Max Planck Institute for Biophysical Chemistry

Research News

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Insights into the earliest stages of spliceosome assembly

The spliceosome is a complex macromolecular RNP enzyme that catalyzes pre-mRNA splicing. During splicing noncoding introns are removed from the pre-mRNA and the remaining coding regions (exons) are ligated together to form mRNA. Pre-mRNA splicing is an essential step in the complex pathway of gene expression. Multiple mRNAs can be generated by alternative splicing from a single pre-mRNA and thus alternative splicing events play a central role in expanding the complexity of the proteomes of higher organisms. Furthermore, errors in the splicing process or defects in splicing regulation are the cause, or act as a severity modifier, of a growing number of pathological conditions, including cancer and neuro-degenerative diseases.

Spliceosomes are assembled anew onto each pre-mRNA intron in a multi-step process during which the snRNPs U1, U2, U4, U5 and U6, plus a multitude of additional splicing factors, interact stepwise with the pre-mRNA. Most mammalian pre-mRNAs contain multiple introns whose sizes vary from several hundred to several thousand nucleotides, whereas their exons have a rather fixed length of only ~120 nucleotides (nts) on average. When an intron's length exceeds ~200-250 nts (which is the case for most introns in higher eukaryotes), splicing complexes first form across an exon, a process called exon definition. During the latter, the U2 snRNP binds the branch site/3'splice site (ss) upstream of the exon and the U1 snRNP binds the 5′ss downstream of it (see Figure, step 1). As the chemical steps of splicing occur solely in complexes formed across an intron, subsequent to exon definition the 3'ss must be paired across the adjacent intron with an upstream 5'ss (see Figure, step 3). Exon definition and the switch from an exon-defined to intron-defined splicing complex has long remained poorly understood. This step is decisive in determining which 5' and 3' exon will ultimately be spliced together and thus which kind of mRNA will be produced from a given pre-mRNA. Indeed recent data indicate that regulation of exon inclusion or skipping during several alternative splicing events occurs during the switch from a cross-exon to cross-intron complex.

Now, members of Reinhard Lührmann's research group (Department of Cellular Biochemistry, Max Planck Institute for Biophysical Chemistry), in collaboration with Henning Urlaub (Bioanalytical Mass Spectrometry, Max Planck Institute for Biophysical Chemistry) and Jamal Tazi (University of Montpellier), have succeeded in dissecting the switch from a cross-exon to cross-intron spliceosomal complex. By affinity purifying cross-exon splicing complexes and characterizing their composition,

they first demonstrated that, in addition to the U1 and U2 snRNPs, these complexes also contain stoichiometric amounts of the U4/U6.U5 tri-snRNP. The latter is recruited in part by a base pairing interaction between the U2 snRNA and U6 snRNAs, the RNA components of the U2 and U6 snRNPs (Figure, step 2). They then demonstrated that the tri-snRNP interaction with the cross-exon complex is stabilized by a 5' splice site-containing oligonucleotide. The latter binds the tri-snRNP and converts the cross-exon complex into a spliceosomal complex containing all of the spliceosomal snRNPs which mimicks a cross-intron spliceosomal B complex.

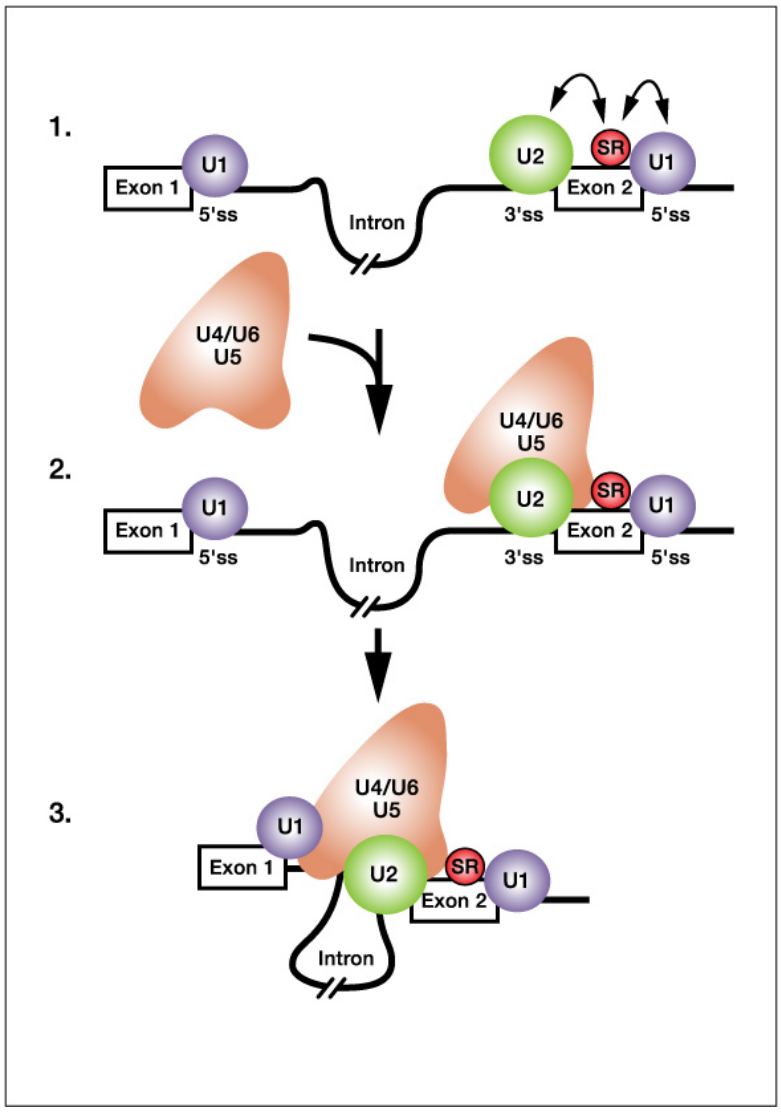

Illustration of exon definition during the earliest stages of spliceosome assembly (steps 1 and 2) and the subsequent conversion of a cross-exon splicing complex to a complex spanning an upstream intron (step 3). *Picture: Lührmann / MPIbpc). Adapted from Schneider et al., 2010.*

These data suggest that the switch from cross-exon to cross-intron complexes can occur directly when an exon-bound tri-snRNP interacts with an upstream 5'ss (Figure step 3), without prior formation of a cross-intron complex containing solely the U1 and U2 snRNPs (which was previously postulated to be formed first). They also indicate that the pairing of the 5'ss and 3'ss across an intron can first occur after all of the snRNPs have assembled on the intron. The results of these studies, which recently were published in the journal *Molecular Cell*, provide new insights into the assembly pathway of spliceosomes generated initially via exon definition and represent a major step forward in our understanding of the earliest stages of spliceosome assembly. [cw/rl]

Original publication:

M. Schneider, C.L. Will, M. Anokhina, J. Tazi, H. Urlaub, and R. Lührmann:
Exon definition complexes contain the tri-snRNP and can be directly converted into B-like precatalytic splicing complexes. *Mol. Cell* **38**, 223-235 (2010).

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