of these genes and regulatory elements occurs as a consequence of dedifferentiation associated epigenetic changes and may drive further differentiation of tumours. Moreover, there is compelling evidence that dedifferentiation can contribute to invasion and metastasis. The specificity of these elements to the placenta and cancers means they could to provide valuable diagnostic and therapeutic targets, and may help to identify cancers at a higher risk of metastasis.

id #1164

FUBP1/Psi functions in the niche to non-autonomously control neural stem cell fate

Damien Muckle[1], Olga Zaytseva[1, 2], Nan-Hee Kim[1], Naomi Mitchell[1, 2], Caroline Delandre[3], David Levens[4], Owen Marshall[3], Leonie Quinn[1, 2]

1. John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia

2. University of Melbourne, Melbourne, VIC, Australia

3. Menzies Institute for Medical Research, Hobart, TAS, Australia

4. National Cancer Institute, National Institute of Health, Bethesda, Maryland, United States of America

The ssDNA binding protein FUBP1 was originally characterised as a transcriptional activator of the *MYC* oncogene. Surprisingly, however, FUBP1 loss-of-function has been identified as a frequent driver mutation in oligodendroglioma. To understand the networks associated with the unexpected tumour suppressive role for FUBP1 in the brain, we have taken advantage of the conservation of FUBP1 (Psi) in *Drosophila.* A major impediment to improved glioma treatment is our lack of understanding of the interaction between glioma stem cells and their glial microenvironment. Thus, we aim to elucidate FUBP1/Psi function not only in neural stem cells but also in the cortical glial microenvironment, or niche, providing the structural support and secreted signals required for stemness and differentiation. Our preliminary data demonstrate the importance of FUBP1/Psi in the microenvironment, where specific depletion of Psi in the cortical glia non-autonomously drives neural stem cell expansion. To better understand how FUBP1/Psi non-autonomously controls neural stem cell fate we used Targeted DamID (TaDa) to identify direct, genome-wide, targets in the cortical glia. Intriguingly, targets included secreted proteins predicted to signal from the cortical glia to control fate of neighbouring neural stem cells. Furthermore, analysis of TCGA datasets revealed 82% of the human orthologues of FUBP1/Psi targets are frequently dysregulated in low grade glioma. Together, our data demonstrate Psi normally acts extrinsically in the glial microenvironment to prevent neural stem cell expansion. We therefore hypothesise that FUBP1 loss-of-function in glioma drives stem cell expansion and tumourigenesis, at least in part, by indirectly disrupting the glial microenvironment.

id #1155

E2A-PBX1 functions as a coactivator for RUNX1 in acute lymphoblastic leukemia

Wen-Chieh Pi[1], Jun Wang[2], Miho Shimada[3], Huimin Geng[4], Yu-Ling Lee[3], Dongxu Li[2], Gang G. Wang[2], Robert G. Roeder[3], Wei-Yi Chen[1]

1. Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei, Taiwan

2. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, USA

3. Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY, USA

4. Laboratory Medicine, UCSF School of Medicine, San Francisco, CA, USA

E2A, a basic helix-loop-helix (bHLH) transcription factor, plays a crucial role in determining tissue-specific cell fate, including differentiation of B cell lineages. In 5% of childhood acute lymphoblastic leukemia (ALL), the t(1,19) chromosomal translocation specifically targets the *E2A* gene and produces an oncogenic E2A-PBX1 fusion protein. While previous studies have demonstrated oncogenic functions of E2A-PBX1 in cell and animal models, the E2A-PBX1-enforced cistrome, the E2A-PBX1 interactome, and related mechanisms underlying leukemogenesis remain unclear. Here, by unbiased genomic profiling approaches, we identify the direct target sites of E2A-PBX1 in t(1,19)-positive pre-B ALL cells and show that, compared to normal E2A, E2A-PBX1 preferentially binds to a subset of gene loci co-bound by RUNX1 and gene-activating machineries (p300, MED1, and H3K27 acetylation). Using biochemical analyses, we further document a direct interaction between E2A-PBX1 and RUNX1 and show that E2A-PBX1 binding to gene enhancers is dependent on RUNX1, but not the DNA-binding activity harbored within the PBX1 homeodomain of E2A-PBX1. Transcriptome analyses and cell transformation assays further establish a significant RUNX1 requirement for E2A-PBX1-mediated target gene

activation and leukemogenesis. Notably, the RUNX1 locus itself is also directly activated by E2A-PBX1, indicating a multilayered interplay between E2A-PBX1 and RUNX1. Collectively, our study provides the first unbiased profiling of the E2A-PBX1 cistrome in pre-B ALL cells and reveals a previously unappreciated pathway in which E2A-PBX1 acts in concert with RUNX1 to enforce transcriptome alterations for the development of pre-B ALL.

id #1173

WDR5 is a novel partner of KLF3 and is important in KLF3 genomic localisation and gene regulation

Lu YaNG[1], Tanit Cavalit[1], Manan Shah[1], Wooi F Lim[1], Ling Zhong[1], Mark J Raftery[1], Marc R Wilkins[1], Kate G R Quinlan[1], Merlin Crossley[1]

1. University of New South Wales, Sydney, NSW, Australia

Transcription factors are generally considered to be comprised of two main components; a DNA-binding domain (DBD) and a functional domain (FD). Unsurprisingly, it is typically thought that the DBD is responsible for localisation and DNA binding whereas the FD is involved in transcriptional regulation. However, we have previously shown that the zinc finger transcription factor Krüppel like factor 3 (KLF3) does not solely rely on the DBD and that the FD also plays a role in genome-wide localisation. We hypothesised that this may be due to the interaction of the FD with a cofactor(s).

To identify potential candidates, we performed co-immunoprecipitation coupled mass spectrometry (CoIP/MS) using a cell line stably expressing the KLF3 FD. WDR5, a member of the WD repeat protein family was identified as a novel KLF3 partner protein and was investigated further. We mapped the interaction interface between WDR5 and the KLF3 FD, identifying a key residue and generating a KLF3 mutant construct that was unable to bind WDR5. To study the role of this interaction, we generated MEF cell lines overexpressing either WT KLF3 or Mutant KLF3 and performed KLF3 and WDR5 ChIP-seq and RNA-seq.

The mutation which disrupted the KLF3/WDR5 interaction caused genome-wide changes to KLF3 binding as well as significant transcriptome changes suggesting that the interaction is both important for KLF3 genomic localisation and gene regulation. Most genes where KLF3 binding is lost due to the mutation are downregulated, suggesting that the KLF3/WDR5 interaction is particularly important in gene activation.

id #1127

High-energy initiation complex retaining σ70 identified as complex hypersensitive to pyrophosphate

Nobuo Shimamoto[1, 2], Masahiko Imashimizu[1, 3]

1. National Institute of Genetics, Japan, Kyoto, JAPAN, Japan

2. Veritas Kitayama, Kyoto, Japan

3. Molecular Profiling Research Center for Drug Discovery, National Institute of Advanced Industrial Science and Technology, Tokyo, JAPAN

During transition from initiation to elongation in *E. coli* transcription, σ^{70} is supposed to be released from transcription complex to remove a steric clash of the 5' end of nascent RNA with

the σ^{70} regions 4 and 3.2 loop. The existence of such high-energy complex has been proposed in abortive initiation but which does not lead to productive elongation.

In this study, we have found a high-energy transcription complex retaining σ^{70} and 9 nt nascent RNA, that is long enough to reach σ^{70} region 4. It catalyzes the reverse reaction of elongation, pyrophosphorolysis, in the presence of low concentrations of PPi which does not inhibit elongation by most transcription complexes. We also demonstrated that the hypersensitivity to PPi was attributed to the complex performing productive RNA synthesis but not abortive RNA synthesis. The high-energy complex is only formed through the transcription initiation from a promoter but not from the reconstitution with RNA polymerase, DNA, and 9nt RNA, consistent with the interpretation that the energy originates from the breakage of β-γ phosphodiester bond of NTP. These results provide energetic evidence for a high-energy initiation complex retaining σ^{70} during the initiation-to-elongation transition. Cellular PPi as well as the high-energy conformation may accelerate the transition and the consequent productive elongation.

Imashimizu M. et al., JMB (2019) 431, 2528-42

id #1122

RNAP-bound factor Rho finely regulates premature transcription termination in prokaryote

Eunho Song[1], Heesoo Uhm[2, 1], Palinda Munasingha[3], Yeon-Soo Seo[3], Kook Sun Ha[3, 4], Jin Young Kang[5], Changwon Kang[3], Sungchul Hohng[1]

1. Department of Physics and Astronomy, and Institute of Applied Physics, Seoul National University, Seoul

2. Department of Physics, University of Oxford, Oxford

3. Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon

4. Department of Life Science, University of Suwon, Gyeonggi-do

5. Department of Chemistry, Korea Advanced Institute of Science and Technology, Daejeon

Factor Rho is key factor in factor-dependent termination in prokaryote. Rho-dependent termination happens in the regions identified as Rho-terminator, many of which position in downstream of open reading frame (ORF). In recent, it was reported that Rho-terminator locates the leader region of ORF and that some of them shows varying termination efficiency depending on the change of external environment. The representative case is *mgtA* terminator, combined with magnesium-sensing riboswitch only to regulates termination efficiency. To study the regulation mechanism of Rho-dependent premature transcription termination, we developed single-molecule fluorescence assay for observing transcription termination. We found that there are two distinct termination pathways, RNAP-dependent pathway and RNA-dependent pathway in *mgtA* terminator. RNAP-dependent pathway solely includes termination caused by RNAP-bound Rho, but RNA-dependent pathway means termination caused by factor Rho directly binding to the RNA. RNAP-dependent pathway showed more dependence on magnesium condition than RNA-dependent pathway, which means RNAP-dependent pathway mainly bring about magnesium-sensing property of *mgtA*. To understand the difference in sensitivity to ion of two pathways, we examined several different DNA substrates with mutants in pausing site. These examinations showed that RNAP-dependent pathway was slower than RNA-dependent pathway, so this pathway more sensitively influenced by b narrower time window in disruption of pausing site. These results suggested that slow termination pathway caused by RNAP-bound Rho finely tune premature transcription termination in response to external change.

id #1097

Haploinsufficiency of LIM Homeobox 9 (*Lhx9*) during genital ridge development impacts ovarian function and infertility

Stephanie Workman[1], Megan Wilson[1]

1. The University of Otago, Dunedin, OTAGO, New Zealand

The network of transcription factors that regulate the development of the reproductive progenitor tissue, the genital ridge, is complex, with only a handful of genes identified as being vital for its formation. Given that the genital ridge will form a majority of the tissues of both the male and female reproductive systems, it is imperative that we characterise the transcriptional regulation of its development if we are to understand abnormalities that arise in disorders of sex development and infertility.

One transcription factor identified as being vital for genital ridge development is LIM Homeobox 9 (Lhx9). Knockout mouse models fail to form gonads, while heterozygous models exhibit reduced fertility and altered ovarian structure. RNA-sequencing and RT-qPCR analysis show altered expression of important cell markers and transcriptional regulators of ovarian function in heterozygous mice compared to wildtype littermates.

Interestingly *Lhx9* displays sex dimorphic expression in the genital ridge prior to *Sry* gene expression. We hypothesised that this differential mRNA expression was due to methylation patterns established early in development, as previous publications have identified methylation differences at these sites in other tissues. Targeted methylation analysis of three CpG islands associated with the *Lhx9* promoter revealed that the average methylation level was significantly greater in males, correlating with mRNA expression being higher in females.

Future characterisation of the differences in *Lhx9* function between males and females will be carried out using ChIP sequencing. A more in depth analysis of the impact on ovarian function via oocyte counting is underway.

id #1126

Psi, the *Drosophila* ortholog of the FUBP1 single stranded DNA binding protein, fine-tunes transcription of developmental patterning genes

Olga Zaytseva[1, 2], Linna Guo[1, 2], Naomi C Mitchell[1], Caroline Delandre[3], Owen J Marshall[3], Ross D Hannan[1, 4], David L Levens[5], Leonie M Quinn[1, 2]

1. John Curtin School of Medical Research, Canberra, ACT, Australia

2. The University of Melbourne, Parkville, VIC, Australia

3. Menzies Institute for Medical Research, Hobart, TAS, Australia

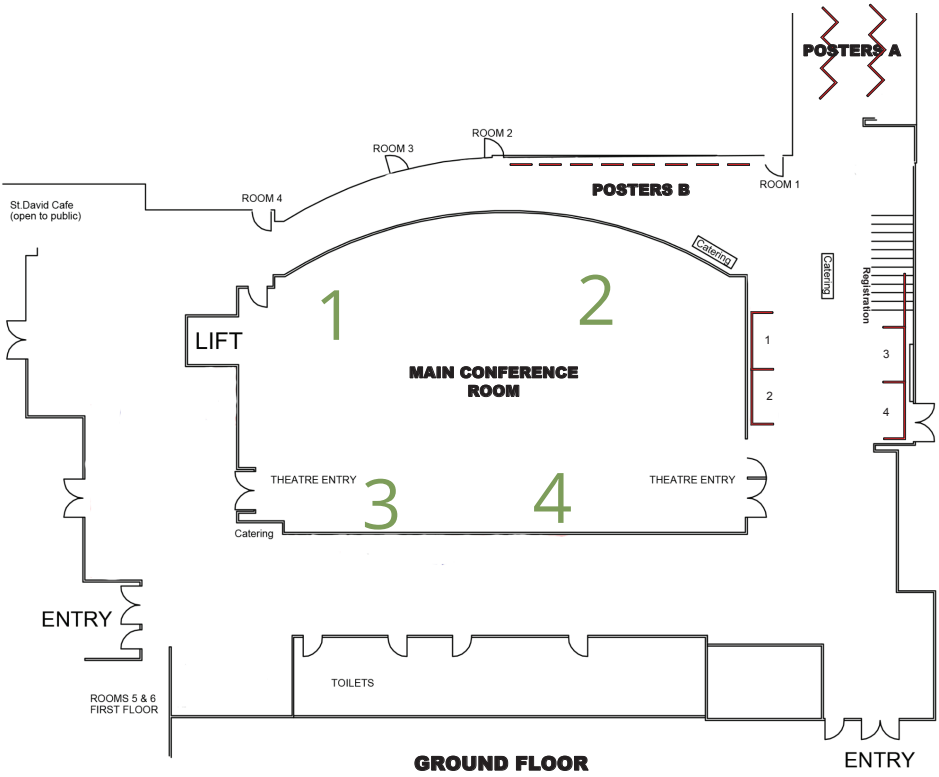
4. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

5. National Cancer Institute, NIH, Bethesda, Maryland, USA

The human Far Upstream Binding Protein 1 (FUBP1) was isolated over a quarter of a century ago due to capacity to bind the active *MYC* promoter, remodel single stranded DNA architecture, and enable maximal activation of *MYC* transcription. Consistent with this function, FUBP1 is upregulated in many cancers, including breast, liver, bladder, kidney and lung. However, the broader significance of FUBP family proteins in genome-wide transcriptional

control and how such functions might regulate animal development has remained unclear. Our recent *Drosophila* studies revealed conserved functions for the FUBP1 ortholog (Psi) in controlling *Myc* transcription and promoting cell and tissue growth in the wing epithelium. We further demonstrated physical and genetic interaction with the Mediator (MED) complex, which likely enables integration of developmental signals at multiple promoters. Indeed, our exciting new data demonstrate that, in addition to *Myc*, Psi binds to multiple transcriptional targets in the wing epithelium *in vivo*. Intersection of RNA-seq to detect differentially expressed genes following Psi depletion, and wing-specific direct binding sites identified genome-wide by Targeted DamID, revealed a developmental patterning signature for Psi including Wnt, Notch and TGFβ cell fate determinant pathways. Moreover, analysis of direct Psi targets identified several novel growth regulators, including the TGFβ signalling regulator *Tolkin* and the RhoGEF *Ephexin*. Together our data suggest Psi integrates cellular signalling inputs to directly modulate transcriptional outputs and fine-tune development. Our current and future work aims to elucidate the mechanisms by which Psi remodels single-stranded DNA and restructures chromatin *in vivo*.

Conference Venue Floor Plan



KEY

1 **Lab Supply**
labsupply.co.nz

2 **Custom Science**
customscience.co.nz

3 **Macrogen**
macrogen.com.au

4 **Genesearch**
genesearch.co.nz