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Structural and functional analyses of the spliceosome requires a multi-disciplinary approach

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Although the human genome contains ~25,000 genes [1], it is estimated we make over 90,000 proteins [2]. The disparity between our genome and our proteome can be explained by the activity of the spliceosome, a macromolecular machine composed of RNAs and proteins [3]. The spliceosome precisely removes introns from pre-mRNA to generate mature messages (mRNA), a process referred to as pre-mRNA splicing. The spliceosome is essential for cell function and defective pre-mRNA splicing causes disease [4–10].

The ~3 MDa spliceosome is composed of snRNPs (small nuclear ribonucleoprotein particles; U1, U2, U5 and U4/U6) and additional pre-mRNA splicing factors. Core components of the spliceosome are highly conserved across species. In contrast to other cellular RNPs such as the ribosome, none of the snRNPs contain a preformed catalytic site, and thus the formation of an active spliceosome requires numerous conformational rearrangements. *In vitro* studies show that snRNPs interact with the pre-mRNA in a stepwise and dynamic manner [11–16]. The transitions between spliceosome intermediates include both the global addition and/or removal of entire snRNPs and local rearrangements of protein-protein, protein-RNA, and/or RNA-RNA interactions. The large size and dynamic nature of the spliceosome has made it a challenging target for structure-function analyses. Although the hallmark of spliceosome function is its dynamicity, the structural changes required for the spliceosome to assemble, transition from a pre- to post-activated complex, and disassemble are still active topics of investigation.

To understand the molecular mechanism of this complex machine, researchers have embraced a multi-disciplinary approach spanning genetics, biochemistry, biophysical techniques, and structural biology. For many years difficulties in isolating large quantities of pure, stable spliceosomal complexes limited structural analysis to studies of individual components (examples include [17–27]), portions of snRNPs (notably the U1 snRNP [28]) and snRNAs (examples include [29–32]) using X-ray crystallography and NMR (Nuclear Magnetic Resonance) spectroscopy. With the recent “resolution revolution” in single-particle cryo-electron microscopy (cryo-EM) [33–37], it is now possible to routinely calculate sub-5 Å resolution structures of dynamic complexes that were previously intractable to mid- to

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high-resolution structural analysis. Thus, freed from the need to coax dilute and heterogeneous spliceosomal complexes into crystalline arrays, the use of single particle cryo-EM has led to a recent spate of sub-5 Å structures of multi-snRNP complexes that have provided the exciting first molecular snapshots of functional spliceosomes at various stages of the splicing reaction (reviewed in [38]). However, these structures do not have homogenous resolutions across their entire three-dimensional (3D) density maps, with various regions of each complex better resolved than others. This means that we still lack an atomic resolution map of any spliceosome complex. The cryo-EM structures represent a huge leap forward in our understanding of spliceosome organization, but do not provide the entire picture of spliceosome function. Thus, these structures serve as important “spring boards” for multi-disciplinary studies that will allow the field to delve deeper into the mechanistic details of spliceosome function.

This special issue aims to provide an overview into the current methods used to probe the structure and function of the dynamic spliceosome. The issue includes both reviews and original research papers that highlight the distinct structural and biophysical methodologies currently being used to address spliceosome function. Mayerle and Guthrie contribute a review that discusses the continuing importance of biochemistry and genetics for testing and extending the functional insights suggested by the mid- to high-resolution cryo-EM structures of multi-snRNP complexes [39]. Wiryaman and Toor present a review article that focuses on the methodologies used to determine the structure of a group II intron lariat [40]. Group II introns are self-splicing catalytic RNAs that use the same chemical reaction used by the spliceosome and thus can provide mechanistic insight into the active site required for eukaryotic pre-mRNA splicing. MacMillan and colleagues describe the methods used to synthesize a directed hydroxyl radical probe tethered to an mRNA substrate that can be used to probe the structures sampled during the spliceosome assembly process [41]. Dr. Pomeraz Krummel and colleagues present a research article on methods that can be used to covalently and reversibly capture the spliceosome at specific stages of spliceosome function [42]. Fairbrother and colleagues discuss the importance of pre-mRNA structure in the splicing reaction and the challenges associated with predicting RNA structure using computational approaches [43]. van der Feltz and Hoskins describe the methods they have used to probe spliceosome RNA dynamics using single-molecule fluorescence resonance energy transfer (smFRET), which when combined with fluorescent co-localization can link conformational dynamics with the presence of specific splicing factors during distinct stages of the splicing reaction [44]. Zhao and colleagues present a timely review on the continued strengths of using X-ray crystallography to determine atomic resolution structures of spliceosome components [45]. The spliceosome depends on a number of RNA helicases during spliceosome assembly, activation, and disassembly. Ficner, Dickmanns, and Neumann contribute a review discussing the combination of structural and biochemical methods that have been used to study the mechanisms of RNA helicase function during the splicing reaction [46]. Finally, Dr. Sperling presents a review of the structural studies that have been used to characterize a 21 MDa supraspliceosome purified from mammalian nuclei [47].

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