doi:10.1017/S1431927621005110
Microsc. Microanal. 27 (Suppl 1), 2021

Understanding the structure and function of spliceosome through cryo-EM

Xueni Li[1], Shiheng Liu[2], Hong Zhou[2] and Rui Zhao[1]

[1]University of Colorado Anschutz Medical Campus, United States, [2]University of California Los Angeles, United States

Pre-mRNA splicing is catalyzed by the spliceosome, a huge protein-RNA complex composed of the U1, U2, U4, U5, U6 snRNPs and many non-snRNP related proteins [1]. U1 snRNP plays a critical role in 5′ splice site recognition and is a frequent target of alternative splicing factors. These factors transiently associate with human U1 snRNP and are not amenable for structural studies, while their *Saccharomyces cerevisiae* (yeast) homologs are stable components of U1 snRNP. We have determined the structure of yeast U1 snRNP at 3.6Å resolution using cryo-EM [2]. The structure reveals common features as well as important differences from the human U1 snRNP. It provides atomic models of nearly all essential domains of U1 snRNA, all core proteins, and five auxiliary proteins. The structure offers a framework to integrate a wealth of existing genetic and biochemical data regarding the structure and function of yeast U1 snRNP and the mechanism of 5' ss recognition. In addition, the yeast U1 snRNP structure and biochemical analyses based on the structure provided intriguing insight into the structure and function of these auxiliary human U1 snRNP proteins in alternative splicing in higher eukaryotes.

In addition to the U1 snRNP, several other proteins recognize the BPS and 3' ss, forming the spliceosomal E complex that initiates the splicing cycle. Due to the lack of structural and mechanistic understanding of the E complex formation, how the splicing machinery accurately defines introns and exons remains a fundamental unanswered question. In yeast which typically contain small introns and large exons, intron definition, where the spliceosome initially recognizes and assembles across an intron, seems to dominate [3]. On the other hand, exon definition (the spliceosome recognizes and assembles across an exon first) [4] prevails in vertebrate, where small exons and large introns are prevalent. In addition to canonical splicing, a peculiar back-splicing reaction generates a class of circular RNAs (circRNAs) observed in diverse eukaryotic species [5]. We recently determined the cryo-EM structures of the yeast spliceosomal E complex assembled on introns, providing a view of the earliest event in the splicing cycle that commits pre-mRNAs to splicing. The E complex architecture suggests that the same spliceosome can assemble across an exon, and that it either remodels to span an intron for canonical linear splicing (typically on short exons) or catalyzes back-splicing to generate circular RNA (on long exons). The model is supported by our experiments, which show that an E complex assembled on the middle exon of yeast EFM5 or HMRA1 can be chased into circular RNA when the exon is sufficiently long. This simple model unifies intron definition, exon definition, and back-splicing through the same spliceosome in all eukaryotes and should inspire experiments in many other systems to understand the mechanism and regulation of these processes.

References

1 Wahl, M. C., Will, C. L. & Luhrmann, R. The spliceosome: design principles of a dynamic RNP machine. *Cell* **136**, 701-718, doi:10.1016/j.cell.2009.02.009 (2009).

2 Li, X. *et al.* CryoEM structure of Saccharomyces cerevisiae U1 snRNP offers insight into alternative splicing. *Nature communications* **8**, 1035, doi:10.1038/s41467-017-01241-9 (2017).

3 De Conti, L., Baralle, M. & Buratti, E. Exon and intron definition in pre-mRNA splicing. *Wiley interdisciplinary reviews. RNA* **4**, 49-60, doi:10.1002/wrna.1140 (2013).

4 Berget, S. M. Exon recognition in vertebrate splicing. *J Biol Chem* **270**, 2411-2414 (1995).

5 Wang, P. L. *et al.* Circular RNA is expressed across the eukaryotic tree of life. *PLoS One* **9**, e90859, doi:10.1371/journal.pone.0090859 (2014).

https://doi.org/10.1017/S1431927621005110 Published online by Cambridge University Press