Tsinghua-*Science* Workshops

Spliceosome and RNA

Session 3: Spliceosome and RNA Function

Tuesday, March 2nd, 2021 8-10 pm (GMT +08:00, Beijing)

8:00pm-8:45pm

Ling-Ling Chen, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, China

Processing, conformation and function of circular and long ncRNAs

8:45pm-9:00pm Q&A

9:00pm-9:45pm

Reinhard Lührmann, Max Planck Institute for Biophysical Chemistry, Germany

Structural insights into U2 RNP remodelling facilitated by Prp5 helicase and the assembly pathway of the human activated spliceosome

9:45pm-10:00pm Q&A

Host

Prof. Yigong Shi

Born in Zhengzhou, Henan Province in 1967, professor Yigong Shi is an Academician of the Chinese Academy of Sciences, a Foreign Associate of the US National Academy of Sciences and an Honorary Foreign Member of the American Academy of Arts and Sciences. As a structural biologist, he is also a recipient of Cheung Kong Scholar, National Distinguished Scholar and Outstanding Youth Talent of National Science Foundation for China. In 1985, he was recommended for admission to Tsinghua University. In 1989, he graduated one year ahead of schedule with a bachelor's degree. In 1995, he received his Ph.D. in molecular biophysics from Johns Hopkins University School of Medicine, and later did postdoctoral research at Memorial Sloan-Kettering Cancer Center. From 1998 to 2008, he

was Assistant Professor, Associate Professor, Professor and Warner-Lambert/Parke-Davis Professor in the Department of Molecular Biology, Princeton University, USA. In 2008, he declined the invitation of a researcher from Howard Hughes Medical Center (HHMI) and returned to Tsinghua University full-time. He served as the Dean of the School of Life Sciences of Tsinghua University until 2016 and is now the President of Westlake University. His research group is devoted to combining structural biology and biochemistry to elucidate the molecular mechanisms of fundamental cellular events, with a focus on apoptosis, regulated intramembrane proteolysis related to Alzheimer's disease, and pre-mRNA splicing.

Speakers

Dr. Ling-Ling Chen

Ling-Ling Chen carried out doctoral and post-doctoral work in Biomedical Science at UConn Health, USA with Gordon G. Carmichael from 2004 and 2010. She also completed an MBA degree in Management at the UConn Business School in 2009 and was promoted to Assistant Professor in Residence at UConn in 2010. Chen moved to the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences as an independent PI in 2011 and was promoted to Senior PI in 2017. She was selected as the Howard Hughes Medical Institute (HHMI) International Research Scholar in 2017.

Chen primarily studies long noncoding RNAs (lncRNAs), a giant class of RNA molecules that are emerging as important regulators in gene expression networks. Her group has pioneered methods for studying non-polyadenylated RNAs and discovered widespread expressed snoRNA-related lncRNAs and circular RNAs. In addition to the characterization of their unusual biogenesis pathways, her group discovered that some sno-lncRNAs are conspicuously absent from patients with Prader-Willi Syndrome and circular RNAs are involved in innate immunity regulation and related to the autoimmune disease systemic lupus erythematosus. Her group now continues efforts to elucidate the biogenesis and function of these unconventional regulatory RNAs in different cellular contexts and in

relevant human diseases.

Chen serves the community as Editorial Boards of several journals: Genome Biol, Mobile DNA, RNA, RNA Biol, Transcription and Trends Genet; and as an organizer including CSHA on RNA Biology (2018/2020) the Annual Meeting of the RNA Society (2020), CSHL on Regulatory RNA (2020) and Keystone Symposium on Noncoding RNAs (2021). She is the recipient of several awards including being named as a Chinese Biological Investigators Society (CBIS) Young Investigator, an Asian-Pacific Molecular Biology Network (A-IMBN) Research Young Investigator, and the L'OREAL China for Women in Science and Young Investigator Award of CAS.

Processing, conformation and function of circular and long ncRNAs

Circular and long noncoding RNAs (ncRNAs) are emerging as new regulators in gene expression networks and exhibit a surprising range of shapes and sizes. Many lncRNAs are transcribed by RNA polymerase II and are capped, polyadenylated, and spliced like mRNAs. Others are processed from long primary Pol II transcripts and are stabilized by different mechanisms, including capping by small nucleolar RNA (snoRNA)–protein (snoRNP) complexes at their ends or forming circular structures. We have shown that some circular and long ncRNAs are involved in gene regulation and also implicated in human diseases. Of an important note, despite the fact that circular and long ncRNAs are produced from distinct pathways, their subcellular localization and structural conformation determine their modes of action. In this talk, I will discuss how we investigate the processing, conformation and subcellular fate of circular and long ncRNAs, which have provided new insights into their biogenesis and function.

Prof. Reinhard Lührmann

Reinhard Lührmann received a diploma in chemistry in 1973 and PhD in Chemistry in 1975, from the University of Münster, Germany. From 1976 to 1980, he was a senior investigator at the Max Planck Institute for Molecular Genetics (West Berlin) and in 1981 he established an independent research group at this institute. In 1988, he took up the chair of Physiological Chemistry and Molecular Biology at the Institute of Molecular and Tumour Biology at the University of Marburg, Germany. In 1999, he was granted a Directorship at the Max Planck Institute for Biophysical Chemistry (Göttingen, Germany), where he founded the Department of Cellular Biochemistry. This enabled him to assemble a multi-disciplinary research group necessary to tackle the structure and function of the spliceosome. Since 2019, he heads an Emeritus research group, continuing his structural work on the spliceosome. Reinhard Lührmann has made seminal contributions to the field of pre-mRNA splicing for almost four decades, making key discoveries concerning the composition, structure and function of the spliceosomal snRNPs, and also of the spliceosome during its assembly and catalytic action, both in higher and lower eukaryotes. He has garnered numerous awards and named lectureships including, most notably, the Max Planck Research Prize, the highly-endowed Gottfried-Wilhelm-Leibniz-Prize of the German Research Foundation, the Feldberg Prize, the Ernst Jung Prize for Medicine, the Cozzarelli Prize, and the Lifetime Achievement in Science Award of the RNA Society. He received Honorary Doctorates from the Adam Mickiewicz University of Poznan, Poland, and the Free University of Berlin. He is an elected member of EMBO, the German Scientific Research Academy Leopoldina, and the Academia Europaea.

Structural insights into U2 RNP remodelling facilitated by Prp5 helicase and the assembly pathway of the human activated spliceosome

The spliceosome is a highly dynamic, large RNP machine that catalyses pre-mRNA splicing. Spliceosomes assemble de novo by the stepwise recruitment of the snRNPs U1, U2 and U4/U6.U5 to the intron, yielding the pre-catalytic B complex. For the transformation of the B complex into an

activated (Bact) complex, the spliceosome undergoes dramatic structural rearrangements, initiated by the Brr2 RNA helicase, which involve the formation of a catalytically active U2/U6 RNA structure, and the exchange of about 50 proteins. The assembly pathway of the U2/U6 RNA active site and the mechanism whereby proteins aid its proper folding remains poorly understood. We have blocked spliceosome assembly at novel intermediate stages of activation and, in collaboration with Holger Stark, determined the cryo-EM structures of two distinct, human pre-Bact complexes that lack a mature, catalytic U2/U6 RNA structure. These pre-Bact structures, coupled with biochemical analyses, provide new insights into the order of protein recruitment and release during Bact formation. They also elucidate how spliceosomal proteins and their mutually exclusive interactions, ensure the correct order of RNP rearrangements needed to generate the U2/U6 catalytic RNA, as well as the molecular mechanism whereby spliceosomal proteins facilitate the formation of the latter, with a conformational change in Prp8 playing a key role in the final 3D folding of the active site RNA.

We have also determined the cryo-EM structure of a major building block of the spliceosome, the human 17S U2 snRNP. Stable addition of U2 during early spliceosome formation requires the helicase Prp5. Our structure reveals that U2 snRNA nucleotides that form base pairs with the pre-mRNA branch-site are sequestered in the branch point interacting stem loop (BSL), and that the BSL is sandwiched between the proteins Prp5, TAT-SF1 and SF3B1. Thus, our studies provide a structural explanation of why TAT-SF1 must be displaced to allow the stable addition of U2 to the spliceosome, and identify RNP rearrangements facilitated by Prp5 that are required for stable interaction between U2 and the branch site.