TKK Science Award in Life Sciences

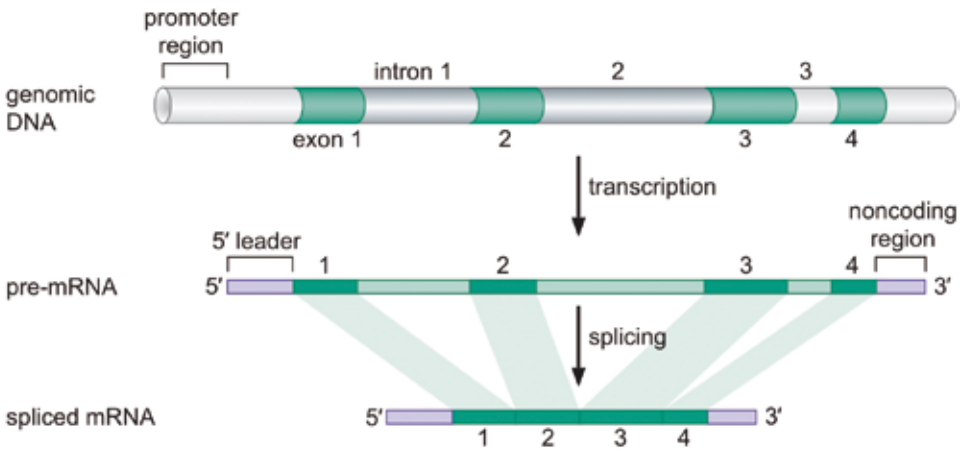
Splicing Cycle Visualized in Atomic Detail

By YAN Fusheng (Staff Reporter)

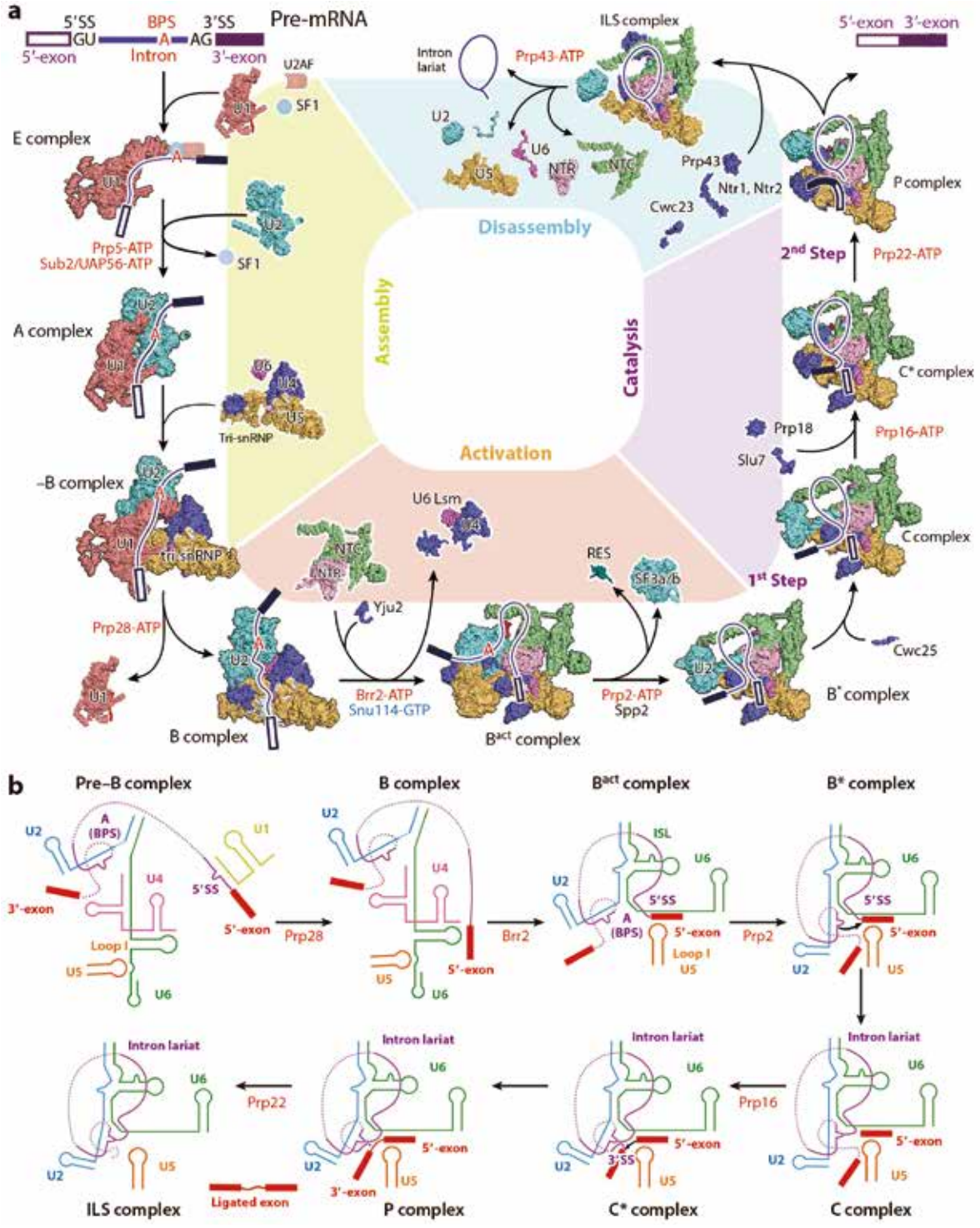
CAS member Prof. SHI Yigong, a distinguished structural biologist and President of Westlake University

The 2020 TKK Science Award in Life Sciences went to CAS member SHI Yigong, a distinguished structural biologist, for his achievements in elucidating the yeast and human splicing cycle in atomic detail, which allows us to visually appreciate the dynamic process of the supermolecular machinery of spliceosome performing molecular surgery on the chain of a pre-message RNA (pre-mRNA) to give rise to a mature mRNA.

Pre-mRNA splicing, the removal of non-coding introns and ligation of coding exons, a process similar to the old fashion film editing by cutting and splicing, is an essential step in gene expression. The splicing of a pre-mRNA together with modifications added to the terminals of the spliced mRNA give rise to a mature mRNA, which is then exported out of the nucleus to cytoplasm to make proteins. When things go wrong



A typical eukaryotic gene. The depicted gene contains four coding exons separated by three introns. The genomic DNA is transcribed into a pre-mRNA that contains all of the exons and introns. Splicing removes the introns and fuses the exons to generate the mature mRNA. (Adapted from *Molecular Biology of the Gene*, 7th edition, by J.D. Watson, et al., p468)



The current understanding of the 'molecular surgery' on pre-mRNA by the spliceosome. (a) A pre-mRNA splicing cycle consists of four major steps, which are spliceosome assembly, activation, catalysis, and disassembly. (b) Choreography of the RNA elements during the splicing cycle. The five RNA-protein complexes (U1, U2, U4, U5, and U6 snRNPs) sit at the core of the splicing machinery that fold the pre-mRNA to right place, so that the removal of the intron and ligation of the exons can properly occur. (Image by SHI's Lab)

during splicing, such as skipping one exon that is supposed to be presented in the mature mRNA or retaining an intron that ought to be spliced out, it leads to mistaken production of proteins. These abnormal scenarios of splicing contribute to approximately one third of human genetic diseases.

RNA splicing is performed by a large molecular machinery called spliceosome, which consists of five small nuclear ribonucleoprotein particles (U1, U2, U4, U5, and U6 snRNPs) and a large number of associated enzymes and cofactors. The splicing process is highly dynamic and the spliceosome keeps changing in composition and conformation along the splicing process.

To gain insights into how the splicing machinery changes over the course and get the job done, SHI and his co-workers sought to capture snapshots of the splicing machinery when it splices a simple pre-mRNA molecule (two exons spaced by one intron). The technology they used to take these snapshots is called cryo-electron microscopy (cryoEM), which applies electron beams to pass through the specimen at different angels and thereby produce many 2D projections on the sensor. These 2D data will later be used to reconstruct the specimen's 3D fine structure. These snapshots, put in the right order, form a choreography that allows us to appreciate the dynamic process of pre-mRNA splicing.

After decades of efforts, SHI' Lab has been successful in illustrating the compositional and conformational structures of the splicing machinery over the splicing cycle, as shown in the figure. Essentially, the pre-mRNA splicing can be seen as a molecular surgery on the chain of pre-mRNA performed by a dynamic group of surgeons (the active core formed mainly by the snRNPs) and nurses (a bunch of proteins and cofactors).

The first breakthrough was made in 2015. SHI and his co-workers reported the three-dimensional structure of a yeast spliceosome at 3.6-Å resolution (1 Å = 10^{-10} m), which marks the first atomic model of an intact spliceosome. During the following two years, they successfully obtained the refined structures of different complexes, including the pre-B and B complexes before activation, the catalytically activated B^{act} complex, the catalytic C and C^{*} complexes that accounts for the two critical reactions of splicing, and the post-catalysis P and ILS complexes during the disassembly stage. By taking all these snapshots, they gradually solve the jigsaw puzzle of how the huge machinery of yeast spliceosome coordinates its components to fold the pre-mRNA into place and wield its final blow to cut out the intron and put the two exons together. Moreover, SHI and his co-workers also revealed the key complexes of the human spliceosome in atomic detail.

"Elucidation of the molecular mechanism of pre-mRNA splicing has direct implications in potential therapeutic intervention of diseases. The discovery of the magic drug Spinraza against crippling spinal muscular atrophy (SMA) serves as a vivid reminder of how impactful basic research can be in real life. Spinraza is an antisense oligonucleotide that enhances production of the functional SMN protein through alternative splicing of the SMN2 gene," wrote SHI et al. in a review appeared in the renowned *Annual Review of Biochemistry*, "…how to harness the rich structural information on the spliceosome toward rational drug discovery represents both a challenge and an opportunity."

The revealing of these refined structures of the yeast and human spliceosomes and how they dynamically change their composition and conformation to splice a simplest model pre-mRNA, has been considered to be a milestone, allowing us to appreciate the whole cycle of pre-mRNA splicing step by step in atomic detail. With the access to this 'surgical manual' on the pre-mRNA, scientists may be able to design new drugs or strategies to precisely fix the glitches, and thereby prevent abnormal splicing events that cause human diseases.