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The spliceosome: caught in a web of shifting interactions

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Splicing is a crucial, ubiquitous and highly complex step in eukaryotic gene expression. The daunting complexity of the splicing reaction, although fascinating, has severely limited our understanding of its mechanistic details. Recent advances have begun to provide exciting new insights into the dynamic interactions that govern the function of the spliceosome, the multi-megadalton complex that performs splicing. An emerging paradigm is the presence of a succession of distinct conformational states, which are stabilized by an intricate network of interactions. Recent data suggest that even subtle changes in the composition of the interaction network can result in interconversion of the different conformational states, providing opportunities for regulation and proofreading of spliceosome function. Significant progress in proteomics has elucidated the protein composition of the spliceosome at different stages of assembly. Also, the increased sophistication and resolution of cryo-electron microscopy techniques, combined with high-resolution structural studies on a smaller scale, promise to create detailed images of the global structure of the spliceosome and its main components, which in turn will provide a plethora of mechanistic insights. Overall, the past two years have seen a convergence of data from different lines of research into what promises to become a holistic picture of spliceosome function.

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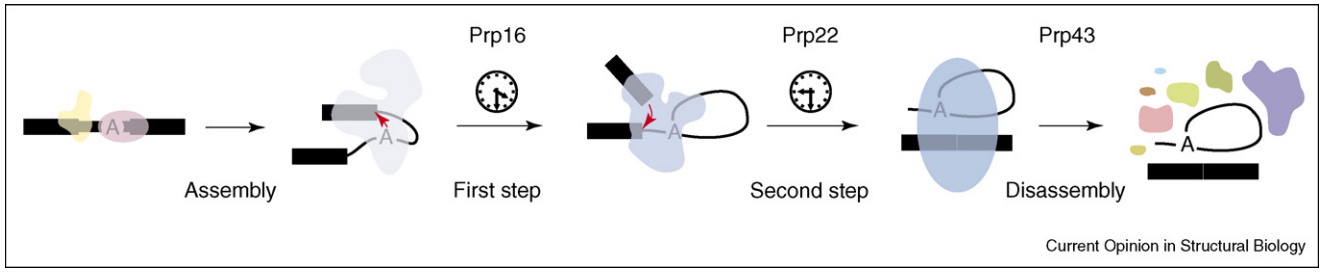
Introduction

A fascinating feature of eukaryotic genomes is the abundance of non-coding intervening sequences, or introns, which frequently interrupt functional genes and thus need to be removed before genetic information can be used by the cell. Any mistake in the process of recognition and removal of introns, or splicing, would lead to an altered genetic message and thus has potentially catastrophic consequences. Commensurate with its critical role, the spliceosome, the molecular assembly that performs the splicing reaction, is one of the largest and most sophisticated cellular machines, consisting of over 200 different proteins and five small nuclear RNA molecules (snRNAs) [1,2]. The spliceosomal components create a highly complex and dynamic network of RNA–RNA, RNA–protein and protein–protein interactions, which undergo extensive remodeling during each splicing cycle. Splicing is performed through two consecutive transesterification reactions (Figure 1). During the first step, the 2′-hydroxyl of a conserved adenosine residue, the branch site adenosine, carries out a nucleophilic attack on the upstream boundary of the intron — the 5′-splice site. The result of this reaction is the release of the upstream coding region, or exon, and formation of a lariat intron. The 3′-hydroxyl group of the newly released exon carries out a nucleophilic attack on the downstream boundary of the intron (the 3′-splice site), resulting in release of the lariat intron and ligation of the two exons. Although three decades of intense research has provided us with an outline of several of the interactions involved in spliceosome assembly, splice site fidelity and regulation of spliceosome function, it has previously not been possible to develop a unified picture from these seemingly disparate data. This review focuses on the advances made in the past two years, which have helped coalesce a large body of information into a single model for spliceosomal function. It also touches upon the recent progress in structural biology efforts that aim to complement the genetic and biochemical approaches by defining the global structure of the spliceosome in various conformational states.

Kinetic proofreading: a recurring theme in spliceosomal quality control

A crucial insight into spliceosomal function came from genetic studies indicating that mutations that slowed the rate of ATP hydrolysis of a spliceosomal RNA-dependent helicase, Prp16, resulted in a decrease in fidelity of the first step of splicing [3,4]. This result was best explained by a kinetic proofreading model that stated that Prp16 acts as a timer for the first step of splicing by changing the conformation of the spliceosome active site from one suitable for catalysis of the first step to another that favors second step catalysis. As suboptimal splicing substrates would take longer than optimal substrates to perform the first step of splicing, they would not be able to undergo the first step before the Prp16-mediated conformational change and thus would be discarded. Mutations that slow ATP hydrolysis by Prp16 would allow the suboptimal substrates to undergo the first step of splicing, thereby reducing fidelity. Although this was a very attractive model for the role of spliceosomal

Figure 1



The spliceosomal cycle. The identity of the factors responsible for the fidelity of each step is indicated. The shape of the spliceosomal complexes has been taken from cryo-EM studies [1,27•,31].

helicases, until recently no other examples of such a mechanism were known.

Recently, Staley and co-workers [5••] provided evidence of a very similar mechanism controlling the fidelity of the second step of splicing. Mutants of another spliceosomal helicase, Prp22, not only allowed mutant 3′-splice sites to undergo the second step, but they also helped aberrant intermediates resulting from mutations in the 5′-splice site or branch site undergo the second step more efficiently, indicating a general improvement in the efficiency of the second step of splicing. Interestingly, Prp22 alleles that showed this effect had impaired ATPase or RNA 'unwindase' activity, suggesting that an ATP-dependent conformational switch mechanism is probably involved. Similar to the role of Prp16 in the first step, wild-type Prp22 probably causes a conformational switch in the spliceosome that occurs before the slow-reacting, suboptimal substrates undergo the second step. Thus, there is kinetic competition between the Prp22-induced conformational change and the speed of catalysis of the second step of splicing; this ensures the fidelity of this step [6]. Mutants of another spliceosomal helicase, Prp43, which is involved in disassembly of the spliceosome, also suppress spliceosome activation defects in an ATP-dependent way, consistent with a general role for helicases in spliceosomal quality control (Figure 1) [7,8].

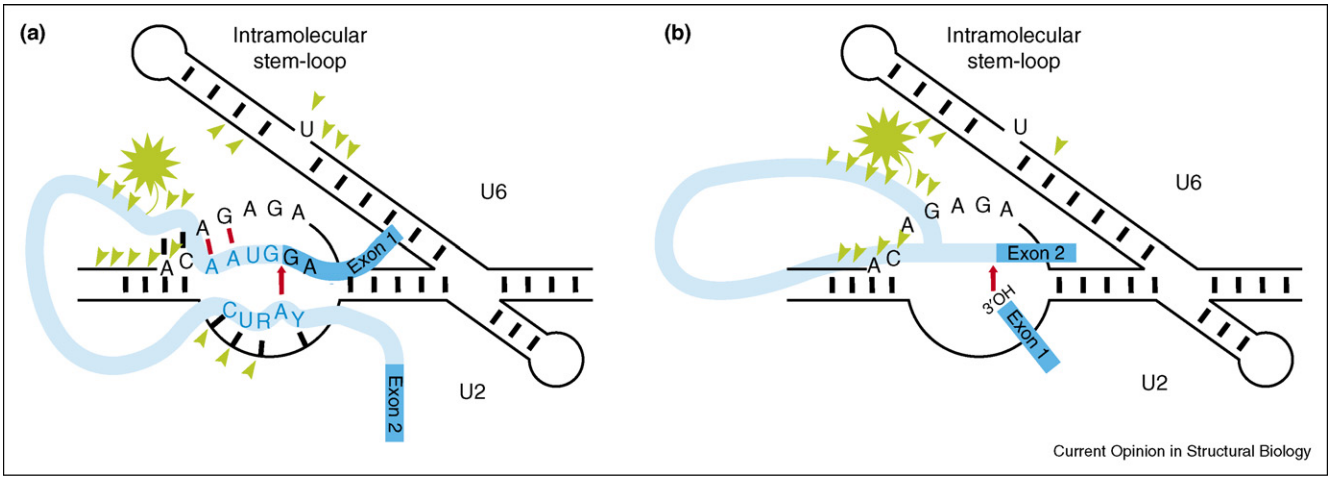
A Yin/Yang model for the spliceosome active site

Consistent with the above model, two recent studies [9,10•] have shown that several mutations of spliceosomal factors Prp8, Prp16, ISY1 (a component of the Prp19 complex) and U6 snRNA can result in a general improvement in the rate of either the first or the second step of splicing, at the expense of repressing the other step. Many of these mutants were able to suppress the splicing defects of a wide variety of suboptimal substrates, indicating lack of allele specificity. These results, which recapitulate a large body of previous data from individual mutants, are consistent with the presence of two mutually exclusive conformational states of the active site during the two steps of splicing. Mutations of splicing factors can result in the disruption of interactions that stabilize one of these two states, resulting in an altered balance between them. This can result in the spliceosome active site being selectively stabilized at the first or second step conformation, over-riding the function of the kinetic proofreading timers and resulting in a general increase in the rate of that step for suboptimal substrates. Konarska and Query [9,11] suggest that the effect of these spliceosomal mutations mimics mutations that affect the decoding of tRNA by the ribosome, which similarly depends on a balance between open and closed conformations. A large number of mutations of ribosomal factors seem to modulate this balance [11]. Thus, kinetic proofreading by altering the balance between different conformational states might be a common quality control mechanism for large cellular machines.

Remodeling the active site during the transition between the two splicing steps

The above-mentioned model is highly appealing because it provides an explanation for a large number of apparently unrelated data and ties them together in a unified theory for the function of the spliceosome active site. Recently, two groups have provided tangible evidence for the existence of mutually incompatible conformational states of the active site. Using a genetic approach, Konarska *et al.* [12••] showed that, whereas base-pairing interactions between the 5′-splice site and the catalytically crucial ACAGAGA domain of U6 snRNA (Figure 2) are required for the first step of splicing, they cause a decrease in the efficiency of the second step. Mutations that reduced the complementarity between U6 and positions +3 and +4 of the 5′-splice site (Figure 2) resulted in a general increase in the efficiency of the second step of splicing for a wide variety of suboptimal second step substrates. These results suggest that the interactions that position the 5′-splice site in the spliceosome active site for the first step of splicing have to be broken before the second step can take place; thus, at least part of the remodeling between the first and second step confor-

Figure 2



The active site conformational change during the transition between the first and second steps of splicing. The U6 and U2 snRNAs are shown as black lines. The ACAGAGA box and the conserved uridine residue in the intramolecular stem-loop of U6 are shown as letters. The pre-mRNA is shown in dark blue (exons) and light blue (introns). The base-pairing interactions are shown by short bars. The interactions between positions +3 and +4 of the 5′-splice site and U6 [12••] are shown by red bars. Red arrows mark the direction of the nucleophilic attack in **(a)** the first or **(b)** the second step of splicing. The green star marks the location of the tethered hydroxyl radical source [13••]. The sites of hydroxyl radical cleavage are shown by green arrowheads.

mations can be attributed to the differential positioning of the substrates in the active site.

An interesting remaining question is whether, in addition to a shift in the positioning of the substrate, other elements of the active site are also significantly remodeled during the transition from the first to the second step beyond the changes necessary to accommodate the reactants of the second step in the active site. Recent evidence against large-scale remodeling is provided by results from hydroxyl radical probing of the vicinity of nucleotide +10 of the 5′-splice site in fully assembled spliceosomes and spliceosomes stalled immediately before the second step of splicing, which have most probably undergone the second step conformational change [13••]. These experiments indicated that, whereas the conserved ACAGAGA box and intramolecular stem-loop of U6 (Figure 2) were cleaved both before and after the first step, there is a minor shift in the area targeted by hydroxyl radical cleavage (Figure 2). Together with previous data [2,14], these results suggest that, although clear remodeling occurs in the spliceosomal active site between the two steps of splicing, the global architecture of the active site is likely to be preserved.

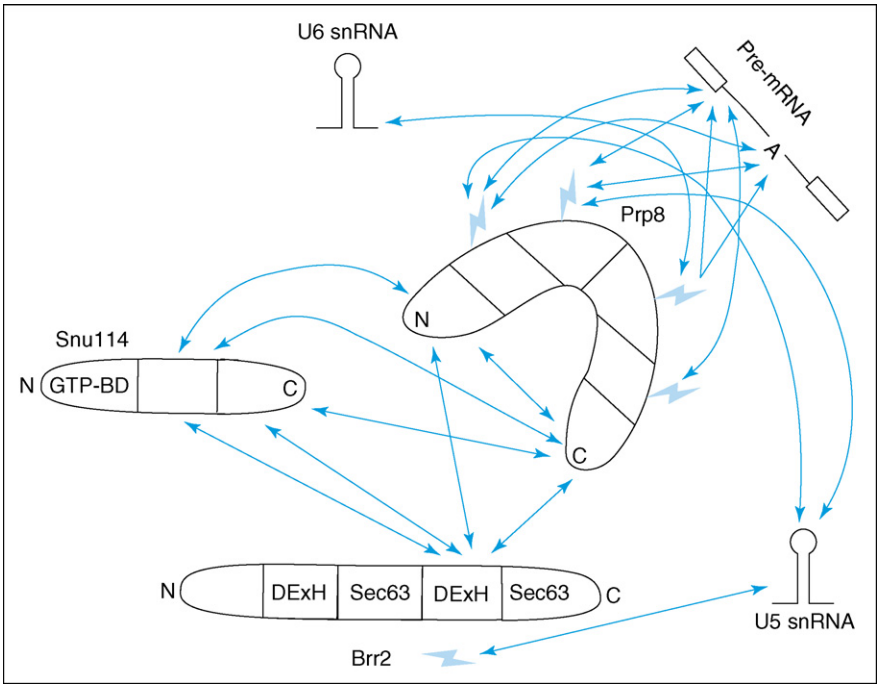
Orchestration of the spliceosomal cycle: an intricate web of protein–protein interactions

Recent data from Staley and co-workers [15••] suggest that two spliceosomal proteins, Snu114 and Brr2, act as regulatory switches during two remodeling steps in the splicing reaction: the dissociation of di-snRNP U4/U6 (which contains snRNAs U4 and U6 along with their associated proteins) before spliceosome activation and the disassembly step after the second step of splicing. In fact, Snu114 seems to act as a classic regulatory G protein, as both U4/U6 dissociation and spliceosomal disassembly are blocked when Snu114 is bound to GDP, whereas GTP binding releases the block. The exact role of GTP hydrolysis in these reactions remains to be established [15••,16,17]. Snu114 probably exerts its effect through Brr2, a U5-associated helicase that is a strong candidate for directly executing dissociation and disassembly. However, there is a strong possibility that several other spliceosomal factors, including Prp8, play a role in this regulatory pathway [1,17,18]. The highly elaborate network of protein–protein interactions uncovered by recent studies is indicative of a complexity far beyond a simple linear regulatory network [1,17–19,20•,21•]. Rather, current data suggest the presence of highly complex feedback loops, two-way signaling, signal convergence and integration, and a high level of cooperativity in the active site (Figure 3). Recent results indicating the functional importance of a ubiquitin-binding domain of Prp8 suggest a role for post-translational modifications in splicing, which may add yet another level of complexity to splicing regulation [15••,17,22].

An intricate web of RNA-mediated interactions

Genetic and biochemical analyses *in vitro* and *in vivo* have indicated the presence of an equally elaborate RNA–RNA and RNA–protein interaction network in the spliceosome (Figure 3) [1,2]. The crucial role of snRNAs in splicing, and mechanistic and structural

Figure 3



The network of interactions between several elements of the spliceosomal active site. The fragmentation pattern used in yeast two-hybrid assays [20•] is indicated for each protein. The C and N termini of each protein are shown. The identity of protein domains present in protein fragments is indicated. The thunderbolts refer to interactions proven by UV cross-linking [25•,32]. Adapted from [20•] with permission.

similarities to self-splicing group II introns suggest that the snRNAs form most, if not all, of the spliceosomal active site ([1,23]; M Mohammadi and S Valadkhan, unpublished). Thus, the net effect of many of the spliceosomal conformational rearrangements is likely to be the remodeling of the RNA elements, which will result in regulation of catalysis. A good candidate for mediating the transmission of regulatory signals to the RNA elements of the active site is the highly conserved protein Prp8, which forms extensive interactions with all active site RNA elements and several spliceosomal proteins (Figure 3) [21•,24]. In a recent study, Newman and colleagues [25•] used a novel transposon-mediated protein dissection method to map the areas in Prp8 that directly contact several active site RNA elements. Interestingly, most of these contact sites are clustered in three regions in the middle third of Prp8 (Figure 3). It is conceivable that these sites might be the end point of the spliceosomal rearrangements, where the regulatory signals converge and are communicated to the spliceosomal active site.

Toward a global structure of the spliceosome

Structural studies of the spliceosome are greatly hindered by its dynamic nature and very large size; however, in recent years, cryo-electron microscopy (cryo-EM) studies of the spliceosome have provided a first glimpse of the global structure of the snRNPs and the spliceosomal complexes, albeit at low resolution [26]. A recent cryo-EM structure of the U4/U6–U5 tri-snRNP (which contains U4, U5 and U6 snRNAs plus many crucial spliceosomal proteins, including Prp8, Snu114 and Brr2) not only made it possible to determine the position of U4/U6 and U5 and their mode of interaction with each other in the tri-snRNP complex, but also permitted the identification of the position of structural features of the U5 snRNA in the tri-snRNP complex via labeling studies [27•]. Further development of the labeling methods, combined with the availability of high-resolution structures of an increasing number of spliceosomal components [28,29], promises to provide a much more detailed global structure of the spliceosome; this will, in turn, provide invaluable insights into spliceosomal dynamics and function.

Conclusions

The past two years have seen the spliceosome field moving toward developing a global paradigm for spliceosome function. The new models, in addition to explaining the existing data, create a framework for future mechanistic studies of the spliceosome. Significant progress in the purification of spliceosomes in various functional states, combined with advances in proteomics, has permitted us to define the spliceosomal factors recruited at each stage and has made it possible to perform *in vitro* complementation studies [5••,15••,30]. These technical advances have made it possible to address a wide range of questions on spliceosome function, including the requirements for

spliceosome activation and progression through the spliceosomal cycle. Together with the increasing sophistication and resolution of structural studies on the spliceosome, the next few years will witness major advances in our understanding of this highly complex cellular machine.

Update

Two recent papers from the Ares [33•] and Staley [34•] laboratories indicate that the U2 sequences downstream of the branch-binding site undergo a number of functionally important conformational rearrangements between two mutually exclusive stem structures throughout the splicing cycle, thus proving the presence of yet another set of dynamic interactions in the spliceosome. In addition, Pena *et al.* [35•] recently obtained high-resolution structural data for an hPrp8 fragment that encompasses a Jab1/MPN-like domain. Yeast two-hybrid analyses suggest that mutations in the C-terminal amino acids of hPrp8, which lead to hereditary blindness in humans, alter the interaction of Prp8 with hBrr2 and hSnu114.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Will CL, Luhrmann R: **Spliceosome structure and function**. In *The RNA World.* Edited by Gesteland RF, Cech TR, Atkins JF. Cold Spring Harbor Laboratory Press; 2006:369-400.

2. Nilsen TW: **RNA-RNA interactions in nuclear pre-mRNA splicing**. In *RNA Structure and Function.* Edited by Simons RW, Grunberg-Manago M. Cold Spring Harbor Laboratory Press; 1998: 279-307.

3. Staley JP, Guthrie C: **Mechanical devices of the spliceosome: motors, clocks, springs, and things**. *Cell* 1998, **92**:315-326.

4. Burgess SM, Guthrie C: **A mechanism to enhance mRNA splicing fidelity: the RNA-dependent ATPase Prp16 governs usage of a discard pathway for aberrant lariat intermediates**. *Cell* 1993, **73**:1377-1391.

5. •• Mayas RM, Maita H, Staley JP: **Exon ligation is proofread by the DExD/H-box ATPase Prp22p**. *Nat Struct Mol Biol* 2006, **13**:482-490.
Using yeast spliceosomes stalled after the first step on a substrate with a mutated 3′-splice site, the authors provide evidence for an ATP-dependent mechanism that blocks the second step of splicing for mutant 3′-splice sites. Mutations of a spliceosomal helicase, Prp22, disable this mechanism, indicating a role for this helicase in splicing fidelity.

6. Query CC, Konarska MM: **Splicing fidelity revisited**. *Nat Struct Mol Biol* 2006, **13**:472-474.

7. Tsai RT, Fu RH, Yeh FL, Tseng CK, Lin YC, Huang YH, Cheng SC: **Spliceosome disassembly catalyzed by Prp43 and its associated components Ntr1 and Ntr2**. *Genes Dev* 2005, **19**:2991-3003.

8. Pandit S, Lynn B, Rymond BC: **Inhibition of a spliceosome turnover pathway suppresses splicing defects**. *Proc Natl Acad Sci USA* 2006, **103**:13700-13705.

9. Query CC, Konarska MM: **Suppression of multiple substrate mutations by spliceosomal prp8 alleles suggests functional correlations with ribosomal ambiguity mutants**. *Mol Cell* 2004, **14**:343-354.

10. • Villa T, Guthrie C: **The Isy1p component of the NineTeen complex interacts with the ATPase Prp16p to regulate the fidelity of pre-mRNA splicing**. *Genes Dev* 2005, **19**:1894-1904.
In a screen for an extragenic suppressor of Prp16 in yeast, the authors find a single suppressor, a truncation mutant of ISY1. Deletion mutations of ISY1 led to a general increase in the fidelity of the first step and a more efficient second step with reduced fidelity.

11. Konarska MM, Query CC: **Insights into the mechanisms of splicing: more lessons from the ribosome**. *Genes Dev* 2005, **19**:2255-2260.

12. •• Konarska MM, Vilardell J, Query CC: **Repositioning of the reaction intermediate within the catalytic center of the spliceosome**. *Mol Cell* 2006, **21**:543-553.
Using a combination of genetic and biochemical analyses, the authors prove that mutations of non-conserved nucleotides of the 5′-splice site (positions +3 and +4) that increase complementarity to U6 result in a decrease in the efficiency of the second step of splicing. Mutations that reduce this complementarity lead to a general improvement in the efficiency of the second step.

13. •• Rhode BM, Hartmuth K, Westhof E, Luhrmann R: **Proximity of conserved U6 and U2 snRNA elements to the 5′ splice site region in activated spliceosomes**. *EMBO J* 2006, **25**:2475-2486.
The authors probe the vicinity of the 5′-splice site by placing a site-tethered hydroxyl radical source at nucleotide +10 in human spliceosomes immediately before the first and second steps. Although the global pattern of cleavage of snRNAs and the pre-mRNA remains the same, there is a clear change in the cleavage pattern, consistent with a conformational rearrangement between the two steps.

14. Umen JG, Guthrie C: **The second catalytic step of pre-mRNA splicing**. *RNA* 1995, **1**:869-885.

15. •• Small EC, Leggett SR, Winans AA, Staley JP: **The EF-G-like GTPase Snu114p regulates spliceosome dynamics mediated by Brr2p, a DExD/H box ATPase**. *Mol Cell* 2006, **23**:389-399.
Using purified yeast spliceosomes poised for disassembly, the authors prove a role for Snu114 and Brr2 in this step. Whereas adding GDP blocks disassembly, addition of GTP relieves this block. Using mutants that change the specificity of the GTP-binding domain of Snu114 to XTP, they prove that this effect is mediated by Snu114. In addition, the authors show an ATP-dependent role for Brr2 in spliceosome dissociation. Furthermore, both these proteins are involved in spliceosome activation, indicating a regulated function for these two remodeling factors in multiple steps of the spliceosomal cycle.

16. Bartels C, Urlaub H, Luhrmann R, Fabrizio P: **Mutagenesis suggests several roles of Snu114p in pre-mRNA splicing**. *J Biol Chem* 2003, **278**:28324-28334.

17. Brenner TJ, Guthrie C: **Genetic analysis reveals a role for the C terminus of the *Saccharomyces cerevisiae* GTPase Snu114 during spliceosome activation**. *Genetics* 2005, **170**:1063-1080.

18. Brenner TJ, Guthrie C: **Assembly of Snu114 into U5 snRNP requires Prp8 and a functional GTPase domain**. *RNA* 2006, **12**:862-871.

19. Bottner CA, Schmidt H, Vogel S, Michele M, Kaufer NF: **Multiple genetic and biochemical interactions of Brr2, Prp8, Prp31, Prp1 and Prp4 kinase suggest a function in the control of the activation of spliceosomes in *Schizosaccharomyces pombe***. *Curr Genet* 2005, **48**:151-161.

20. • Liu S, Rauhut R, Vornlocher HP, Luhrmann R: **The network of protein-protein interactions within the human U4/U6.U5 tri-snRNP**. *RNA* 2006, **12**:1418-1430.
Using co-immunoprecipitation and yeast two-hybrid assays, the authors map the interactions between the various proteins found in human tri-snRNP. They elucidate a highly complex pattern of interactions between these proteins, which provides the basis for tri-snRNP stability and protein–protein interactions in the assembled spliceosome.

21. • Boon KL, Norman CM, Grainger RJ, Newman AJ, Beggs JD: **Prp8p dissection reveals domain structure and protein interaction sites**. *RNA* 2006, **12**:198-205.

A novel transposon-mediated approach is used by the authors to show that the highly conserved U5-specific protein Prp8 can be divided into two pieces at certain positions and that the two pieces are functional when both are provided *in trans*. In addition, the authors use the same approach to determine the regions of Prp8 that interact with Snu114 and Aar2.

22. Bellare P, Kutach AK, Rines AK, Guthrie C, Sontheimer EJ: **Ubiquitin binding by a variant Jab1/MPN domain in the essential pre-mRNA splicing factor Prp8p**. *RNA* 2006, **12**:292-302.

23. Valadkhan S, Manley JL: **Splicing-related catalysis by protein-free snRNAs**. *Nature* 2001, **413**:701-707.

24. Grainger RJ, Beggs JD: **Prp8 protein: at the heart of the spliceosome**. *RNA* 2005, **11**:533-557.

25. • Turner IA, Norman CM, Churcher MJ, Newman AJ: **Dissection of Prp8 protein defines multiple interactions with crucial RNA sequences in the catalytic core of the spliceosome**. *RNA* 2006, **12**:375-386.

To map the regions of Prp8 that directly contact the spliceosomal snRNAs and the pre-mRNA, the authors use a novel transposon-mediated approach. Interestingly, the authors show that there are certain areas in Prp8 that tolerate the transposon insertions, whereas others do not. They also show that most protein–RNA interactions cluster in three regions in the central third of the protein.

26. Stark H, Luhrmann R: **Cryo-electron microscopy of spliceosomal components**. *Annu Rev Biophys Biomol Struct* 2006, **35**:435-457.

27. • Sander B, Golas MM, Makarov EM, Brahms H, Kastner B, Luhrmann R, Stark H: **Organization of core spliceosomal components U5 snRNA loop I and U4/U6 di-snRNP within U4/U6.U5 tri-snRNP as revealed by electron cryomicroscopy**. *Mol Cell* 2006, **24**:267-278.

Purified human tri-snRNP, di-snRNP and U5 snRNP are subjected to cryo-EM analysis. Furthermore, the authors used a labeling approach to determine the site of conserved loop 1 and the tri-methylguanine cap of U5 snRNA in U5 snRNP and the tri-snRNP particle. The authors successfully fit the individual structures of U5 snRNP and di-snRNP into the deduced tri-snRNP structure.

28. Schellenberg MJ, Edwards RA, Ritchie DB, Kent OA, Golas MM, Stark H, Luhrmann R, Glover JN, MacMillan AM: **Crystal structure of a core spliceosomal protein interface**. *Proc Natl Acad Sci USA* 2006, **103**:1266-1271.

29. Spadaccini R, Reidt U, Dybkov O, Will C, Frank R, Stier G, Corsini L, Wahl MC, Luhrmann R, Sattler M: **Biochemical and NMR analyses of an SF3b155-p14-U2AF-RNA interaction network involved in branch point definition during pre-mRNA splicing**. *RNA* 2006, **12**:410-425.

30. Deckert J, Hartmuth K, Boehringer D, Behzadnia N, Will CL, Kastner B, Stark H, Urlaub H, Luhrmann R: **Protein composition and electron microscopy structure of affinity-purified human spliceosomal B complexes isolated under physiological conditions**. *Mol Cell Biol* 2006, **26**:5528-5543.

31. Jurica MS, Sousa D, Moore MJ, Grigorieff N: **Three-dimensional structure of C complex spliceosomes by electron microscopy**. *Nat Struct Mol Biol* 2004, **11**:265-269.

32. Reyes JL, Gustafson EH, Luo HR, Moore MJ, Konarska MM: **The C-terminal region of hPrp8 interacts with the conserved GU dinucleotide at the 5′ splice site**. *RNA* 1999, **5**:167-179.

33. • Perriman RJ, Ares M Jr: **Rearrangement of competing U2 RNA helices within the spliceosome promotes multiple steps in splicing**. *Genes Dev* 2007, **21**:811-820.

Using yeast genetic analyses, the authors prove the presence a dynamic rearrangement in yeast U2 snRNA, in which a switch between the mutually exclusive stems IIa and IIc promotes different splicing steps.

34. • Hilliker AK, Mefford MA, Staley JP: **U2 toggles iteratively between the stem IIa and stem IIc conformations to promote pre-mRNA splicing**. *Genes Dev* 2007, **21**:821-834.

The authors show that U2 snRNA toggles between stem IIc, which seems to exist at the time of splicing catalysis, and stem IIa, which seems to promote the binding and release of substrates. The authors indicate that this switch is facilitated by Prp16.

35. • Pena V, Liu S, Bujnicki JM, Luhrmann R, Wahl MC: **Structure of a multipartite protein-protein interaction domain in splicing factor prp8 and its link to retinitis pigmentosa**. *Mol Cell* 2007, **25**:615-624.

This work provides the first high-resolution structure of an hPrp8 fragment, proving the presence of a predicted Jab1/MPN domain. The authors also show that, in yeast two-hybrid assays, the mutations in the C terminus of hPrp8 that cause *Retinitis pigmentosa* diminish the interaction of Prp8 with hBrr2 and hSnu114 under stringent conditions.