**Splicing process under cryo-EM: structure of U4/U6.U5 tri-snRNP**

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Splicing of pre-mRNA is executed by the spliceosomes which contains five small nuclear ribonucleoproteins andlarge numbers of associated enzymes and cofactors. Using cryo-EM, structures can be clearly analyzied and process could be indicated. This article foucuses on researches of Y.Shi’s group on the splicing process, especially the down to date discovery: the 3.8 Å structure of the U4/U6.U5 tri-snRNP and the role it palys in spliceosome assembly and catalysis. This article also discussed cryo-EM and compared it with X-ray crystallography, for its decisive function in the whole study

**Introduction**

The central dogma has also been described as “DNA makes RNA and RNA makes protein” and it contains three essential steps: the first is transcription from DNA into pre-mRNA, then splicing from pre-mRNA into mature mRNA, and translation from mature mRNA into protein. As a vital part, splicing of pre-mRNA is executed by the spliceosomes which have been thought to be a protein-directed metalloenzyme. With two catalytic magnesium (Mg2+) ions as a reaction center, five small nuclear ribonucleoprotein particles (snRNPs)，that is U1, U2, U5, U4 and U6 snRNP, and large numbers of associated enzymes and cofactors involve in splicing of pre-mRNA, including the removal of introns from pre-mRNA and the post-transcriptional modification.

In 2015, Y.Shi’s group published two associated articles in the top international journal “science” named “Structure of a Yeast Spliceosome at 3.6 Angstrom Resolution” and “Structural basis of pre-mRNA splicing”. The first article reported the three-dimensional structure of yeast spliceosome near atomic resolution by single particle cryo electron microscopy (cryo-EM) analysis. The second article did detailed analysis on its base, describing the basic working mechanism of splicing of pre-RNA. These two articles forged ahead with the “central dogma” of molecular biology in the study of the molecular mechanism and Y.Shi said that they would continue to promote the basic research work and get a series of structure to clearly describe the whole process of gene splicing.

The down to date article is “The 3.8 Å structure of the U4/U6.U5 tri-snRNP: Insights into spliceosome assembly and catalysis”, published in January 2016 by Y.Shi’s group, which revealed the arrangements of the tri-snRNP in the activation process of the spliceosomal ribozyme by constructing a refined atomic mode of the the core regions of tri-snRNP under the 3.0-3.5 Å resolution of cryo-EM.

**Recent Progress**

To explain why the U4/U6.U5 tri-snRNP plays an indispensable role in the series of concerted steps of the assembly of the catalytically active spliceosome, this article showed and explained all parts of the tri-snRNP including the the cryo-EM maps of the yeast U4/U6.U5 tri-snRNP and its cartoon representative, structure of U4 and U6 snRNAs and their duplex together with spliceosomal proteins, structures of U5 snRNP and U5 snRNA, and how pre-mRNA was recognized by U6 snRNA and Loop I of U5 snRNA in the U4/U6.U5 tri-snRNP, indicating that pre-mRNA in the U4/U6.U5 tri-snRNP is poised for the splicing reaction.

Y.Shi’s group first drew a structure of the structure of the spliceosomal U4/U6.U5 tri-snRNP from S. cerevisiae which is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, and from the cartoon representative we can see a pre-mRNA molecule, three snRNA, and 30 proteins, including Prp 8 Core, Prp 3, Snu13, Prp 31, Prp6, Brr2 and so on. These proteins are working together to maintain the inactive structure of the tri-snRNP. During the catalytic activating progress of the spliceosome, these proteins should be disengaged to release the U4/U6 duplex and loose its winding for splicing. The protein that promotes the loose of helix is Brr2, which is a RNA-dependent ATPase. The group then shows the structure of the U4/U6 snRNA duplex and Base-pairing interactions between four nucleotides at the 5’-end of U4 snRNA and the nucleotides G78-A81 of U6 snRNA. They also compared the sequence alignment of U6 snRNA from S.cerevisiae, S.pombe, and Homo sapiens (H. sapiens), which reveals that this is a highly conserved catalytic sequence from yeast to human. Another statistic, a translocation of up to 100 Å from the nucleotides in U6 snRNA of the tri-snRNP to their corresponding positions in the active S.Pombe spliceosome, indicating that U6 snRNA undergoes a drastic conformational switch during assembly of a functional spliceosome.

After showing how the U4/U6 duplex is recognized by surrounding proteins (mainly by hydrogen bonds and structure fitness between helix and loop) and the structure of U5 snRNP and U5 snRNA, the group found evidence to conjecture that pre-mRNA is recognized by U6 snRNA and Loop I of U5 snRNA in the U4/U6.U5 tri-snRNP. The pre-mRNA is located in the center of tri-snRNP and forms duplexes with both U6 snRNA and U5 snRNA by base-pairing interactions between the exon sequences and Loop I of U5 snRNA. The group also indicated that the pre-mRNA in the U4/U6.U5 tri-snRNP is poised for the splicing reaction, because the tri-snRNP and the spliceosome showed a perfect alignment which proved that part of tri-snRNP had been adopted the morphology in the splicing reaction, including Prp8/Spp 42,Snu114/Cwf10 and U5.

**Discussion**

The most significant part in this work is the use of cryo-EM. Without cryo-EM, they cannot reach a sub-nanometer or near-atomic resolution to allow new insights into the structure and biology of these large assemblies, like spliceosome in Y.Shi’s work.

As we know, cryo-EM has been gaining popularity in structural biology in the last few years, however, as a landmark of the EM 3D reconstruction method, cryo-EM was born in 1968, about 10 years later than protein X-ray crystallography. It was even once mocked as “blobology” for its relatively low resolution compared with X-ray crystallography due to the bottleneck of technology method. But the most important revolutionary event occurred two or three years ago and pushed the cryo-EM technology to the peak, and to some extent could greatly exceed the crystallography, which mainly reflected in the following aspects:

First, with no need to crystallize, the scope of the research object has been greatly expanded, and research speed has been greatly improved. For small molecules, for example, small and stable protein is not difficult to crystallize, but as the research object of structural biology is becoming much more gigantic and complex, crystallization would be highly impracticable and almost impossible to apply; and even if it can be crystallized, it may not necessarily be diffracted and to obtain atomic resolution structure. However, cryo-EM method has been used to skip the crystallization of the super molecular complex and the direct target is structure of the solution state of the complex. Using this technology, scientists can analyze a new ribosomal structure in a week. For example, the Royal Society president, MRC-LMB structure center director Venki Ramakrishnan Professor, won the 2009 Nobel Prize in Chemistry for study on the crystal structure of the ribosome. His lab published the last crystal structure in 2014, and thereafter all of the articles were dominated by cryo-EM.

Second, sample preparation is fast, and only needs a small quantity for analysis, while shows a high repeatability. Important biological samples are very precious, but protein crystals generally require high concentration of large volume while the same amount of protein can be diluted to prepare a number of cryo-EM samples, each sample has hundreds of thousands of regions, each region has hundreds of small holes, each hole can collect more than one photo. Third, we can study the natural and dynamic structure. X ray crystallography can only try different conditions to obtain biological macromolecules a or some fixed state, and prone to crystal packing caused by the real interaction and one major weakness of the crystallography is its incapability to get a natural dynamic structure because researchers can desperately bypass the crystallization process. But cryo-EM is to do such thing----direct analysis of natural, liquid state, dynamic, and even in situ structure, in order to understand how living molecules execute their functions in both space and time scale.

By using cryo-EM, Y.Shi’s group has been analyzed some structures of some complex in the splicing process like the U4/U6.U5 tri-snRNP. But there is still a long way to go, for the large numbers of associated enzymes and cofactors have not been analyzed and their biological function still need to be studied. Furthermore, after finishing the structural analysis, it may be applied to medical research because many human diseases can be attributed to the error of the gene or the regulation of the splicing.

Re**ferences**

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