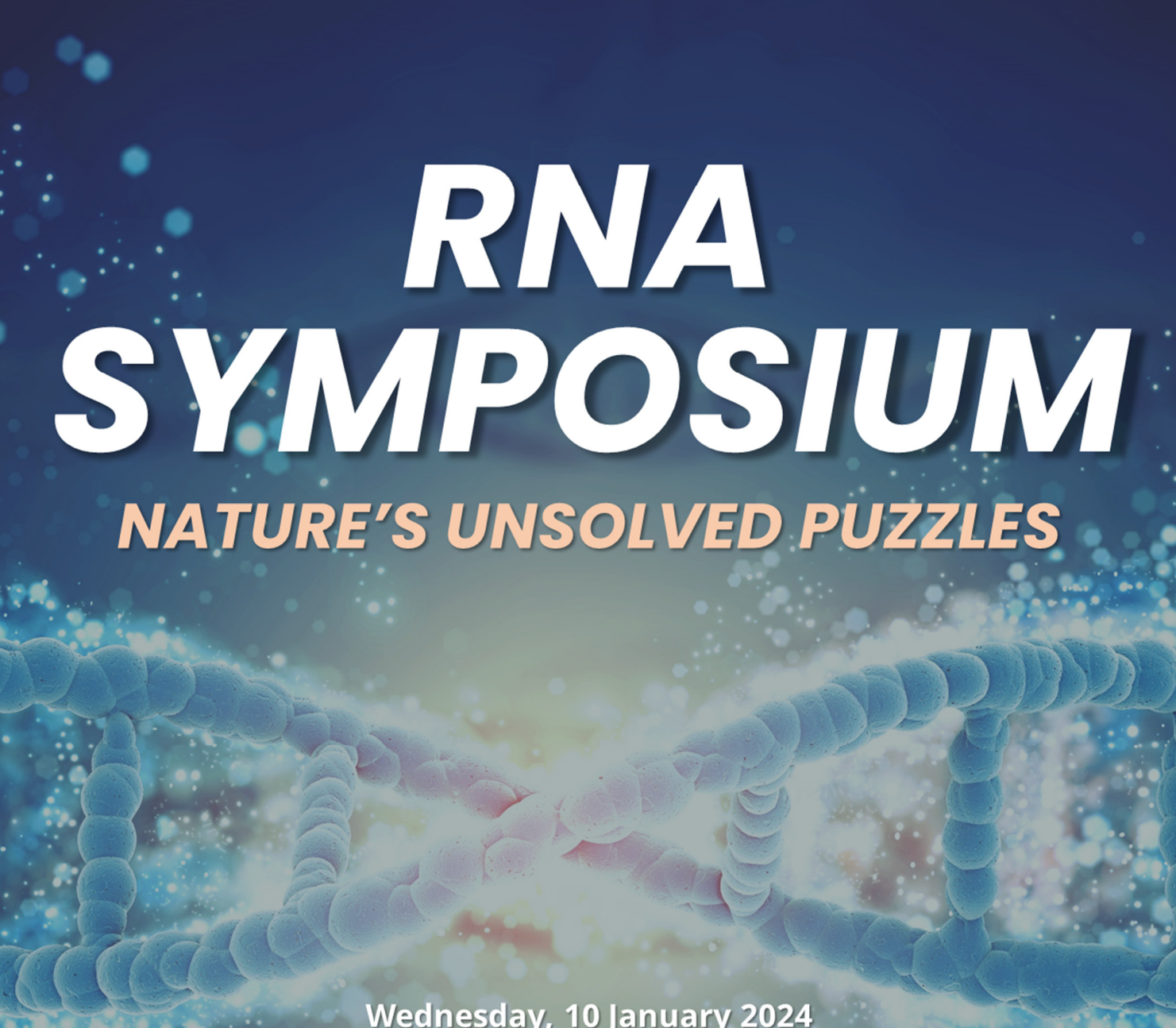
RNA SYMPOSIUM

NATURE'S UNSOLVED PUZZLES



Wednesday, 10 January 2024
9:00 AM – 5:30 PM

Learning Studio, Experimental Medicine Building
Nanyang Technological University
59 Nanyang Drive, Singapore 636921

PROGRAMME BOOKLET

JOINTLY ORGANISED BY

Agency for Science, Technology and Research SINGAPORE

NANYANG TECHNOLOGICAL UNIVERSITY SINGAPORE

NUS National University of Singapore

NATIONAL RESEARCH FOUNDATION
PRIME MINISTER'S OFFICE
SINGAPORE

GYSS SINGAPORE

RNA SOCIETY

LEXOGEN
Enabling complete transcriptome sequencing

Contents

WELCOME MESSAGE

Dear Colleagues,

On behalf of the organising committee, we would like to express our sincere gratitude and welcome you to this RNA Symposium. Jointly organised with and as part of the National Research Foundation's Global Young Scientists Summit (GYSS) this year, we are especially privileged to kick off the year with three very distinguished keynote speakers – Prof. Sir Richard J. Roberts, Prof. Narry Kim and Prof. Ling-Ling Chen.

With a wonderful lineup of keynotes and speakers, we trust you will have a great time interacting with one another and learning about the latest science and developments in the RNA community. It is our hope that this symposium will continue to spark your interest in RNA and inspire you on all the fun things this molecule can bring – from gaining new fundamental knowledge about the world to improving our health.

We hope you will enjoy this RNA symposium. Wishing you an enriching time and a wonderful year ahead discovering the science behind RNA!

Yue Wan, Polly Chen and Xavier Roca
Committee Co-Chairs

COMMITTEE CO-CHAIRS

- Dr. Yue WAN, Genome Institute of Singapore, A*STAR
- Assoc. Prof. Leilei Polly CHEN, Cancer Science Institute of Singapore, National University of Singapore
- Assoc. Prof. Xavier ROCA, School of Biological Sciences, Nanyang Technological University

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- Dr. Xue Yan YAM, National University of Singapore
- Ms. Hema CHANDRAMOHAN, National University of Singapore

PROGRAMME

9:00AM	9:10AM	**OPENING ADDRESS**
		Chair: Dr. Yue WAN, A*STAR
9:10AM	9:50AM	**KEYNOTE: Prof. Narry KIM** **Institute for Basic Science and Seoul National University** *Control of Viral and Cellular mRNA Stability*
9:50AM	10:30AM	**KEYNOTE: Prof. Ling-Ling CHEN** **Shanghai Institute of Biochemistry and Cell Biology** *Biogenesis, Function and Potential Application of Circular RNAs*
10:30AM	11:00AM	**TEA BREAK**
		Session 1 | RNA Technologies **Session Chair: Dr. Yue WAN, A*STAR**
11:00AM	11:20AM	**Dr. Ru-Yi ZHU** **Department of Chemistry, National University of Singapore** *Reprogram RNA with Small Molecules*
11:20AM	11:40AM	**Dr. Leslie BEH** **Institute of Molecular and Cell Biology (IMCB), A*STAR** *Identifying and Engineering Nucleic Acid Modifying Enzymes for Biology and Biotechnology*
11:40AM	12:00PM	**Dr. Jiaxu WANG** **Genome Institute of Singapore (GIS), A*STAR** *Development of A Single-Cell RNA Structure Sequencing Approach to Study Neurogenesis*
12:00PM	12:20PM	**Dr. Beverly MOK** **Institute of Molecular and Cell Biology (IMCB), A*STAR** *Continuous Evolution of T7 RNA Polymerase for Enhanced Properties*
12:20PM	1:50PM	**LUNCH**
		Session 2 | RNA Biology **Session Chair: Assoc. Prof. Xavier ROCA, NTU**
1:50PM	2:10PM	**Dr. Anthony KHONG** **Cancer Science Institute (CSI) Singapore, Dept. of Physiology, NUS** *Principles of Stress Granule Assemblies*
2:10PM	2:30PM	**Dr. Jia Jia CHAN** **Cancer Science Institute (CSI) Singapore, NUS** *Pan-Cancer Pervasive Upregulation of 3'UTR Splicing Drives Tumorigenesis*
2:30PM	2:50PM	**Mr Jia Wei Joel HENG** **Year 4 PhD Student (Supervised by Assoc. Prof. Tan Meng How),** **Chemical Engineering & Biotechnology, School of Chemistry, NTU** *An Atlas of A-To-I RNA Editing in Xenopus*
2:50PM	3:10PM	**Mr Donald Yuhui SIM** **Year 4 PhD Student (Supervised by Assoc. Prof. Xavier Roca),** **School of Biological Sciences, NTU** *Alternative Splicing Profiles in Myeloid Cell Maturation*
3:10PM	3:40PM	**TEA BREAK**

		Session 3 | RNA: From Discovery to Application **Session Chair: Assoc. Prof. Polly CHEN, NUS**
3:40PM	4:00PM	**Assoc. Prof. Dahai LUO** **Lee Kong Chian School of Medicine, NTU** *Towards Virus-Inspired RNA Therapeutics: Immunomodulatory RNA and Self-Amplifying RNA*
4:00PM	4:20PM	**Dr. Li Ren KONG** **NUS Centre for Cancer Research, Dept. of Pharmacology, NUS** *Advancing Prognostication and Personalised Treatment through Proteogenomics in NSCLC*
4:20PM	4:40PM	**Ms Natalie Bao Ying LIM** **Year 3 PhD Student (Supervised by Prof Phan Anh Tuan),** **School of Physical and Mathematical Sciences, NTU** *Telomere Shortening with the Use of Antisense Oligonucleotides Against Telomerase RNA*
4:40PM	5:00PM	**Dr. Minghao CHIA** **Genome Institute of Singapore (GIS), A*STAR** *Metatranscriptomics Reveals Variations in Core Microbes and Gene Expression Across Healthy Human Skin*
5:00PM	6:00PM	**KEYNOTE: Nobel Laureate Prof. Sir Richard ROBERTS** **New England BioLabs** *The Discovery of RNA Splicing*
6:00PM	6:10PM	**Award Ceremony** *Best Graduate Student/ Postdoc Prize*
6:10PM	6:15PM	**Closing**

SPEAKERS' PROFILE

Prof. Sir Richard J. ROBERTS

- Chief Scientific Officer, New England Biolabs
- Nobel Laureate – Nobel Prize Physiology/Medicine (1993)
- Research interest: Split genes and RNA splicing

Ever since he was a young child, puzzles have dazzled Sir Richard Roberts, now the Chief Scientific Officer at New England Biolabs, Ipswich, Massachusetts. His journey from a curious child to a Nobel laureate is punctuated by numerous collaborations and partnerships that catalysed his scientific explorations.

Born in Derby, England, Sir Richard's inquisitive nature was nurtured by mentors who recognised his knack for problem-solving. His academic pursuits took him to Sheffield University, where the allure of organic chemistry seized his imagination and earned him a PhD in the discipline.

Fundamental to his work is the belief that understanding the structure of molecules we work with is pivotal to understanding how they function. After his postdoctoral research at Harvard University, Sir Richard transitioned to Cold Spring Harbor Laboratory, where his interests gravitated from pure chemistry toward molecular biology under the tutelage of Dr James Watson, who played a crucial role in the discovery of DNA.

At the laboratory, Sir Richard delved into the genes of the common cold virus and by comparing viral DNA with its complementary DNA, he unravelled the complex structure of genes and DNA. His pioneering work on DNA sequencing and genetic engineering, coupled with his discovery of the alternative splicing of genes, has had a profound impact on molecular biology. Without these insights, decoding the human genome would have been impossible. This body of work culminated in Sir Richard being honoured with the Nobel Prize in Physiology or Medicine in 1993.

Since then, Sir Richard has continued his work, focusing on the restriction and modification of genes. One project in particular sees his group collaborating with the US National Cancer Institute to study over a thousand strains of Helicobacter pylori, a common bacterium that attacks the stomach lining, to ascertain if they play any role in gastric cancer.

Reflecting on the importance of collaboration, Sir Richard says that working with diverse groups has been instrumental in enabling his work. From analysing gene sequences to unearthing patterns amidst seas of data, many facets of his work thrive on the collective endeavour of individuals hailing from various backgrounds and disciplines, all working towards solving a grand puzzle.

Throughout his career, Sir Richard has navigated numerous hurdles, including scepticism around his Nobel-Prize-winning work. "When we were first exploring the field that led to the discovery of split genes and RNA splicing, almost everyone thought we were pursuing an artefact, that we were wasting our time and money instead of doing something useful," said Sir Richard.

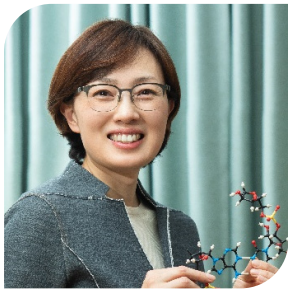
Still embodying the spirit of curiosity, Sir Richard is looking forward to meeting many students at the Global Young Scientists Summit 2024, hoping they will ask him important questions that he doesn't know the answer to.

In recognition of his contributions to the scientific community, Sir Richard was knighted in 2008. He also received the Sir Hans Krebs Medal in 2013.

Lecture Topic: ***The Discovery of RNA Splicing***

Synopsis:
In this talk I will describe my personal path into science, how I became a molecular biologist and the work that led to the discovery of RNA splicing. It is a tale of a clever experiment that went wrong, but when we explored the reasons, we discovered that Nature was trying to tell us that our hypothesis about transcription and the production on mRNAs in eukaryotes was wrong. We had assumed that the process would be identical to that found in bacteria and bacteriophages. I will describe the initial experiment, which aimed to characterize a eukaryotic promoter and how its failure led us to spend more than a year trying to find out what had gone wrong and to explain the strange results we obtained during the post-mortem.

SPEAKERS' PROFILE



Prof. Narry KIM

- Professor, School of Biological Sciences, Seoul National University, Republic of Korea
- Director, Center for RNA Research, Institute for Basic Science, Republic of Korea

Narry Kim is a Professor in the School of Biological Sciences at Seoul National University and a founding Director of the RNA Research Center at Institute for Basic Science. Kim graduated from Seoul National University in 1992 and received her Ph.D. in 1998 from the University of Oxford where she studied lentiviruses and gene delivery. For postdoctoral training, she joined the Gideon Dreyfuss lab at the University of Pennsylvania to study mRNA surveillance. Kim moved back to Seoul National University in 2001 to set up her own group and has been investigating how genes are regulated at the RNA level. The Kim lab delineated the microRNA pathway by identifying key factors including DROSHA and revealing their action mechanisms and structures. Kim also uncovered the role of noncanonical RNA tailing such as uridylation and mixed tailing in the control of microRNA, mRNA, and viral RNA. She is a recipient of the L'Oreal-UNESCO Women in Science Award, Hoam Prize, and Asan Prize, and a member of KAS, NAS, EMBO, and the Royal Society.

Lecture Topic: ***Control of Viral and Cellular mRNA Stability***

Synopsis:
I would like to present two latest projects. The first part is about the stability control of viral and cellular mRNAs. Viruses harbour diverse regulatory modules, yet understanding of their functions lags behind the rapidly expanding universe of sequenced viral genomes. We screened the activities of 30,367 viral segments from 143 species representing 36 viral families, uncovering hundreds of regulatory RNA elements. In a case study on a prominent positive element, K5, we discovered a previously uncharacterized protein, ZCCHC2, as a host factor. ZCCHC2 recruits terminal nucleotidyl transferases TENT4A and TENT4B to induce mixed tailing, resulting in delayed deadenylation. K5 enhances mRNA stability and translation in all contexts tested, including adeno-associated viral vectors and synthetic mRNAs. This study illustrates the importance of mixed tailing in RNA activation and the potential of the virosphere for identifying novel regulatory mechanisms.

The second project explores the dynamics of mRNA-protein complex (mRNP) remodeling throughout the mRNA life cycle. mRNAs continually change their protein partners, yet a lack of temporal data has constrained our understanding of mRNP remodeling. We tackled this problem by generating time-resolved mRNA interactome data by performing pulse metabolic labeling with photoactivatable ribonucleoside, UVA crosslinking, poly(A)+ RNA isolation, and mass spectrometry. This longitudinal approach allowed quantification of over 700 RNA-binding proteins (RBPs) across ten distinct time points. Overall, the mRNA binding dynamics aligns well with known functions, localizations, and molecular interactions. Our data further revealed numerous RBPs with unexpected dynamics, helping us to better understand the mRNA life cycle.

SPEAKERS' PROFILE

Prof. Ling-Ling CHEN

- Director, Key Laboratory of RNA Science and Engineering, Chinese Academy of Sciences (CAS), China
- Principal Investigator (Lab Head), Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, China

Ling-Ling Chen carried out doctoral and post-doctoral work at the UConn Health, USA from 2004 to 2010. She also completed an MBA degree at the UConn Business School in 2009 and was promoted to Assistant Professor in Residence at UConn in 2010. Chen moved to Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (CAS) as an independent PI in 2011. She was selected as a Howard Hughes Medical Institute (HHMI) International Research Scholar in 2017, and as a New Cornerstone Investigator in 2023. She has been appointed as the Associate Director, State Key Laboratory of Molecular Biology since 2017, and as the Director of the Key Laboratory of RNA Science and Engineering, Chinese Academy of Sciences since 2022. Her research has made important contributions to our understanding of the diversity, biogenesis and function of long coding RNAs (lncRNAs), in particular, the biogenesis of circular RNAs and their impact on innate immunity, and the snoRNA-related lncRNAs in the assembly and function of nuclear bodies.

Lecture Topic: ***Biogenesis, Function and Potential Application Of Circular RNAs***

Synopsis:
Many genes in higher eukaryotes can produce circular RNAs (circRNAs) through back-splicing of exons. CircRNAs differ from linear RNAs in their production, structure and turnover and thereby have unique cellular functions and potential biomedical applications. In this talk, I will discuss recent progress in our understanding of the biogenesis, turnover and function of circRNAs. I will also discuss our efforts on developing circular aptamers that can effectively suppress the activation of dsRNA-activated Protein Kinase R (PKR). This has the potential to alleviate inflammation in certain pathophysiological contexts.

SPEAKERS' PROFILE

Dr. Ru-Yi ZHU

- Assistant Professor, Department of Chemistry, National University of Singapore (NUS)

Ru-Yi obtained his BS with honors in chemistry under the supervision of Prof. Zhang-Jie Shi at Peking University in July 2013. Then he moved to the Scripps Research Institute and received his PhD in chemistry with Prof. Jin-Quan Yu in November 2018, focusing on tackling challenging aliphatic carbon-hydrogen (C-H) bond activation with novel ligands and directing groups. After that, he joined Prof. Eric T. Kool's lab at Stanford University as a postdoc to develop chemical tools to study DNA, RNA, and associated proteins for therapeutic applications. Recently, Ru-Yi was awarded the prestigious NUS Presidential Young Professorship (PYP) in April 2021. He joined the Department of Chemistry as a NUS PYP Assistant Professor in September 2021. His research focuses on new chemistry development for RNA manipulation and finding selective RNA-binding small molecules as well as DNA catalysis.

Lecture Topic: ***Reprogram RNA with Small Molecules***

Synopsis:
Incorporating stimuli-responsive components into RNA constructs provides precise spatiotemporal control over RNA structures and functions. Despite considerable advancements, the utilization of redox-responsive stimuli for the activation of caged RNAs remains largely underexplored. In this context, we present a novel strategy that leverages post-synthetic acylation coupled with redox-responsive chemistry to exert reversible control over RNA. To accomplish this, we design and synthesize a series of acylating reagents specifically tailored for introducing disulfide-containing acyl adducts into the 2'-OH groups of RNA. Our data reveal that these acyl moieties can be readily appended, effectively blocking RNA hybridization and folding. We demonstrate the traceless release and reactivation of RNAs through reducing stimuli. Employing this strategy, RNA exhibits rapid cellular uptake and effective distribution within the cytosol without succumbing to lysosomal entrapment. Notably, small interfering RNA targeting the green fluorescent protein (GFP) could be reactivated in the cytosol, demonstrating its efficacy in silencing the GFP gene. We anticipate that our methodology will be accessible to laboratories engaged in RNA biology and holds promise as a versatile platform for various RNA-based applications.

SPEAKERS' PROFILE

Dr. Leslie BEH

- Principal Investigator, Institute of Molecular and Cell Biology (IMCB), A*STAR

Leslie earned an A.B. in Biology at Harvard University as a John Harvard scholar, where he trained with Nicole Francis to uncover sequence features underlying Polycomb Group protein activity. Under her tutelage, Leslie gained a lasting appreciation for biochemical approaches to understand protein function. He then completed an A.M. and Ph.D. in Biology from Princeton University with Laura Landweber and Tom Muir as a Petrie fellow, where he developed methods for assembling synthetic chromosomes with custom epigenetic modifications. Through genomics and biochemical fractionation approaches, he identified a novel DNA methyltransferase complex that is homologous to the RNA m6A methyltransferase, METTL3-14. Leslie then embarked on a short postdoctoral stint with Sam Sternberg at Columbia University, where he studied CRISPR-Cas systems that mediate RNA-guided DNA integration. Following this, Leslie pursued a career in industry, joining Illumina to lead a research group for developing novel epigenetics assays. His work led to the filing of multiple technology disclosures, patent applications, and trade secrets in < 2 years.

Driven by the desire to do biological research and make an impact on students, Leslie returned to A*STAR / IMCB in September 2022 as a Principal Investigator. Leslie is a recipient of the Young Individual Research Grant (2023) and the National Research Foundation Fellowship (Class of 2024).

Lecture Topic: ***Identifying and Engineering Nucleic Acid Modifying Enzymes For Biology And Biotechnology***

Synopsis:
The natural world harbours a vast diversity of enzymes, many of which remain uncharacterised. My research focuses on discovering novel DNA and RNA modifying enzymes, dissecting their mechanistic basis of function, and repurposing them for biotechnological applications. Here, I will describe a new effort in my lab, focused on single-celled eukaryotes that orchestrate precise genome rearrangements during their sexual cycle. These genome rearrangements are guided by long and short RNAs, presumably in complex with yet-unknown enzymes. We aim to identify these RNA-guided enzymes and engineer them to reverse disease-causing structural variants in human cells.

SPEAKERS' PROFILE

Dr. Jiaxu WANG

- Fellow, Genome Institute of Singapore (GIS), A*STAR

Jiaxu Wang has a diverse scientific background, which stemmed from his Ph.D. training at the University of Science and Technology of China. His work focused on the mechanism of somatic reprogramming and stem cell differentiation. Upon completing his Ph.D. training, he started his post-doctoral fellowship in Prof. Lawrence W. Stanton's lab at the Genome Institute of Singapore. His project is to study neurogenesis using single-cell RNA-sequencing technology. Jiaxu Wang then joined Wan Yue's lab to start his second post-doctoral fellowship in 2017, since then, he has been very interested in RNA biology, and his work mainly focuses on the roles of RNA secondary structure in human cells especially during neurogenesis. Their work shows that RNA structures have different dynamics compared to RNA expression during neurogenesis, which suggests RNA structures play roles beyond their expression. Because of his experience in both single-cell technologies and RNA structure study, he recently developed a single-cell RNA-structure-sequencing approach to study neurogenesis, this approach could study both RNA expression and RNA structure simultaneously at a single-cell level.

Lecture Topic: ***Development of a Single-Cell RNA Structure Sequencing Approach to Study Neurogenesis***

Synopsis:
The relationship between structure and function is the heart of modern cell biology. However, previous studies mainly focus on protein structure. Unlike protein structure, the studies of flexible RNA structure are still in the early stages due to technique limitations. In this talk, I will share our recent work on RNA structure studies at a single-cell level. Single-cell technologies have been applied to study transcriptome, genome, and proteins but RNA structure because current RNA structure studies require millions of cells as start materials. We recently developed a single-cell RNA structure sequencing approach to study RNA structure heterogeneity during neurogenesis, our data shows that RNA structure has different heterogeneity in different cell types/stages, suggesting RNA structure provides an additional layer of information during neurogenesis beyond RNA expression. Human ES cells are more homogeneous than differentiating neuron cells, and translation and RBP binding are 2 major reasons for the RNA structure heterogeneity. In summary, we developed a single-cell RNA structure sequencing approach which allows us to study the roles of RNA structure with high resolution in various cellular processes and diseases.

SPEAKERS' PROFILE

Dr. Beverly Yin Leng MOK

- Senior Scientist, Institute of Molecular and Cell Biology (IMCB), A*STAR

Dr. Beverly Mok is a senior scientist at the Molecular Engineering Lab in IMCB. She received her B.A from the University of Cambridge in 2015 and her PhD from Harvard University in 2022. During her PhD, she developed the first reported genome editing agent (DdCBE) that performs precise DNA edits in human mitochondrial DNA. As a postdoctoral researcher at IMCB, Beverly is interested in exploring different classes of DNA and RNA-modifying enzymes for applications in human therapeutics and biomanufacturing.

Lecture Topic: ***Continuous Evolution of T7 RNA Polymerase for Enhanced Properties***

Synopsis:
mRNA therapeutics is a rapidly expanding field of medicine with significant applications in vaccine development and cancer immunotherapy. In vitro transcription (IVT) using T7 RNA polymerase is the primary process to synthesize mRNA. IVT yields and purity are heavily dependent on the initiation sequence and the sequence following the initiation site. Templates that deviate from the optimal sequence often suffer from low yields and 3'-inhomogeneity. Additional challenges associated with current T7 RNAP technology include formation of dsRNA impurities, limited nucleotide substrate specificity and high cost of co-transcriptional capping. Here we describe the development of a phage-assisted non-continuous protein evolution (PANCE) platform to evolve for high RNA-yielding T7 RNAPs. Coupled with an optimized automated protein expression system and fluorescence-based IVT assay, we identified T7 variants that exhibit up to 5-fold improvement in RNA yields compared to wild-type T7 RNAP across various DNA templates. Interestingly, these variants contain a combination of 1 to 6 mutations at positions which have not been reported thus far, warranting further characterization of other valuable properties including 3'-homogeneity and dsRNA content.

SPEAKERS' PROFILE

Dr. Anthony KHONG

- Principal Investigator, Cancer Science Institute of Singapore
- Assistant Professor, Department of Physiology, NUS

Dr. Anthony Khong is an Assistant Professor at the Cancer Science Institute of Singapore and the Department of Physiology at the National University of Singapore (NUS). He earned his Ph.D. in Biochemistry and Molecular Biology from the University of British Columbia in 2015. Subsequently, in 2016, he joined the laboratory of Dr. Roy Parker at the University of Colorado Boulder, focusing on the study of messenger ribonucleoprotein granules. In 2023, Anthony assumed his current position at the Cancer Science Institute, where he is dedicated to unravelling RNA-mediated mechanisms that underlie adaptive responses in the context of cancer.

Dr. Khong is known for his postdoctoral investigations into messenger ribonucleoprotein architecture, stress granule assembly mechanisms, and stress granule functions. His research has yielded 19 peer-reviewed articles, seven of which he led. Notably, one of his contributions laid the groundwork proposing that stress granules form as a consequence of RNA aggregation (Khong et al., 2017 Mol Cell).

Lecture Topic: ***Principles of Stress Granule Assemblies***

Synopsis:

Stress granules are transient, non-membrane-bound cytosolic RNA-protein structures that emerge in response to various stresses such as oxidative stress, hypoxia, and proteotoxic stress. Their assembly is mediated by molecular interactions among many non-translating messenger ribonucleoprotein that accumulate during stress-induced translational shutoff. Stress granules are believed to play a crucial role in stress adaption, a mechanism potentially exploited by cancers to survive, proliferate, and metastasize.

Multiple lines of evidence underscore the significance of stress granules in cancer biology. Firstly, stress granules are observed in patient tumours. Secondly, elevated expression of key genes promoting stress granules in tumours correlates with poorer patient outcomes. Thirdly, oncogenic signalling pathways sensitizes cells to forming stress granules. Finally, depletion of key stress granule scaffold proteins impedes critical cancer processes, such as tumour initiation, immune evasion, and metastasis.

Despite their likely importance in stress adaptation and cancer, the biochemical processes governing stress granule formation and functions are poorly understood. In this seminar, I will present a novel model model for stress granule assembly arising from my postdoctoral research in Roy Parker's laboratory at the University of Colorado Boulder. Challenging conventional views that regard stress granules as sites for RNA triage, our research suggests that the accumulation of RNAs in stress granules results from the biophysical tendency of RNAs to aggregate. This raises the intriguing question of whether stress granules are incidental RNA aggregates and, if so, are they functionless? The presentation will conclude with a discussion on how stress granules may indeed possess functions that emerge when considered as RNA aggregates.

SPEAKERS' PROFILE

Dr. Jia Jia CHAN

- Senior Research Scientist, Cancer Science Institute of Singapore, NUS

Jia Jia obtained her BSc in Biotechnology from University College London (UCL) and started her PhD at the Institute of Cancer Research, London, before moving back to UCL to complete her training. In 2015, she joined Yvonne Tay's group at the Cancer Science Institute of Singapore as a postdoctoral fellow and is currently there as a senior research scientist. Her work focuses on understanding the role of untranslated regions (UTRs) of protein-coding mRNAs, how dysregulated UTR processing contributes to oncogenesis, and the potential crosstalk between RNA:RNA and RNA:protein networks, as well as RNA processing pathways, such as spicing, alternative polyadenylation and editing.

Lecture Topic: ***Pan-cancer Pervasive Upregulation Of 3'UTR Splicing Drives Tumorigenesis***

Synopsis:
The majority of mammalian genes generate mRNAs with different 3' untranslated regions (3'UTRs). Alternative 3'UTRs are produced via multiple mechanisms, and function as important post-transcriptional regulators. They are widespread and have been implicated in disease pathogenesis through their differential modulation of gene expression and function. In cancer, most known alternative 3'UTRs are derived from alternative polyadenylation, whereas 3'UTR splicing remains poorly understood as splicing studies have traditionally focused on protein-coding alterations. Here, we systematically mapped the pan-cancer landscape of 3'UTR splicing to reveal that it is widespread, upregulated in cancers, correlated with poor prognosis, and more prevalent in oncogenes. We demonstrate that targeted inhibition of 3'UTR splicing efficiently reduces oncogene expression and impedes tumor progression. Notably, CTNNB1 3'UTR splicing is the most consistently dysregulated event across multiple cancers. We validate its upregulation in hepatocellular carcinoma and colon adenocarcinoma, and show that the spliced 3'UTR variant is the predominant contributor to its oncogenic functions. Overall, our findings highlight the significance of 3'UTR splicing in cancer and may launch new avenues for RNA-based anti-cancer therapeutics.

SPEAKERS' PROFILE

Mr Jia Wei Joel HENG

- Year 4 PhD Student (Supervised by Assoc. Prof. Tan Meng How)
  Chemical Engineering & Biotechnology, School of Chemistry, NTU

Joel is a PhD student in his 4th year, under the supervision of A/P Tan Meng How. He is interested in developing methods to detect and analyse RNA modifications by leveraging both second- and third-generation sequencing technologies. To that end, he splits his time between culturing cells in the biosafety cabinet and designing/debugging pipelines on the computing cluster. Most recently, he was the co-first author of "Deep transcriptome profiling reveals limited conservation of A-to-I RNA editing in Xenopus" published in BMC Biology. Prior to embarking on his PhD, he received a BEng (Biomedical Engineering) from the National University of Singapore. He also worked as a research assistant in the Genome Institute of Singapore under the supervision of A/P Ramanuj Dasgupta, developing binders against immune receptors using yeast surface display and flow cytometry. Outside of the lab, he enjoys noodling on his guitar and going down the rabbit hole of chasing tones.

Lecture Topic: ***An Atlas of A-to-I RNA Editing in Xenopus***

Synopsis:
Xenopus has served as a valuable model system for biomedical research over the past decades. Notably, ADAR was first detected in frog oocytes and embryos as an activity that unwinds RNA duplexes. However, the scope of A-to-I RNA editing by the ADAR enzymes in Xenopus remains under-explored. In this talk, I will present how we identified millions of editing events in Xenopus with high accuracy and systematically mapped the editome across developmental stages, adult organs, and species. We observed diverse spatiotemporal patterns of editing with deamination activity highest in early embryogenesis before zygotic genome activation and in the ovary. Strikingly, editing events are poorly conserved across different Xenopus species. Even sites that are detected in both X. laevis and X. tropicalis show largely divergent editing levels or developmental profiles. In protein-coding regions, only a small subset of sites that are found mostly in the brain are well conserved between frogs and mammals. Collectively, our work provides fresh insights into ADAR activity in vertebrates and suggest that species- specific editing may play a role in each animal's unique physiology or environmental adaptation.

SPEAKERS' PROFILE

Mr Donald Yuhui SIM

- Year 4 PhD Student (Supervised by Assoc. Prof. Xavier Roca)
  School of Biological Sciences, NTU

I am a final-year PhD student under the supervision of Associate Professor Xavier Roca. My primary area of research lies in studying alternative splicing during myeloid cell maturation and the RNA binding proteins that may regulate this process. Additionally, I am also interested in understanding how exonic and intronic properties may influence their capacity to be differentially spliced in the context of immune cells. Through a combination of both wet laboratory and bioinformatics experiments, my current project continues our group's previous works on how alternative splicing regulates this highly plastic group of cells.

Lecture Topic: ***Alternative Splicing Profiles in Myeloid Cell Maturation***

Synopsis:
With the prevalence of splicing defects in acute myeloid leukaemias (AML) that disrupt myelopoiesis, the alternative splicing factors and programmes that govern myeloid cell maturation are of interest. By analysing previously published and newly generated RNA-sequencing data, we identified several alternative splicing trends that occur during granulocyte and/or monocyte-macrophage development.

Additionally, we identified that the multi-functional RNA binding protein, RBM47, was a potential myelopoietic regulatory factor. Among white blood cells, RBM47 is primarily expressed in the neutrophil and monocyte lineages. By overexpressing RBM47 in the AML cell line HL-60, we found that these cells exhibited an improved response to monocytic differentiation induced by vitamin D3, as well as enhanced myeloid cell effector functions.

While the RNA-sequencing of these RBM47-overexpressing cells revealed many transcriptomic changes, many genes were only differentially expressed upon the addition of vitamin D3 in conjunction with RBM47 overexpression. Concomitantly, RBM47 expression alone does not induce myeloid maturation in HL-60, suggesting that RBM47 may regulate specific stages of monocytic development. As a whole, these results indicate a possible role for this RBP in modulating the maturation of myeloid cells of the granulocyte-monocyte branch.

SPEAKERS' PROFILE

Assoc. Prof. Dahai LUO

- Associate Professor, Lee Kong Chian School of Medicine, NTU

Dahai is an Associate Professor of Infection and Immunity and holds the Provost's Chair in Medicine at the Lee Kong Chian School of Medicine. He earned his Bachelor of Science (B.Sc.) in 2006 and his Ph.D. in 2010 from the School of Biological Sciences at Nanyang Technological University (SBS-NTU), Singapore. From 2010 to 2013, he served as a Postdoctoral Research Associate at Yale University. In September 2013, Dahai joined the newly established Lee Kong Chian School of Medicine as a Nanyang Assistant Professor and was granted tenure in 2019. His laboratory focuses on unravelling the molecular mechanisms underlying human infection by RNA viruses such as dengue, Zika, and Chikungunya, as well as the corresponding defense responses of the human immune system.

Lecture Topic: ***Towards Virus-Inspired RNA Therapeutics: Immunomodulatory RNA and Self-Amplifying RNA***

Synopsis:
RNA viruses present both significant challenges and serve as a source of inspiration for novel therapeutics. This intersection of challenge and opportunity in the study of RNA viruses underscores the importance of research in understanding and harnessing these entities. The insights gained from studying RNA viruses not only aid in managing current and future infectious diseases but also propel the development of cutting-edge medical therapies across various fields. Here I give two examples of how we can draw inspiration from their mechanisms, particularly how their RNA-based mechanisms can be utilized for various research and development purposes. Immunomodulatory RNA (immRNAs), designed to mimic key aspects of viral RNA recognized by the immune receptor RIG-I, have been studied as antiviral agents and cancer immune adjuvants. Self-amplifying RNA (saRNA), derived from the RNA genome of an RNA virus such as an alphavirus, has the capability for self-replication and transcription once inside a host cell. This amplification significantly enhances the expression of the encoded protein or antigen. We are studying the molecular mechanisms underlying these functional RNAs with the hope of developing new RNA-based therapeutics.

SPEAKERS' PROFILE

Dr. Li Ren KONG

- Lee Kuan Yew Fellow, NUS Centre for Cancer Research, N2CR, Dept. of Pharmacology, NUS

Dr Li-Ren Kong received his PhD in cancer pharmacology from National University of Singapore, during which he focused on understanding the resistance mechanisms in chemo-refractory tumours. His postdoctoral work at the Cancer Science Institute of Singapore aimed to identify cancer-specific germline mutations that may improve patient outcome through drug repurposing. He further his research training at MRC Cancer Unit, University of Cambridge, under the supervision of Prof Ashok Venkitaraman to elucidate the underlying factors that accelerate the progression of BRCA2-associated carcinogenesis. In 2022, he returned to Singapore after being awarded the prestigious Lee Kuan Yew Research Fellowship. His research focuses on defining the cell-autonomous functions of population-specific, cancer-associated germline mutations to determine their significance in directing cancer therapies in the clinics. By coupling state-of-the-art omics technology with conventional molecular techniques, his research aims to improve early clinical diagnosis and cancer intervention of Asian cancers. His work has been published in several scientific and clinical journals, with successful translation into biomarker-driven clinical trials.

Lecture Topic: ***Advancing Prognostication and Personalised Treatment through Proteogenomics in NSCLC***

Synopsis:
Advances in sequencing and computational techniques have illustrated the heterogeneity of the mutational landscape in cancers, expanding the field of precision oncology with improved outcomes. Yet, molecular testing of somatic drivers only provides actionable targets in 20% of non-small cell lung cancer (NSCLC) patients. Here, we sought to improve molecular diagnostics in NSCLC using integrative analyses to connect somatic mutational signatures with transcriptomic and proteomic profiles.

We collected data from 27 NSCLC tumours with matched normal adjacent tissues from Southeast Asians. NGS-based molecular testing confirmed actionable mutations (EGFR, ALK, RET, MET) in 13/27 tumours, with EGFR mutants found in 7 cases. Through transcriptomic and proteomic profiling, we further classified NSCLC into functionally distinct subgroups, with prediction of immunosuppressive scores based on expressions of interferon genes and immune checkpoint markers. Genome-wide mutational signature analysis annotated tumours driven by tobacco carcinogens and APOBEC3 deaminases. NSCLC with a tobacco-induced base substitution signature (SBS4) were correlated to oxidative and hypoxic stress signals, where a combined anti-VEGF therapy with chemotherapy may have potential benefit. Oncogene-driven tumours detected with APOBEC-associated signatures (SBS2/13) displayed transcriptomic patterns recapitulating those of drug resistant tumours, which may shorten the duration of response to single agent target therapies. Lastly, miRNA profiling revealed strong cancer-associated biomarkers with potential for early diagnosis.

Our findings underscore the clinical utility of multi-omics in diagnosing clinically-relevant cancer hallmarks of NSCLC. This warrants further studies to evaluate such predictive and prognostic values to augment existing molecular testing in oncology.

SPEAKERS' PROFILE

Ms Natalie Bao Ying LIM

- Year 3 PhD Student (Supervised by Prof Phan Anh Tuan)
  School of Physical and Mathematical Sciences, NTU

Natalie Lim, a graduate of the CN Yang Scholars Programme which offers rigorous training across diverse science and engineering disciplines, obtained her Bachelor of Science (Hons) in Chemistry and Biological Chemistry at Nanyang Technological University (NTU). Her research focuses on the area of nucleic acid therapeutics (NAT), with particular emphasis in the aspect of delivery and applications in telomere biology. She is currently a graduate student in the School of Physical and Mathematical Sciences (SPMS), NTU.

Lecture Topic: ***Telomere Shortening with the Use of Antisense Oligonucleotides Against Telomerase RNA***

Synopsis:

Progressive telomere shortening – a result of the "end replication problem" imposes a cap on the number of cell divisions and restricts excessive cellular proliferation. Telomerase is a ribonucleoprotein enzyme complex that helps maintain telomere length by synthesizing telomeric repeats. Approximately 90% of cancer cells possess elevated telomerase activity that brings about the continuous renewal of telomere lengths, which safeguards against replicative senescence and leads to unchecked cellular proliferation. In addition to their primary function in stabilizing chromosome, telomeres also have roles in regulating gene expression. Thus, disrupting the maintenance of telomere length emerges as a potential anti-cancer strategy and opens up avenue for researching telomere biology in the context of cancer. Gapmer antisense oligonucleotides (ASOs) are short, synthetic oligonucleotides that can modulate gene expression by interacting with a target through Watson-Crick complementary base-pairing and triggering RNase H-mediated degradation. Here, we demonstrate the use of ASOs for the efficient degradation of hTR as an approach to inhibit telomerase activity and shorten telomeres in cancer cells. The results show that treatment with our gapmer ASO candidate, hTR ASO-1, efficiently depleted hTR with low nanomolar IC50 values (<50 nM) in K-562 cells and HT1080 cells and successfully inhibited telomerase activity without the use of transfecting reagent. Furthermore, the long-term inhibition of telomerase activity resulted in the progressive shortening of telomeres. This study shows the potential of hTR ASO-1 as a new anti-telomerase agent and paves the way for its utilization as a biological tool for the study of telomere biology.

SPEAKERS' PROFILE

Dr. Minghao CHIA

- Scientist, Genome Institute of Singapore, A*STAR

Dr. Chia Minghao is a senior research fellow in the laboratory of metagenomic systems and microbial technologies at the Genome Institute of Singapore.

Prior to joining GIS, Dr. Chia completed his PhD at the Francis Crick Institute where he performed genome-wide profiling of the exact 5' and 3' ends of mRNA isoforms during cell fate specification in budding yeast and discovered that the use of alternative transcription start sites can tune gene expression levels to regulate cell fate.

His current research interests are themed around uncovering native host-microbial interactions and advancing microbiome research towards clinical and functional relevance. He has driven or supervised multiple published microbiome projects ranging from identifying microbial sharing between caregivers and children in paediatric eczema, to mining bioactive gene clusters in Singaporean gut microbes, to rigorously demonstrating the absence of a core blood microbial community in healthy Singaporeans.

He is particularly interested in methods to perform spatiotemporal RNA mapping for understanding microbiome function. This involves using RNA technologies to study the spatial distribution or role of expressed genes within the microbes that colonize the human body. He is excited to build on scientific infrastructure and capabilities to drive microbiome research in Singapore for the benefit of human health and potential.

Lecture Topic: ***Metatranscriptomics Reveals Variations in Core Microbes and Gene Expression Across Healthy Human Skin***

Synopsis:
The application of metatranscriptomic (RNA seq) technologies in skin microbiome research is hampered by the lack of a robust, non-invasive protocol that can accommodate a range of skin sites which have low microbial biomass, but high host and environmental contamination. Consequently, data from transcriptionally active microbes and microbial pathways across different skin sites is scarce and highlights the need for an optimized experimental and computational workflow to jointly profile skin metagenomes and metatranscriptomes. To address these challenges, we integrated experimental and computational methods specifically designed for non-invasive sampling and systematic capture of DNA and mRNA signals from skin microbes. We present the first multi-site metagenomic and metatranscriptomic survey of healthy human skin (n = 27 subjects) from five different skin sites (n=270 libraries). Our protocol demonstrates relatively even coverage across bacterial and fungal genes and robustness in the face of challenges posed by skin microbial RNA sampling and analysis such as low microbial biomass, poor RNA integrity, the need to deplete unwanted ribosomal RNAs (rRNAs) of diverse eukaryotic and prokaryotic origins and rigorous control of experimental and computational

artifacts. We found that the core metatranscriptome differs from the core metagenome across different skin sites, meaning highly abundant species such as Cutibacterium acnes are not always the most transcriptionally active organisms. We also identified signatures of microbial adaptation to different in vivo nutrient sources such as the upregulation of fungal transcripts involved in phospholipid metabolism on cheek relative to scalp. Finally, we identified diverse classes of antimicrobial genes which are transcribed by skin commensals in situ as well as a poorly characterized protein secreted by the fungi Malassezia restricta, whose transcript levels are negatively correlated with Cutibacterium acnes organismal abundances, suggesting a potential inhibitory interaction. Together, our work showcases the potential of leveraging metatranscriptomics to understand the microbial functions and microbe-microbe interactions occurring in situ on skin and provides a foundation for future identification of expressed microbial pathways or biomarkers associated with human health or disease.

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