Chapter 2
Pre-mRNA Splicing and the Spliceosome: Assembly, Catalysis, and Fidelity

Elizabeth A. Dunn and Stephen D. Rader

Abstract At the level of gene architecture, the widespread presence of interrupting sequences in eukaryotic genes serves as a defining difference between eukaryotic organisms and other domains of life. These interrupting sequences, known as introns, must be precisely removed from pre-messenger RNA (pre-mRNA) transcripts. Concomitantly, the coding regions, or exons, are joined together through a nuclear-localized process known as pre-mRNA splicing. A number of splicing factors, both protein and RNA, assemble into a multimegadalton splicing machine known as the spliceosome, which is responsible for identifying the intronic regions and positioning the pre-mRNA substrate in a favorable orientation for the splicing reactions to occur. While the chemical steps of splicing—two sequential transesterification reactions—are identical in all eukaryotes, the gene architecture and splicing apparatus can differ substantially. Here, we review our current understanding of the splicing process with an emphasis on the model organism *Saccharomyces cerevisiae*. We discuss the key features of introns, along with mechanistic aspects of the splicing cycle, namely spliceosome assembly, catalysis, and spliceosome disassembly. We also highlight recent discoveries supporting the role of kinetic proofreading in ensuring the fidelity of splicing.

S. D. Rader (✉)
Department of Chemistry, University of Northern British Columbia,
Prince George, BC, Canada
e-mail: rader@unbc.ca

E. A. Dunn
Department of Biochemistry and Molecular Biology,
University of British Columbia, Vancouver, BC, Canada

A. Sesma and T. von der Haar (eds.), *Fungal RNA Biology*,
DOI: 10.1007/978-3-319-05687-6_2,

Contents

Gene Architecture

The eukaryotic gene is composed of protein coding sequences, known as exons, which are interrupted by non-protein coding sequences called introns (Fig. 2.1a). Over the last decade and a half, a number of complete fungal genomes have been sequenced, allowing in-depth comparative analyses of intronic features, intron abundance and position, and intron evolution. These analyses have revealed that intron-containing genes are prevalent across fungal species to varying extents, although an intron-poor genome appears to be a common feature in budding yeasts (Neuvéglise et al. 2011). In most of these species, including the well-established model organism *Saccharomyces cerevisiae*, fewer than 10 % of genes contain an intron (Spingola et al. 1999; Bon et al. 2003; Neuvéglise et al. 2011). For example, only 5 % of *S. cerevisiae*'s genes contain an intron, in stark contrast to the intron-rich fission yeast, *Schizosaccharomyces pombe*, in which 43 % of genes contain introns (Wood et al. 2002). *S. pombe* most closely resembles metazoans in this respect. For example, approximately 95 % of human genes contain introns (Venter et al. 2001). Much like mammalian genes, composed of an average of seven introns per gene, *S. pombe* genes tend to contain multiple introns, with 34 genes containing between 7 and 15 introns (Sakharkar et al. 2004; Wood et al. 2002). In fact, only 45 % of intron-containing genes in *S. pombe* contain a single intron while 49 % contain two, three, or four introns (Wood et al. 2002).

In contrast, hemiascomycetous yeast species (including *Saccharomyces* and *Candida* species) possess very few genes with a single intron and no gene with more than two (Spingola et al. 1999; Neuvéglise et al. 2011). Intriguingly, the introns of all budding yeasts show a positional bias toward the 5′ end of the gene (Spingola et al. 1999; Neuvéglise et al. 2011). In *S. pombe*, however, introns in genes that contain seven introns or more are distributed throughout the gene with no positional bias, much like metazoans. In contrast, those genes that contain

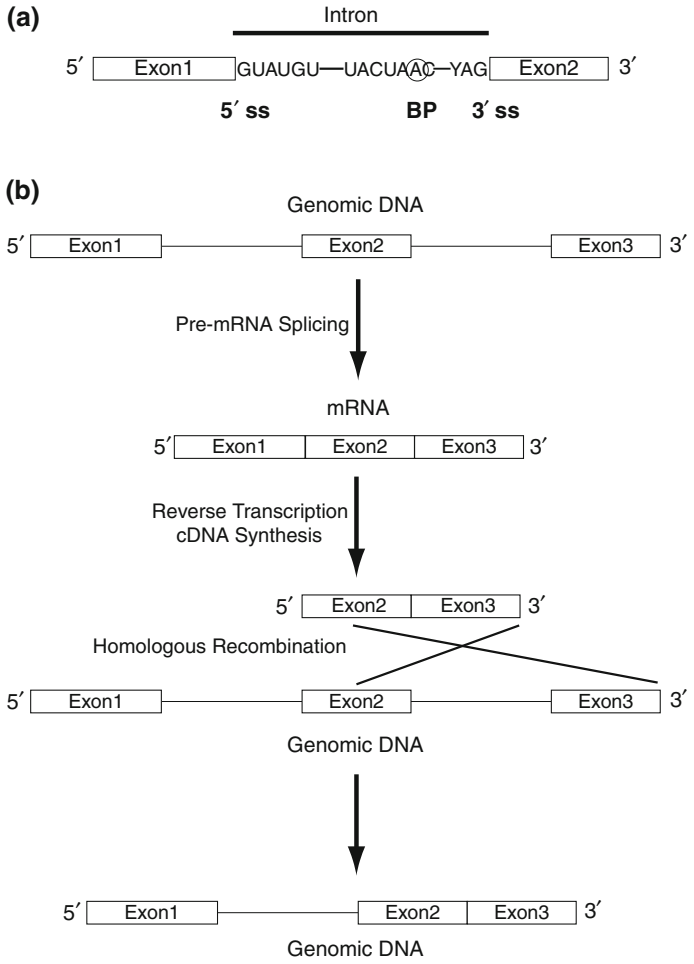

Fig. 2.1 **a** Eukaryotic gene structure, showing introns (*top*) and exons (*boxed*). The 5′ splice site (5′ss), branchpoint sequence (BP, reactive adenosine *circled*), and 3′ splice site (3′ss) are indicated along with the consensus sequence at each of these sites. **b** Homologous recombination model of intron loss. Exons are *boxed*, while *horizontal lines* connecting the exons indicate introns

fewer than seven introns show the same 5′ positional bias observed in budding yeasts (Wood et al. 2002). Fink (1987) attempted to explain this phenomenon through a homologous recombination model that suggested that reverse transcribed cDNAs generated from an mRNA template undergo homologous recombination with the yeast genome at the site of the gene that produced the original mRNA (Fig 2.1b). Since reverse transcriptase often falls off the template before reaching the 5′ end of long mRNAs, Fink (1987) argued that an intronless 3′ end of the gene would be over-represented in the cDNA population, and therefore the

recombination event would be more likely to replace the intron-containing 3′ end of the gene with an intron-less one. Consequently, a higher frequency of 5′ introns would be retained in the original gene.

The homologous recombination model makes three major predictions: retained introns show a 5′ positional bias, intron loss is precise, and adjacent introns are lost simultaneously (Fink 1987). All of these predictions have been substantiated in a number of fungal species, as well as in vertebrates (Coulombe-Huntington and Majewski 2007; Zhu and Niu 2013). Even in intron-rich fission yeasts, 656 out of 660 identified intron losses were precise and located in the 3′ portion of the gene, and 38 different losses of adjacent introns were reported (Zhu and Niu 2013). According to the homologous recombination model, one would expect that more frequently transcribed genes would lose their introns first, since there would be more of these mRNAs present to serve as templates for reverse transcription. However, this is not the case with the ribosomal protein genes that represent about 90 % of all mRNAs derived from intron-containing genes in *S. cerevisiae* (Ares et al. 1999). Among intron-containing genes in budding yeasts, ribosomal protein genes are over-represented, reaching as a high as 41 and 61 % in *S. cerevisiae* and *S. servazzii*, respectively (Bon et al. 2003). The introns of these highly expressed ribosomal protein genes have been retained through selective pressure and they appear to play a functional role in positive regulation of ribosome biogenesis (Vinogradov 2001).

While the homologous recombination model of mass intron loss in many fungal species still dominates today, several alternative models have been put forward in recent years. Under the genomic deletion model, introns are lost individually through the unequal exchange of alleles, often resulting in imprecise intron loss (Soochin et al. 2004). Evidence for such a mechanism has been found in only a small number of multicellular organisms and in only 4 of more than 600 cases of intron loss across 24 fission yeast species (Zhu and Niu 2013 and therein). In a third model, introns can be lost either precisely or imprecisely through nonhomologous end joining repair of double strand breaks (Farlow et al. 2011). While evidence for this model has been presented for only a few metazoan species, there are no supporting examples across fission yeast species (Farlow et al. 2011; Zhu and Niu 2013). It should be noted that intron gain is also common, and, in fungi, the presence of nearly identical introns in up to 500 copies per cell supports a mechanism of intron gain that involves duplication of intron-like elements (Collemare et al. 2013).

Unlike their mammalian counterparts, introns found in the budding yeasts are typically very short, showing a bimodal distribution of intron length with averages of 50–100 nucleotides and 250–400 nucleotides (Spingola et al. 1999; Bon et al. 2003; Neuvéglise et al. 2011). Intriguingly, these two classes of intron length can be assigned to two specific groups of genes: those encoding ribosomal proteins (longer introns) and those encoding nonribosomal proteins (shorter introns) (Spingola et al. 1999). Short introns are common to all known budding yeast, although exceptions have been reported (Bon et al. 2003; Neuvéglise et al. 2011). For example, introns in *Y. lipolytica* range in size from 41 to 3478 nucleotides,

with 16 introns larger than 1 kb (Mekouar et al. 2010). Even more distant fungal relatives, such as *S. pombe*, have short introns with an average length of 50 nucleotides, although the range in size, 29–819 nucleotides, is much smaller than in *Y. lipolytica* (Deutsch and Long 1999; Wood et al. 2002).

Spliceosomal introns have three defining features: the 5′ splice site (5′ss), the branch point (BP), and the 3′ splice site (3′ss) (Fig. 2.1a). The sequences at these sites are generally well conserved from budding yeast to humans, where in almost all cases the intron begins with a GT dinucleotide and ends with an AG dinucleotide (Spingola et al. 1999; Burset et al. 2000). Exceptions to this so-called GT–AG rule can be found both within and across species; for example, five *S. cerevisiae* and three *S. pombe* introns begin with the dinucleotide GC (Spingola et al. 1999; Wood et al. 2002). In addition, the sequence context at the splice sites and branch point is very important in *S. cerevisiae* and other closely related budding yeast, for which the consensus sequences GTATGT, TACTAAC (where A is the branch nucleotide), and YAG (where Y is a pyrimidine), at the 5′ss, BP, and 3′ss, respectively, are adhered to very closely (Bon et al. 2003; Neuvéglise et al. 2011). Some flexibility is observed at the 5′ss and BP in the more distantly related species *D. hansenii*, *P. angusta*, and *Y. lipolytica*, however, the 3′ss sequence in these species conforms to the YAG consensus (Bon et al. 2003). Notably, the sequences GTAAGT and GTGAGT at the 5′ss are the dominant sequences in *P. angusta* and *Y. lipolytica*, respectively, where these sequences represent 69 and 75 % of the introns in these genomes (Bon et al. 2003).

Pre-mRNA Splicing and the Spliceosome

Eukaryotic pre-mRNA splicing is catalyzed by the spliceosome, a large complex of five small nuclear RNAs (snRNAs U1, U2, U4, U5, and U6) and more than 100 core proteins (Jurica and Moore 2003). Many of these proteins associate specifically with an snRNA to form small nuclear ribonucleoprotein (snRNP) particles, while others associate with the spliceosome in an snRNA-independent manner that is often mediated through protein–protein interactions. Assembly of the spliceosome on a newly transcribed pre-mRNA substrate requires the addition of four major splicing subcomplexes: U1 snRNP, U2 snRNP, the pre-formed U4/U6•U5 triple snRNP, and the Prp19 Complex (Cheng and Abelson 1987; Hoskins et al. 2011). Through numerous structural rearrangements, the spliceosome interacts dynamically with the transcript, recognizing the 5′ss, BP, and 3′ss, and positioning the pre-mRNA substrate in a favorable orientation for the splicing reactions to proceed (Brody and Abelson 1985; Grabowski et al. 1985).

Recent real-time kinetic analyses of spliceosome assembly using multiwavelength fluorescence microscopy support a long held view that spliceosome assembly occurs through the highly ordered association of subcomplexes with the transcript (Cheng and Abelson 1987; Hoskins et al. 2011). These studies show that commitment of the transcript to splicing increases as assembly progresses, and that

association of the subcomplexes with the transcript is reversible (Hoskins et al. 2011). Notably, although higher order splicing complexes such as a penta-snRNP have been purified and characterized (Stevens et al. 2002), Crawford et al. (2013) find no evidence for the association of preformed complexes with the pre-mRNA. Thus, such higher-order complexes probably represent a stable association of the constituents already assembled on pre-mRNA substrates. Regardless of whether a *bona fide* penta-snRNP exists independent of substrate in vivo, such a species would presumably have to undergo the same conformational and compositional rearrangements outlined in the stepwise assembly model in order to ensure that all proofreading stages are passed so that high fidelity splicing can be achieved. The details of spliceosome assembly, activation, catalysis, and disassembly will be discussed here, focusing on the findings in the model organism *S. cerevisiae*. Consequently, the nomenclature used will be that of *S. cerevisiae* unless otherwise stated.

Association of U1 snRNP with the pre-mRNA Transcript

Assembly of the spliceosome begins with the association of U1 snRNP with the pre-mRNA transcript through a base-pairing interaction between the 5′ end of U1 snRNA and the 5′ss of the transcript (Fig. 2.2; Siliciano and Guthrie 1988; Crawford et al. 2013). This association proceeds in the absence of ATP hydrolysis, and is dependent on the presence of an intact 5′ss that maintains base pairing at intron positions one and five, but not at position four (Fig. 2.2; Siliciano and Guthrie 1988; Crawford et al. 2013). Notably, even though the 5′ss consensus sequence in mammals is far more degenerate than in yeast, the first ten nucleotides of U1 snRNA are invariant across eukaryotes (Guthrie and Patterson 1988). In mammals, the site of cleavage at the 5′ss is determined by complementarity to U1 rather than by the intron sequence, with specific cleavage occurring opposite the C8–C9 nucleotides of U1 (Weber and Aebi 1988). This is not the case for *S. cerevisiae*, where authentic 5′ss cleavage appears to require a G at position five of the intron, along with the U1 snRNP specific proteins Nam8 and Luc7, which stabilize the 5′ss/U1 interaction through contacts with the intron and 5′ exon, respectively (Siliciano and Guthrie 1988; Puig et al. 1999, 2007).

U1 snRNA is fairly well conserved across eukaryotes, consisting of an almost invariant short single-stranded 5′ end, three stem loop structures (stems I, II, and III) that are closed by a long-range interaction, a single-stranded region containing the Sm protein binding site, and a terminal stem loop (stem IV) (Fig. 2.2; Guthrie and Patterson 1988). Stem III is highly divergent in the hemiascomycetous yeasts, ranging from a short stem of 14 nucleotides in *Y. lipolytica* to a long unbranched stem of 104 nucleotides in the *Candida* species, and a long multibranched stem loop in *S. cerevisiae* (Mitrovich and Guthrie 2007). Intriguingly, this large insertion, referred to as the U1 snRNA fungal domain (Guthrie and Patterson 1988), is accompanied by the presence of several yeast-specific U1 snRNP proteins. Prp42,

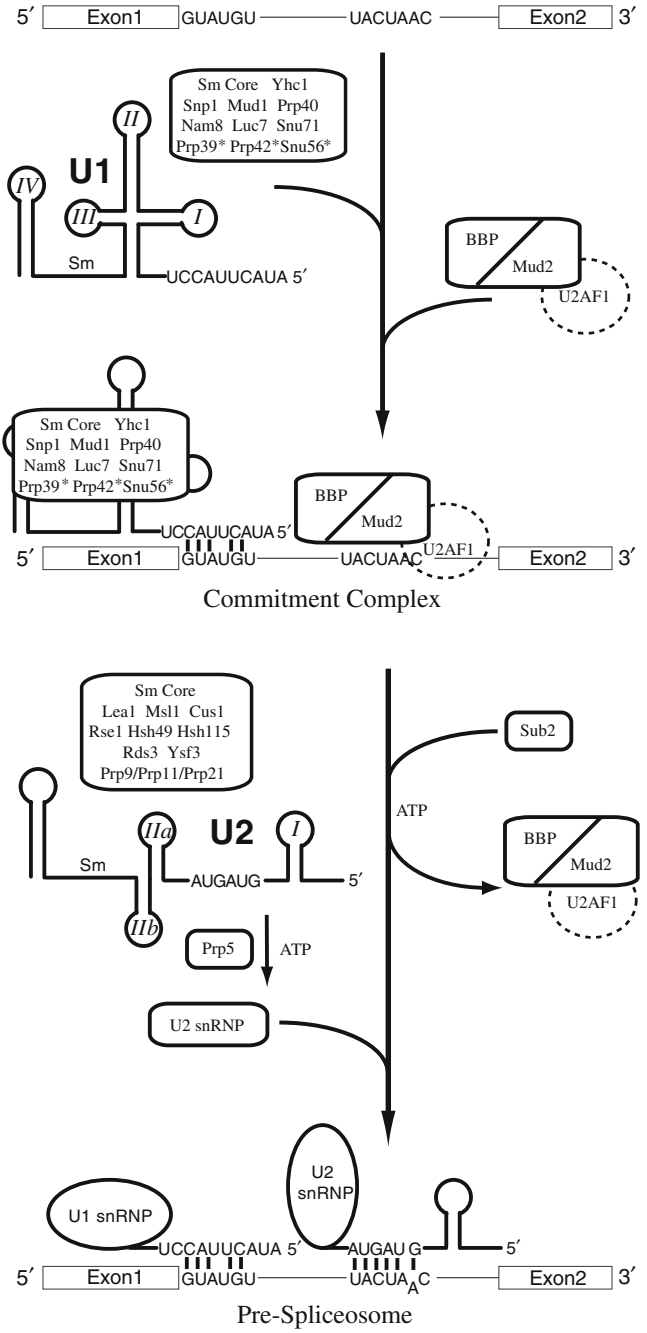

Fig. 2.2 Pre-spliceosome assembly showing ATP-independent addition of U1 snRNP and the BBP/Mud2 dimer to the 5′ss and BP, respectively (*top*), followed by the ATP-dependent addition of U2 snRNP to the BP (*bottom*). Proteins are indicated by *large rectangles*; those unique to *S. cerevisiae* are indicated with an *asterisk*, while those that are absent are indicated with a *dashed oval*. Base pairing interactions between RNA nucleotides are indicated by *vertical lines*

which is thought to have arisen as a duplication of the yeast-specific protein Prp39 in a common ancestor of *S. cerevisiae* and *C. albicans*, might interact with the extended stem III, since a homolog in *Y. lipolytica* does not exist (Mitrovich and Guthrie 2007). Stem III of *Y. lipolytica* is more similar in size to most other eukaryotes that also lack a Prp42 homolog (Fabrizio et al. 2009). Likewise, the *S. cerevisiae* specific protein Snu56 might associate with U1 snRNA through its extended and branched Stem III (Mitrovich and Guthrie 2007).

Of the ten U1 snRNP specific proteins identified in *S. cerevisiae*, seven have mammalian homologs, although not all of the mammalian homologs associate specifically with U1 snRNP, and the mode of interaction between the protein and U1 snRNA has not necessarily been conserved (Mitrovich and Guthrie 2007; Fabrizio et al. 2009). For example, the human homolog of Mud1, U1A, interacts with human U1 snRNA through direct contacts between the amino-terminal RNA recognition motif (RRM) and the loop nucleotides of U1 stem loop II (Oubridge et al. 1994). While this binding interaction appears to be conserved in *C. albicans*, the nucleotide sequence has become quite degenerate in *S. cerevisiae*, accompanied by an insertion of 30 amino acids and degeneration of the surrounding amino acid sequence in the Mud1 RRM, suggesting that the mode of interaction between U1 snRNA and Mud1 in *S. cerevisiae* is different from in humans (Mitrovich and Guthrie 2007). Interestingly, the opposite situation is observed for the human protein homolog of Snp1, U1-70 K, which binds the loop residues of stem loop I in humans (Surowy et al. 1989). In this case, the *S. cerevisiae* interaction is invariant while *C. albicans* shows some sequence degeneration at the site of interaction (Mitrovich and Guthrie 2007).

In addition to recognizing the 5′ss of splicing substrates, U1 snRNP has been proposed to play a role in increasing splicing fidelity. Single molecule fluorescence resonance energy transfer (smFRET) experiments have revealed that the 5′ss and BP are held apart upon U1 snRNP association with the transcript, as demonstrated by a reduction in FRET efficiency upon U1 snRNP binding (Crawford et al. 2013). Furthermore, these sites remain separated during spliceosome assembly up to the point of spliceosome activation (Crawford et al. 2013). These authors propose that this additional role for U1 snRNP—to physically separate chemically reactive groups—is crucial to ensuring that splicing cannot occur until the spliceosome has assembled correctly. Indeed the U5 snRNP protein Prp28 has been shown to play a role in proofreading at the 5′ss, an event that would necessarily occur later in spliceosome assembly, i.e., once the triple-snRNP has assembled onto the splicing substrate, but before spliceosome activation (Yang et al. 2013).

Recognition of the Branchpoint

Prior to recruitment of the other major splicing complexes to the pre-mRNA substrate, the BBP-Mud2 heterodimer binds the BP sequence in an ATP independent manner, making direct contacts with the pre-mRNA in this region

(Fig. 2.2; Abovich et al. 1994; Wang et al. 2008). In most species, including most fungi, the homologous BBP-Mud2 complex contains a third protein, U2AF1, which interacts with the AG dinucleotide located at the 3′ss (Wu et al. 1999). Notably, U2AF1 is not present in *S. cerevisiae*. U2AF1 is highly conserved, when present, with the *S. pombe* and human proteins showing 75 % similarity (Käufer and Potashkin 2000). The presence of U2AF1 appears to correlate with a short distance between the BP and 3′ss (less than 15 nucleotides), suggesting that this heterotrimer is responsible for identifying both the BP and 3′ss (Neuvéglise et al. 2011). In species such as *S. cerevisiae*, where this distance is much longer (on average 30 nucleotides) and contains a conserved tract of polypyrimidines (PPT) near the 3′ss, association of the BBP-Mud2 complex is 3′ss independent, and interactions between the BBP-Mud2 complex and the BP and PPT appear to be stronger (Rymond and Rosbash 1985; Neuvéglise et al. 2011).

Commitment of a splicing substrate to the splicing pathway requires the stable association of U1 snRNP and the BBP–Mud2 complex at the 5′ss and BP regions, respectively, although commitment complex formation is reversible (Legrain et al. 1985; Crawford et al. 2013). One of the key features of the commitment complex is the formation of a bridge connecting the 5′ss and BP through protein-protein contacts that involve a direct physical interaction between BBP and the U1 snRNP specific protein Prp40 (Abovich and Rosbash 1997; Schwer et al. 2013). The presence of homologs of these bridging proteins, Prp40, BBP, and Mud2 in *S. pombe* and humans suggest that the cross-talk between the 5′ and 3′ regions of the intron is important at very early stages of intron recognition and spliceosome assembly across eukaryotes (Käufer and Potashkin 2000). Once this network of contacts has been established, the assembling spliceosome is then ready to accept the U2 snRNP complex.

Stable association of U2 snRNP with the pre-mRNA to form the pre-spliceosome is the first ATP-dependent step in spliceosome assembly (Fig. 2.2; Crawford et al. 2013). Two different ATPases, Sub2 and Prp5, are required at this stage to allow direct base-pairing interactions between U2 snRNA and the intron to form (Parker et al. 1987; Kistler and Guthrie 2001; O'Day et al. 1996). Sub2 is thought to function in the removal of Mud2 and BBP from the pre-mRNA, exposing the BP region of the transcript, while Prp5 appears to play a role in U2 snRNA remodeling to make the U2 snRNA BP-binding sequence more accessible. Interestingly, association of the Prp9/Prp11/Prp21 complex (SF3a complex in humans) with U2 snRNA is required for Prp5 activity, and RNase H treatment of U2 snRNA has shown that prior to assembly onto the transcript, three different Prp9/Prp11/Prp21-dependent and Prp5-dependent U2 conformations exist (Wiest et al. 1996). Association of the Prp9/Prp11/Prp21 complex with U2 converts a more open U2 snRNA that is unable to form pre-spliceosomes into a more closed particle that becomes the Prp5 substrate (Wiest et al. 1996). Association of Prp5 and subsequent ATP hydrolysis then converts U2 snRNP into a second more open conformation that is competent for association with the BP of the pre-mRNA (Wiest et al. 1996).

U2 snRNA is composed of four stem loop structures, the first three of which are separated by short single-stranded regions (Fig. 2.2; Guthrie and Patterson 1988). Like U1 snRNA, U2 snRNA is highly conserved across eukaryotes, except in *S. cerevisiae* where a large insertion of approximately 1 kb, referred to as the U2 fungal domain, replaces the third stem loop (Guthrie and Patterson 1988). Surprisingly, the entire fungal domain can be deleted without affecting growth in yeast, and a yeast U2 snRNA deletion can be complemented with human U2 snRNA (Shuster and Guthrie 1988, 1990). In contrast to U1 snRNP, the U2 snRNP components are much more highly conserved, with eleven proteins specifically associating with U2 snRNA in both yeast and humans in addition to the Sm protein core (Fabrizio et al. 2009). Stable association of U2 snRNP with the pre-mRNA involves direct U2 snRNP protein contacts with the pre-mRNA upstream of the BP in addition to base pairing (Gozani et al. 1996, 1998).

Assembly of the U4/U6•U5 Triple snRNP

In contrast to U5 and U6 snRNPs, which can exist as free particles, U4 is almost always found in association with U6 in either the U4/U6 di-snRNP or in the U4/U6•U5 tri-snRNP (Fig. 2.3; Cheng and Abelson 1987; Fortner et al. 1994). This association involves an extensive base pairing interaction that spans U6 nucleotides 55–80 and U4 nucleotides 1–17 and 57–64, generating U4/U6 stem I and stem II (Fig. 2.3; Brow and Guthrie 1988). The U4/U6 di-snRNP protein complement is small, comprised only of the U6-associated LSm proteins, the U4-associated Sm proteins, and four proteins found in the di-snRNP that are not part of free U6 snRNP (Stevens et al. 2001; Fabrizio et al. 2009). It is not clear whether these four proteins, Prp31, Prp3, Prp4, and Snu13, associate with U4 snRNA in a free U4 snRNP particle since isolation and characterization of free U4 snRNP has not been possible due to its very low abundance. Alternatively, these proteins might recognize and bind the U4/U6 duplex at some point during U4/U6 di-snRNP formation.

The U6 snRNP-specific protein Prp24, consisting largely of four RRMs, is the only other protein found associated with U6 snRNA in free U6 snRNP, aside from the Lsm2-8 protein complex that binds the 3′ uridine-rich tail of U6 (Fig. 2.3; Stevens et al. 2001; Mayes et al. 1999). While Prp24 does not stably associate with the U4/U6 di-snRNP, the rate of base-pair formation between U4 and U6 snRNAs is greatly enhanced in its presence (Shannon and Guthrie 1991; Raghunathan and Guthrie 1998). It is not yet understood how Prp24 facilitates this interaction, but it is known to do so in an ATP-independent manner (Raghunathan and Guthrie 1998). Surprisingly, the structure of yeast Prp24, which consists of four RRMs, is strikingly different from the mammalian homolog, SART3, which is three times as large and consists of only two RRMs and a long amino-terminal extension not present in *S. cerevisiae* (Bell et al. 2002; Rader and Guthrie 2002). The first two RRMs of Prp24 most closely resemble the SART3 RRMs, and in both yeast and

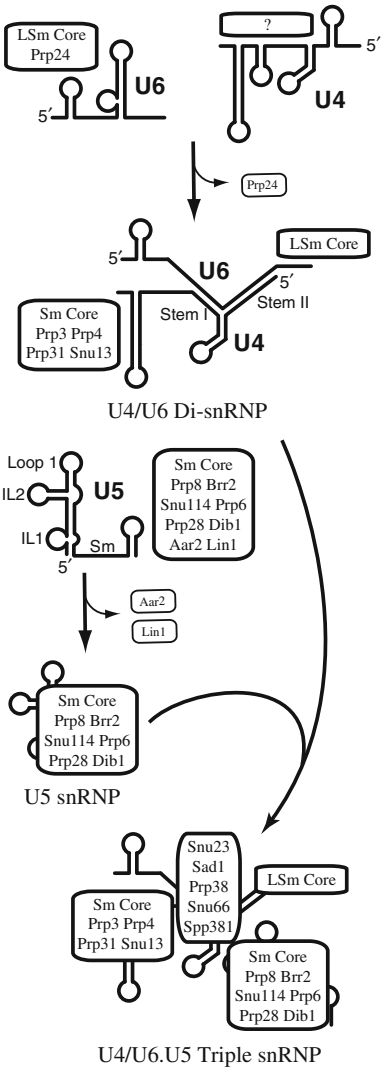

Fig. 2.3 Tri-snRNP assembly showing snRNA secondary structures (U6 and U4, *top*, U5, *middle*, stems and loops labeled as in text), and associated proteins in *large rectangles*

humans have been shown to bind U6 snRNA with high affinity (Bell et al. 2002; Kwan and Brow 2005). The *S. pombe* homolog is similar in size to SART3, consisting of a large amino-terminal extension in addition to the four RRMs that are common among most other fungi (Rader and Guthrie 2002). Whether the additional RRMs in yeast Prp24 perform a similar function to the amino-terminal extension found in other homologs remains to be determined.

Unlike U1 and U2 snRNAs, neither fungal U4 nor U6 snRNAs deviate in size relative to other eukaryotes (Guthrie and Patterson 1988). Essentially all of the very little size variation in U6 snRNA is found in the 5′ stem loop where the length

of the stem can vary by several base pairs (Brow and Guthrie 1988). In addition to the size conservation, U6 snRNA exhibits a striking level of primary sequence conservation with close to 80 % sequence identity across the middle third of the RNA across eukaryotes (Brow and Guthrie 1988). This region of U6 engages in base pairing interactions with U4, and consequently it is not surprising that the corresponding region of U4 snRNA is highly conserved in primary sequence as well. Outside of this region, however, the primary sequence is quite degenerate (Guthrie and Patterson 1988). On the U4 side of the U4/U6 duplex, stems I and II are interrupted by a stem loop structure, the 5′ stem loop, which has been absolutely conserved in structure from yeast to humans even though the nucleotide sequence in the stem differs at almost every position (Fig. 2.3; Guthrie and Patterson 1988). This high level of phylogenetic co-variation argues for an important function for this structure, which has been shown to bind the protein Snu13 (Vidovic et al. 2000). U4 snRNA also contains a 3′ stem loop that varies substantially across eukaryotes, followed by the Sm protein-binding site and, in most eukaryotes excluding *S. cerevisiae*, a final stem loop structure (Guthrie and Patterson 1988).

In order for the U4/U6 di-snRNP to assemble onto the pre-mRNA, it must first associate with U5 snRNP to form the U4/U6•U5 tri-snRNP complex (Fig. 2.3). U5 snRNA can be divided into two major domains: the 5′ domain, which contains a complex stem loop structure, and the 3′ domain, which contains the single-stranded Sm protein binding site followed by a 3′ stem loop that varies in both size and sequence (Fig. 2.3; Guthrie and Patterson 1988). In *S. cerevisiae*, there are two functional forms of U5, a short and long form, with the short form terminating just prior to the 3′ stem loop structure (Patterson and Guthrie 1987). The 5′ stem loop is comprised of a long stem loop (loop 1) that is broken into three segments by two internal loops, IL1 and IL2, and a stem loop on the 5′ side of IL2 that is unique to *S. cerevisiae* (Guthrie and Patterson 1988). Loop 1, which makes direct contacts with the exon junction (Sontheimer and Steitz 1993; Newman et al. 1995), exhibits extreme sequence conservation where nine of eleven nucleotides are invariant across eukaryotes. *C. albicans* is an exception to this where two additional nucleotides have been reported to deviate from the loop 1 consensus sequence (Mitrovich and Guthrie 2007).

Free U5 snRNA associates with eight different proteins—in addition to the heptameric Sm protein ring—to form the free U5 snRNP; all of them have a mammalian homolog (Stevens et al. 2001; Fabrizio et al. 2009). While Brr2, Prp8, and the only known spliceosomal GTPase, Snu114 (Fabrizio et al. 1997), are the key players in spliceosome activation, they also appear to play a major role in U5 snRNP formation and stability (Dix et al. 1998). Prp8 physically contacts U5 snRNA on both sides of IL1 and IL2, as well as loop 1 of the 5′ stem loop, while Snu114 contacts the 5′ side of IL2 (Dix et al. 1998). The stability of Prp8 depends on its ability to interact with Snu114, and stable interaction between these proteins requires the binding, but not hydrolysis, of GTP by Snu114 (Brenner and Guthrie 2006). Stable association of the GTP-bound Snu114/Prp8 dimer with U5 snRNA is required for stable association of Brr2, which interacts directly with Prp8, but not

with U5 snRNA or Snu114 (Dix et al. 1998; Brenner and Guthrie 2006). Once fully formed, this free U5 snRNP particle then associates with the U4/U6 di-snRNP, and, upon addition of five other proteins, generates the U4/U6•U5 tri-snRNP complex (Fig. 2.3; Stevens et al. 2001).

Spliceosome Activation

Activation of the spliceosome requires large conformational and compositional changes that result in the loss of U1 and U4 snRNPs, the acquisition of the Nineteen Complex (NTC), and formation of the catalytic core of the spliceosome (Fig. 2.4; Cheng and Abelson 1987; Hoskins et al. 2011). Each of these occurrences is precisely regulated and has been described as an allosteric cascade, in which the execution of one event is dependent on a conformational change associated with the previous event (Brow 2002). In some cases, this is the exchange of a base-pairing partner for a mutually exclusive partner, and in other cases it involves a structural rearrangement in a protein that changes the accessibility of an interaction domain. The key drivers of these rearrangements during spliceosome activation are the U5 snRNP associated DExD-box RNA helicase proteins Prp28, which acts on the 5′ss/U1 snRNA interaction, and Brr2, which acts on the U4/U6 di-snRNA interaction (Fig. 2.4; Raghunathan and Guthrie 1998). The U5 snRNP proteins Prp8 and Snu114 regulate the activity of both helicases through a delicate and finely tuned feedback system (Brenner and Guthrie 2005; Small et al. 2006).

It is not yet clear how or what recruits the U4/U6•U5 tri-snRNP to the assembling spliceosome, although initial association of this complex probably involves protein–protein interactions between U1 and U5 snRNP proteins. Yeast two hybrid interactions have been reported between the U1 snRNP proteins Prp40 and Snp1 and the U5 snRNP proteins Prp8 and Brr2, respectively, suggesting that these interactions allow the tri-snRNP complex to dock with the pre-spliceosome (Abovich and Rosbash 1997; Fromont-Racine et al. 2000). Stable association of the tri-snRNP with the pre-spliceosome is guided largely by Prp8, which stabilizes an interaction between loop 1 of U5 snRNA and the exons (Dix et al. 1998). Prior to docking with the pre-spliceosome, Prp8 and Snu114 inhibit Prp28 and Brr2 helicase activity through a mechanism that is not well understood; however, contact between the tri-snRNP and U1 snRNP proteins upon docking induces a large structural rearrangement in the C-terminus of Prp8 that results in the activation of both helicases (Kuhn et al. 1999, 2002; Brenner and Guthrie 2005).

The first major rearrangement during spliceosome activation is the exchange of U1 snRNA for U6 snRNA at the 5′ss, a process that requires ATP hydrolysis and Prp28 (Fig. 2.4; Staley and Guthrie 1999). Under normal wild type conditions, the yeast protein yU1C stabilizes the U1 snRNA/5′ss duplex. Mutations that alter either yU1C or U1 snRNA in the 5′ss binding region, however, render Prp28 dispensable for splicing, and, indeed, for cell viability (Chen et al. 2001). Since

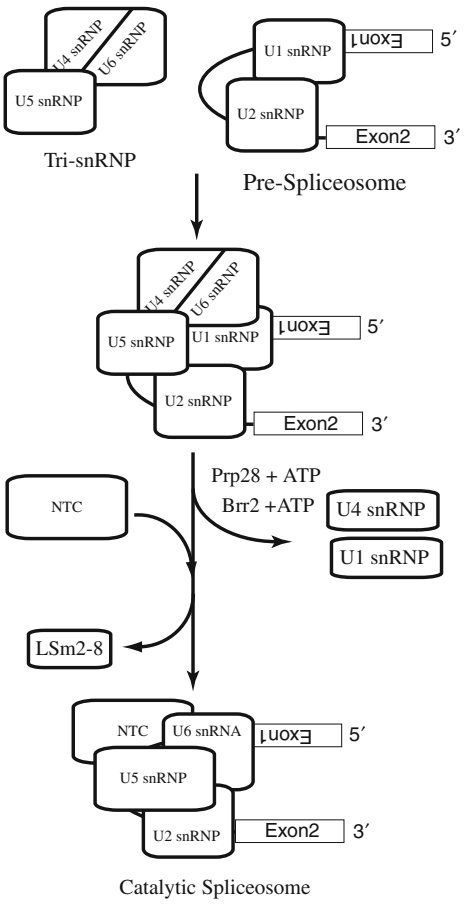

Fig. 2.4 Spliceosome activation, indicating tri-snRNP addition to the pre-spliceosome (*top*), U1 and U4 dissocation and NTC addition (*bottom*). SnRNP particles and protein complexes are indicated by *large rectangles*

these mutations act to destabilize the interaction between U1 snRNP and the 5′ss, Prp28 is thought to function as an antagonist to yU1C, destabilizing its interaction with the pre-mRNA to provide a more suitable environment for U6 snRNA binding to the 5′ ss (Chen et al. 2001). Formation of the U6 snRNA/5′ss duplex promotes the complete dissociation of U1 snRNP from the 5′ss (Kuhn et al. 1999; Chen et al. 2001). Notably, extending the U1 snRNA/5′ss interaction by several base pairs inhibits the switch for U6 snRNA and stalls spliceosome assembly (Staley and Guthrie 1999). This inhibition can be reversed by lengthening the U6 snRNA/5′ss interaction by several base pairs, suggesting that U1 and U6 compete for binding to the 5′ ss, resulting in an equilibrium between the two bound states (Staley and Guthrie 1999). Prp28 appears to play a role in proofreading the stability of the U6 snRNA/5′ss interaction, rejecting suboptimal 5′ss pre-mRNAs by sending them down a discard pathway (Yang et al. 2013).

A second major structural rearrangement during spliceosome activation is the disruption of the U4/U6 di-snRNP. Several lines of evidence suggest that

unwinding of the U4/U6 duplex is tightly coupled to destabilization of U1 snRNP at the 5′ss. First, when U1 snRNA/5′ss unwinding is blocked by extending base pairing, U4/U6 duplex unwinding is also blocked (Staley and Guthrie 1999). Second, in the presence of a mutation that extends stem I of the U4/U6 di-snRNA to include the 5′ss binding region of U6 snRNA, U4/U6 unwinding is impeded and U1 snRNP is retained in a stalled spliceosome assembly intermediate (Li and Brow 1996; Kuhn et al. 1999). A mutation in Prp8 is capable of suppressing the conditional phenotype generated by the stem I-lengthening mutation, suggesting that U4/U6 unwinding is triggered by Prp8 only after stable association of U6 snRNA with the 5′ss (Kuhn et al. 1999; Staley and Guthrie 1999). Such a system would ensure that catalytic structures do not form prior to correct identification of the 5′ss, ensuring splicing fidelity during first step catalysis (Staley and Guthrie 1999).

While Prp8 is involved in regulating U4/U6 unwinding, it is Brr2 that plays an active role in unwinding the duplex (Raghunathan and Guthrie 1998b). In the absence of ATP or in the presence of a mutation in the helicase domain of Brr2, U4/U6 unwinding is inhibited (Raghunathan and Guthrie 1998b; Maeder et al. 2009). Genetic studies have implicated Prp8 as a negative regulator of Brr2, and in recent years some of the details of the mechanism of regulation have begun to surface (Kuhn et al. 2002). Specifically, the RNase H-like domain of Prp8 interacts directly with U4 and U6 snRNAs in single-stranded regions adjacent to U4/U6 stem I, the same region of U4 that is required for loading Brr2 onto the duplex (Mozaffari-Jovin et al. 2012). Prp8 and Brr2 physically interact with the same region of U4 snRNA in a mutually exclusive manner, with Prp8 blocking U4/U6 unwinding by preventing Brr2 from binding (Mozaffari-Jovin et al. 2012). A high-resolution crystal structure has revealed that Prp8 further blocks Brr2 activity by inserting its C-terminal tail into the RNA binding tunnel of Brr2, inhibiting the ATP-dependent helicase activity of Brr2 (Mozaffari-Jovin et al. 2013).

Once Brr2 has loaded onto U4 snRNA, it translocates along U4 to unwind U4/U6 stem I (Hahn et al. 2012; Mozaffari-Jovin et al. 2013). It is not yet clear how stem II is unwound, since Brr2 would encounter the protein-bound U4 snRNA 5′ stem loop before reaching stem II. It is possible that Brr2 continues to translocate along U4 snRNA, displacing proteins as they are encountered, and finally unwinding stem II (Nielsen and Staley 2012). Alternatively, Brr2 might somehow jump the 5′ stem loop to immediately unwind stem II following stem I unwinding, or it might not be involved in stem II unwinding at all (Nielsen and Staley 2012). What is known is that following release of U4 snRNA, Brr2 activity must be turned off to allow formation of the catalytic center of the spliceosome. Snu114 appears to function as a regulator of Brr2, since Brr2 activity is repressed when Snu114 is bound to GDP (Small et al. 2006). Importantly, while GTP hydrolysis is not required for U4/U6 unwinding, it is required for U4 snRNA release from the assembling spliceosome (Bartels et al. 2003; Small et al. 2006). Thus, the hydrolysis of GTP following U4/U6 unwinding might trigger the release of the destabilized U1 and U4 snRNPs from the assembled spliceosome, likely by influencing the physical contacts between proteins at the core of the spliceosome.

Following the release of U1 and U4, the Nineteen Complex (NTC) is recruited to stabilize the assembled spliceosome during spliceosome activation (Fig. 2.4; Chan et al. 2003). The NTC is composed of Prp19 and at least seven other Prp19-associated proteins, which assemble into the pre-formed NTC prior to association with the spliceosome (Chen and Cheng 2012). Binding of the NTC results in the destabilization of the LSm complex of proteins from the 3′ tail of U6 snRNA, allowing these U6 snRNA nucleotides to interact with an intronic region of the substrate near the 5′ss (Chan and Cheng 2005). Crosslinks between U6 snRNA and the NTC component Cwc2 have led to the proposal that Cwc2 serves to link the NTC to the spliceosome (McGrail et al. 2009). Interestingly, Prp19 itself contains a ubiquitin ligase motif at its N-terminus, and might regulate aspects of the splicing cycle through its ability to add ubiquitin to various proteins (Ohi et al. 2003). Indeed, Prp19 has been shown to ubiquitinate the U4/U6-associated protein Prp3 in humans, influencing tri-snRNP stability (Song et al. 2010). Further, ubiquitin is necessary for splicing in yeast, as inhibition of ubiquitin's ability to interact with other proteins through ubiquitin mutation, or the presence of an inhibitory small molecule, reduces splicing by reducing tri-snRNP levels (Bellare et al. 2008).

The catalytic core of the spliceosome is formed through base pairing between the U2 and U6 snRNAs. Notably, these interactions are mutually exclusive with U4/U6 interactions, supporting the proposal that U4 snRNA acts as a negative regulator of U6 snRNA, masking U6 nucleotides so that catalytic features of the active site do not form prematurely (Brow and Guthrie 1989). Specifically, the stem I region of U6 base pairs to U2 snRNA to form U2/U6 helix I, and the stem II region of U6 folds back on itself to generate an intramolecular stem loop structure known as the 3′ ISL (Madhani and Guthrie 1992, Fortner et al. 1994). Interestingly, the C-terminal region of Prp8 influences U6 3′ ISL formation and/or stability, highlighting the importance of Prp8 throughout the splicing cycle (Kuhn et al. 2002). It is not yet clear whether U2/U6 helix I forms before, after, or at the same time as the U6 3′ ISL, however unwinding of U4/U6 stem I prior to stem II suggests that correct association of U2 and U6 snRNAs might be a prerequisite to stem II unwinding and 3′ ISL formation. Once these catalytically important structures have formed, the spliceosome is considered to be fully activated and ready to splice a substrate.

Catalytic Steps

The splicing reaction consists of two sequential transesterification reactions separated by a period of spliceosomal remodeling. In the first reaction, the 2′ hydroxyl of a bulged adenosine found in the branch site consensus sequence of the intron reacts with the phosphodiester bond at the 5′ss (Fig. 2.5a; Padgett et al. 1984; Konarska et al. 1985). This results in the formation of an unusual 2′–5′ phosphodiester linkage joining the 5′ end of the intron to the branch point adenosine,

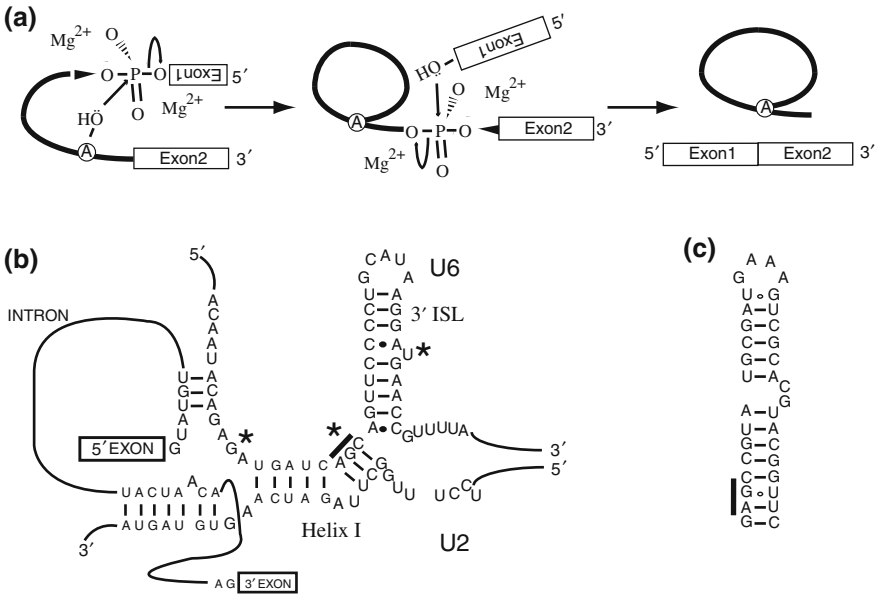

Fig. 2.5 **a** Nucleophillic reaction of the 2′-hydroxyl of the branchpoint adenosine (*circled*) with the 5′ splice site liberates the 5′ exon concomitantly with the lariat intron/3′ exon intermediate (*middle*). Nucleophillic reaction of the 3′-hydroxyl of the 5′ exon with the 3′ splice site results in the ligation of the exons and release of the intron in the form of a lariat (*right*). **b** RNA interactions between U6 (*above*), U2 (*below*), and pre-mRNA (*left*) at the first chemical step. Catalytic Mg^{2+} ion binding sites are indicated with an *asterisk*, and the AGC triad is marked with a *bar*. **c** Catalytic domain V of a group II intron. The AGC triad is marked with a *bar*

with concomitant liberation of the 5′ exon (Padgett et al. 1984; Konarska et al. 1985). In the second step, the 3′ hydroxyl group of the 5′ exon reacts with the phosphodiester bond at the 3′ss, joining the 5′ and 3′ exons through a standard 3′–5′ phosphodiester linkage, with concomitant release of the intron in the form of a lariat (Padgett et al. 1984; Konarska et al. 1985). Both chemical steps are inferred to proceed through an in-line S_N2 nucleophilic reaction based on the inversion of stereochemistry observed at the chiral phosphates (Maschhoff and Padgett 1993; Moore and Sharp 1993). While the spliceosome is composed largely of proteins, a long-held view is that the splicing reactions might actually be catalyzed by the highly conserved snRNAs located at the catalytic core of the spliceosome (Madhani and Guthrie 1992).

A catalytic function for U6 snRNA has been suspected for decades, not only because of its high level of sequence and size conservation, but also because of mechanistic and structural similarities to group II self-splicing introns (Madhani and Guthrie 1992; Peebles et al. 1995). At the active site of the spliceosome, U6 snRNA adopts a conformation that resembles the active domain of group II introns through formation of U2/U6 helix I and the U6 3′ ISL (Fig. 2.5b). U2/U6 helix I contains an invariant AGC triad that is required for exon ligation (Madhani and

Guthrie 1992; Fabrizio and Abelson 1992; Hilliker and Staley 2004; Lee et al. 2010). The AGC triad is also present in a base-paired structure in group II introns (Fig. 2.5c), and like the spliceosome, has a strict requirement for a purine at the second position (Peebles et al. 1995; Hilliker and Staley 2004). Interestingly, in both systems the AGC triad is less tolerant of mutation than the complementary nucleotides to which it is base paired, demonstrating an important role for these nucleotides in addition to base pairing (Madhani and Guthrie 1992; Peebles et al. 1995). Directly adjacent to U2/U6 helix I lies the 3′ISL, which, like domain V of group II introns, contains a small internal bulge on the 3′ side of the stem (Fabrizio and Abelson 1992; Lee et al. 2010; Peebles et al. 1995).

Mechanistically, pre-mRNA splicing and group II self-splicing are identical: both are Mg^{2+}-dependent processes that result in the removal of a lariat intron (Peebles et al. 1995; van der Veen et al. 1986; Cech 1986). Steitz and Steitz (1993) have proposed a two metal ion mechanism for these reactions in which one metal ion activates the sugar hydroxyl, while the other metal ion directly coordinates and stabilizes the oxyanion leaving group. To date, three Mg^{2+} ion binding sites have been identified in U6 snRNA: one in the AGC triad, one at position U80 in the internal loop of the 3′ ISL, and a third in the almost invariant ACAGAGA sequence, which base pairs to the 5′ splice site of the pre-mRNA transcript and is located 5′ of U2/U6 helix I (Fabrizio and Abelson 1992; Lee et al. 2010). In order for these Mg^{2+} ions to work in concert during the splicing reactions, the 3′ ISL must be closely juxtaposed with the 5′ splice site of the pre-mRNA transcript. Chemical structure probing of assembled spliceosomes has shown that this is indeed the case, with all three of these Mg^{2+} binding sites located in close proximity to position ten of the intron prior to the first catalytic step (Rhode et al. 2006). This constrains the structure of the active core in three dimensions, placing all three Mg^{2+} ions close to the reactive groups for the first chemical step. Following the first reaction, the accessibility of the 3′ ISL changes, supporting the view that some level of spliceosomal remodeling occurs between the two splicing reactions (Rhode et al. 2006).

While the exact role of the Mg^{2+} ion that is coordinated at each site has not yet been elucidated, Yean et al. (2000) showed that substitution of a phosphorothioate at position U80 in the 3′ ISL reconstitutes fully assembled, but catalytically inactive spliceosomes. Only in the presence of more thiophilic metal ions does splicing proceed, demonstrating that it is the splicing reaction, not spliceosome assembly, that requires this Mg^{2+} ion. While metal substitution fails to restore splicing in a phosphorothioate-substituted internal loop of a group II intron (Gordon and Piccirilli 2001), Tb^{3+} ion cleavage at this position suggests that this is indeed a site of metal ion coordination (Sigel et al. 2000). Notably, Fica et al. (2013) showed that U6 snRNA catalyzes both splicing reactions by positioning Mg^{2+} ions that are critical to stabilize the leaving groups, confirming Steitz and Steitz's (1993) original proposal. Further, a reaction that resembles pre-mRNA splicing has been performed in the presence of Mg^{2+} ions in a protein-free system consisting of regions of U2 and U6 snRNA that make up the proposed catalytic domain (Valadkhan and Manley 2001). Thus, the spliceosome

can be considered a metallo-enzyme in which U6 snRNA plays a key role in coordinating these metal ions.

In addition to metal ion coordination by snRNAs at the active site of the spliceosome, Mg^{2+} ions are coordinated by protein components, although a direct role in catalysis for these metal ions has not been shown. Prp8 is the largest spliceosomal protein (260 kDa), containing RNase H-like, endonuclease-like, and reverse transcriptase-like domains, none of which are catalytically active (Jackson et al. 1988; Pena et al. 2008; Dlakic and Mushegian 2011). Different first and second step conformations for Prp8 have been suspected for some time based on genetic findings, and recent structural work with human Prp8 has revealed a subtle difference in Prp8 conformation in which one state, an open form, allows Mg^{2+} ion coordination in the RNase H-like domain, while the other, the closed form, does not (Schellenberg et al. 2013). The Mg^{2+}-bound open state functions during the second catalytic step, where Mg^{2+} ion coordination was shown to promote exon ligation (Schellenberg et al. 2013). Schellenberg et al. (2013) suggest that Prp8 might present its Mg^{2+} ion at the active site along with two other metal ions presented by the snRNAs to generate a three-metal spliceosomal active site as observed for other enzymes that catalyze phosphoryl transfer reactions. In contrast, Abelson (2013) favors a role for this Mg^{2+} ion in stabilizing the second step active site conformation rather than a direct role in catalysis, given that the RNase H-like domain of Prp8 is catalytically inert.

Spliceosome Remodeling Between Catalytic Steps I and II

A general theme in spliceosome remodeling between the catalytic steps is beginning to emerge in which the components that are required for each step are present throughout both splicing reactions, but with substantial 'toggling' of these components to generate the appropriate active site for each step. For example, U2 snRNA toggles between two mutually exclusive stem structures: stem IIa and stem IIc (Fig. 2.6a). Stem IIc is required for catalysis of both steps of the splicing reaction, while stem IIa is required for spliceosome assembly and substrate rearrangement between the two catalytic steps (Hilliker et al. 2007). Thus, U2 toggles between these two conformations to allow spliceosome assembly and catalysis to proceed, and there is evidence to suggest that the RNA-dependent helicase Prp16 plays a role in this interchange (Fig. 2.6b; Hilliker et al. 2007). Similar events have been reported for active site protein components in which the affinity for protein binding in the spliceosome toggles between low and high affinity states. For example, Prp16 and Slu7 bind the activated spliceosome through low affinity entry sites that are converted to high affinity binding sites following the first catalytic step, when the action of these proteins is required (Ohrt et al. 2013).

As in spliceosome assembly and activation, several RNA-dependent ATPases are required to promote each splicing reaction, probably by facilitating the formation of the step one and step two active sites. Interaction of Prp2 with the intron

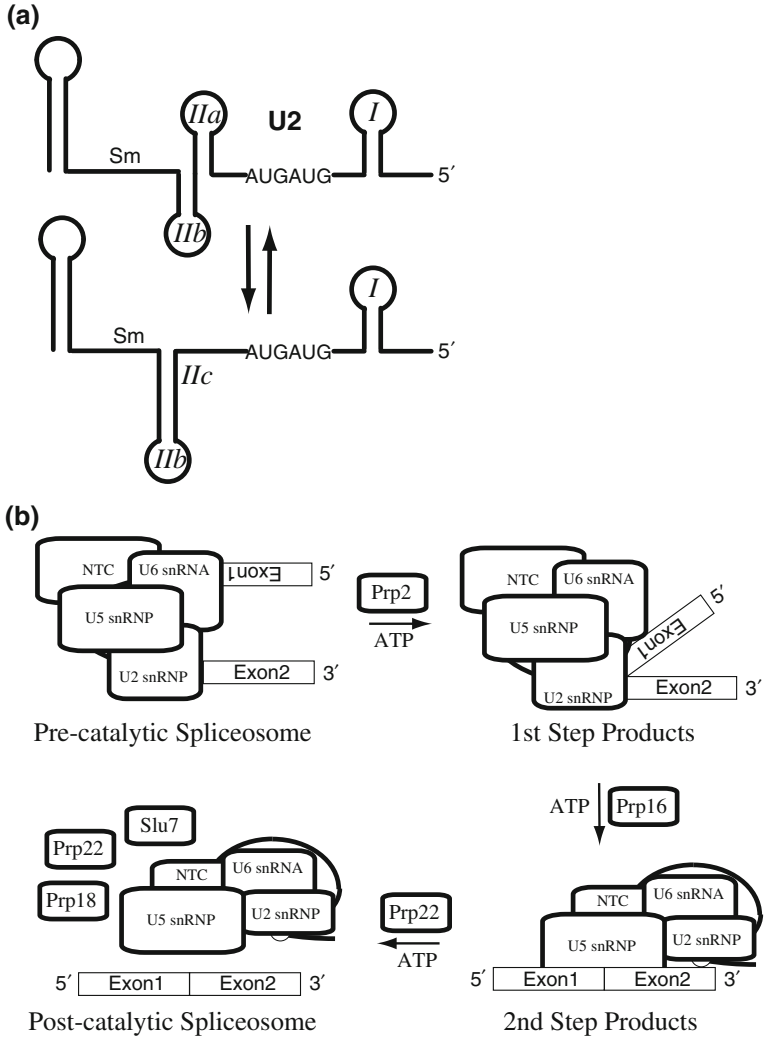

Fig. 2.6 **a** Conformational toggling in U2 snRNA, showing the switch between stem-loop IIa (*top*) and the mutually exclusive stem IIc (*bottom*), which lengthens stem-loop IIb. **b** ATP hydrolases of the helicase family associated with the chemical steps of splicing and spliceosome disassembly

prior to the first catalytic step is required for the splicing reactions to proceed (Fig. 2.6b), and, in addition to making direct contacts with the pre-mRNA, Prp2 interacts with the carboxyl-terminus of Brr2 (Liu and Cheng 2012). This interaction has been proposed to allow recruitment of Prp2 to the pre-catalytic spliceosome (Liu and Cheng 2012). Contact between Prp2 and Brr2 promotes the ATPase activity of Prp2, which results in the displacement of nine of eleven U2

snRNP-associated proteins (the SF3a and SF3b complexes in humans) through a mechanism that is not yet understood (Warkocki et al. 2009; Lardelli et al. 2010; Liu and Cheng 2012). The presence of the U2 snRNP-associated proteins at the BP region of the pre-mRNA may mask the reactive 2′-hydroxyl of the branchpoint adenosine until the spliceosome has correctly formed the step one active site. Removal of these proteins by Prp2 exposes the 2′-hydroxyl in a conformation that is compatible with in-line reaction with the phosphodiester bond at the 5′ss (Lardelli et al. 2010). Notably, these U2 snRNP proteins can be isolated in a particle containing U2 snRNA when purified spliceosomes are disassembled, suggesting that the U2 snRNP proteins, while displaced from the branchpoint for the first catalytic step, might remain loosely associated with the spliceosome throughout the splicing reactions (Fourmann et al. 2013).

Following the first catalytic step of splicing, the spliceosome re-positions the substrate for the second catalytic step, and the key driver of this remodeling event is the RNA-dependent ATPase, Prp16 (Fig. 2.6b; Schwer and Guthrie 1992). Prp16 is required specifically for the second catalytic step where it influences 3′ss cleavage and exon ligation, however, it has been shown to associate with the spliceosome in an ATP-independent manner prior to the first catalytic step to stabilize binding of the protein Cwc25 at the branchpoint (Schwer and Guthrie 1991; Tseng et al. 2011). Following the first catalytic step, Prp16 functions in an ATP-dependent manner to displace Yju2 and Cwc25 to allow for the association of the second step splicing factors Slu7, Prp18, and Prp22 (Tseng et al. 2011). Notably, Cwc25 is not displaced by Prp16 alone, but requires the stable association of Slu7 and Prp18, which are required to dock the 3′ss into the step two active site of the spliceosome (Ohrt et al. 2013). Interestingly, exon ligation can occur in the absence of Slu7 and Prp18 when the distance between the branchpoint and 3′ss is short, however, both proteins are required when this distance is longer than seven nucleotides (Brys and Schwer 1996; Ohrt et al. 2013).

In a genetic study, Mefford and Staley (2009) showed that Prp16 acts to destabilize U2/U6 helix I after the first catalytic step. However, since helix I integrity is important for both catalytic steps, they proposed that helix I reforms prior to second step catalysis. This is reminiscent of a second region of U2 snRNA discussed previously that undergoes toggling between the stem IIa and stem IIc conformations throughout the splicing cycle (Hilliker et al. 2007). Following 5′ss cleavage, Prp16 has been proposed to disrupt the stem IIc catalytic conformation by destabilizing stem IIc itself, as well as to destabilize interactions that are mutually exclusive with stem IIa, thereby promoting stem IIa formation (Hilliker et al. 2007). While the specific Prp16 substrate has yet to be identified, it is tempting to speculate that Prp16's role in displacing Yju2 and Cwc25 is an indirect consequence of Prp16 unwinding various U2 snRNA duplexes. Unwinding these structures would relax the catalytic core of the spliceosome, allowing for substrate re-positioning, while reformation of the snRNA structures would result in stable formation of the step two active site.

Once the splicing reactions have been completed, the mature mRNA product must be released from the spliceosome, and Prp22 is the RNA-dependent ATPase

responsible for promoting this event (Fig. 2.6b; Company et al. 1991; Schwer and Gross 1998). Like many of the ATPases encountered so far, Prp22 performs both an ATP-independent and an ATP-dependent function in splicing. The ATP-independent function is not well characterized, but is only required when the distance between the BP and the 3′ss is greater than 20 nucleotides (Schwer and Gross 1998; Schwer 2008). This function is required prior to execution of the second step, when Prp22 has been proposed to act in concert with Slu7 and Prp18 to position the 3′ss and 3′-hydroxyl of the 5′-exon for catalysis (Schwer 2008). Site-specific crosslinks and RNase H protection of the mRNA downstream of the exon-exon junction in the presence of Prp22 suggest that a conformational rearrangement following the second catalytic step places Prp22 on the mRNA at this location (Schwer 2008). Prp22 then acts to unwind the mRNA/U5 snRNA duplex, releasing the mRNA from the spliceosome using the energy generated through ATP hydrolysis (Schwer and Gross 1998; Schwer 2008). Following mRNA release, Slu7, Prp18, and Prp22 dissociate from the spliceosome (James et al. 2002).

Spliceosome Disassembly

After a substrate has been spliced, the spliceosome undergoes disassembly, resulting in the separation of U2, U5, U6, the NTC, and the lariat intron, and thereby allowing the spliceosomal components to be recycled for subsequent rounds of splicing (Fig. 2.7; Tsai et al. 2005). The DExD/H box RNA helicase Prp43, which associates with Ntr1 and Ntr2 to form the NTR complex, is responsible for promoting spliceosome disassembly in an ATP-dependent manner following mRNA release (Arenas and Abelson 1997; Tsai et al. 2005). Prp43 helicase activity is greatly enhanced through its interaction with Ntr1, demonstrating that Ntr1 is an accessory factor that is required to regulate Prp43 activity (Tanaka et al. 2007). Prp43 is recruited to the spliceosome through an interaction between Ntr2 and the U5 snRNP-component Brr2 (Tsai et al. 2007). Since Brr2 is present early in spliceosome assembly and throughout both catalytic steps, it is notable that binding of Ntr2 is competitively inhibited by the presence of Prp16 and Slu7, ensuring that spliceosome disassembly is not prematurely triggered through early association of the NTR complex with Brr2 (Chen et al. 2013).

Whether or not Brr2 helicase activity is required during intron release and spliceosome disassembly is up for debate. Small et al. (2006) reported that in a GTP-bound state, Snu114 derepresses Brr2 activity after the second catalytic step, resulting in intron release and spliceosome disassembly in much the same way as observed for U4/U6 unwinding during spliceosome assembly. This model presents another example of toggling throughout the splicing cycle, whereby hydrolysis of GTP to GDP results in repression of Brr2 activity following U4/U6 unwinding; subsequent exchange of the GDP for a new GTP following the splicing reactions derepresses Brr2 to allow spliceosome disassembly. Indeed, the RNA-dependent

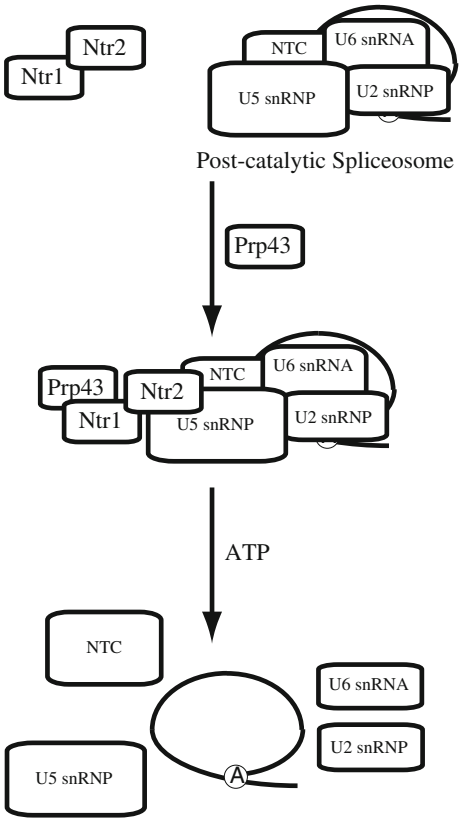

Fig. 2.7 Spliceosome disassembly mediated by Prp43 and the NTR complex. The excised intron is shown as a lariat with the BP adenosine *circled*. Proteins and snRNP particles are indicated by *rectangles*

ATPase activity of Brr2 is preferentially stimulated by annealed U2/U6, suggesting that the U2/U6 duplex could be a Brr2 substrate (Xu et al. 1996). However, Fourmann et al. (2013) recently showed that while Prp43 is necessary and sufficient for spliceosome disassembly, Brr2 is not required. Since Brr2 activity is specifically dependent on ATP hydrolysis, the fact that spliceosome disassembly proceeded as efficiently in the presence of UTP, CTP, and GTP as it did in the presence of ATP strengthens the argument that Brr2 activity is not required at this step (Fourmann et al. 2013).

The conflicting results reported by Small et al. (2006) and Fourmann et al. (2013) could reflect the different study systems used by the two groups. Fourmann et al. (2013) devised a purified splicing system with which stalled activated spliceosomes were isolated from an extract, followed by addition of recombinantly expressed and purified first and second step protein factors. The consequences of protein addition were then observed. In contrast, Small et al. (2006) used a tagged Prp43 to pull the Prp43-containing complex out of whole cell extract where potential endogenous factors reside that might play a role in splicing but have not yet been identified. It is possible that Prp43 activity destabilizes the spliceosome substantially, enough so that in the purified system, Brr2 activity is dispensable. In

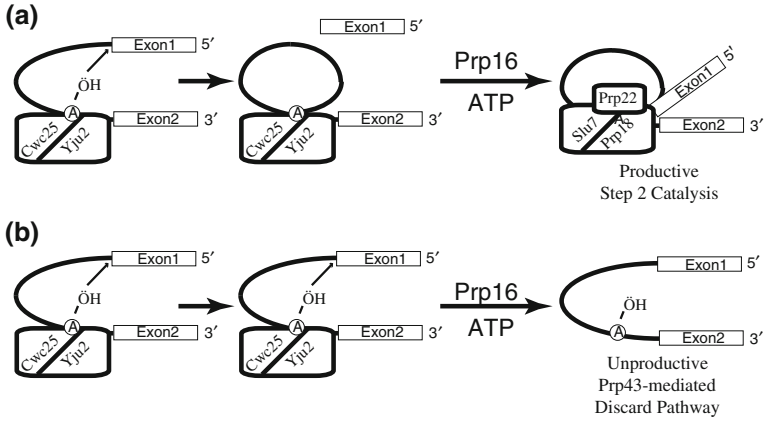

Fig. 2.8 Kinetic proofreading of the first catalytic step by Prp16. **a** An optimal substrate, in which reaction of the BP adenosine (*circled*) with the 5′ss is faster than ATP hydrolysis by Prp16, leading to dissociation of Cwc25 and Yju2 after the first chemical step has occurred. **b** A suboptimal pre-mRNA in which Prp16 hydrolysis occurs before the first chemical step, leading to premature dissociation of Cwc25 and Yju2, and subsequent Prp43-mediated disassembly

the absence of Brr2 activity, for example in the presence of UTP, the workload for Prp43 might increase to complete disassembly. In the Small et al. (2006) complex, other factors might contribute to the stability of the disassembling spliceosome such that Brr2 activity is required for efficient disassembly. Further experimentation will be required to reconcile these differences.

Splicing Fidelity

Since pre-mRNA splicing involves the removal of intervening sequences and ligation of protein coding sequences to generate a continuous translation template, splicing must proceed with single nucleotide precision to avoid introducing nucleotide insertions or deletions that would result in the translation of frame-shifted, aberrant products. The spliceosome has evolved a number of proofreading mechanisms to ensure fidelity throughout assembly and catalysis. In these, the spliceosome acts to promote splicing of optimal substrates, while antagonizing splicing of suboptimal substrates (Semlow and Staley 2012). One proofreading mechanism for which there is support in splicing is kinetic proofreading, originally described independently by Hopfield (1974) and Ninio (1975) in the translation field. In splicing, kinetic proofreading has been observed in early spliceosome assembly, where U2 snRNP association with the branchpoint, and exchange of U1 for U6 at the 5′ss, are proofread by Prp5 and Prp28, respectively. Other examples have been found through the first and second catalytic steps (Xu and Query 2007; Yang et al. 2013; Burgess and Guthrie 1993; Mayas et al. 2006).

In the kinetic proofreading model, energy is expended to allow for inspection of the substrate before allowing the substrate to proceed down a productive pathway. Optimal substrates undergo reaction quickly, while the time required for reaction of suboptimal substrates is longer (Fig. 2.8). Several splicing ATPases, such as Prp16 and Prp22, have been implicated as "timers" during these proofreading stages, in which splicing of optimal substrates proceeds more rapidly than the ATPase acts (Fig. 2.8a; Burgess and Guthrie 1993; Mayas et al. 2006). As a consequence, hydrolysis of ATP promotes a conformational change that shuffles the substrate down a productive pathway. However, when suboptimal substrates are encountered, ATP hydrolysis occurs more rapidly than the splicing reaction (Fig. 2.8b). This results in a conformational change in the spliceosome that promotes the rejection of the substrate through a discard pathway. Discrimination between fast and slow substrates may be based in part on the spliceosome's ability to discriminate between substrates that are positioned correctly for the chemical steps and those that are not (Chua and Reed 1999).

The role of Prp16 in kinetic proofreading during the first catalytic step has been well characterized and serves as an excellent example of proofreading by the spliceosome. Proofreading at this stage involves kinetic competition between the Prp16-dependent release of Cwc25 and the first transesterification reaction (Fig. 2.8a; Tseng et al. 2011). When the splicing machinery encounters an optimal substrate, the transesterification reaction proceeds more rapidly than the removal of Cwc25, and thus Cwc25 is displaced by Prp16 after the first catalytic step, thereby making way for second-step splicing factors. In the case of a suboptimal substrate containing branchpoint mutations, however, ATP hydrolysis by Prp16 occurs more rapidly than the transesterification reaction, resulting in the premature release of Yju2 and Cwc25 from the spliceosome prior to completion of the first transesterification reaction (Fig. 2.8b; Tseng et al. 2011). Discard of the suboptimal substrate at this point involves the disassembly factor Prp43 (Koodathingal et al. 2013). In fact, Prp43-mediated spliceosome disassembly can be initiated after the action of Prp2, Prp16, or Prp22, following their dissociation from the spliceosome when a suboptimal substrate is encountered. This suggests that Prp43 plays a more general role in discarding suboptimal substrates throughout catalysis, in addition to disassembling the spliceosome following splicing of an optimal substrate (Chen et al. 2013).

Concluding Remarks

Over the last several decades, much work has been completed to understand the chemical mechanism of the splicing reactions and the composition of the spliceosome, which is responsible for catalyzing these reactions. Despite this wealth of information, very little is known about the exact role of many splicing factors, and even less is known about the mechanisms through which these factors function. With recent advances in the technology used to study splicing, we now have an

opportunity to investigate and explore questions that could not be addressed previously. For example, we are seeing a shift from analyzing bulk splicing in whole cell extract to monitoring the fate of individual substrates by fluorescence microscopy. As a result of this transition, we are already beginning to understand the kinetics of individual steps, and the order of association and dissociation events, with greater depth. These types of inquiries, along with progress in atomic-resolution structure determination of splicing complexes, will lead to a better understanding of the intricate details of the splicing cycle.

Acknowledgments This work was supported by NSERC Discovery Grant 298521 to SDR and an NSERC PGS award to EAD, as well as by awards from UNBC's Office of Research.

References

Abelson J (2013) Toggling in the spliceosome. Nat Struct Mol Biol 20:645–647

Abovich N, Liao XC, Robash M (1994) The yeast MUD2 protein: an interaction with PRP11 defines a bridge between commitment complexes and U2 snRNP addition. Genes Dev 8:843–854

Abovich N, Rosbash M (1997) Cross-intron bridging interactions in the yeast commitment complex are conserved in mammals. Cell 89:403–412

Arenas JE, Abelson J (1997) Prp43: an RNA helicase-like factor involved in spliceosome disassembly. PNAS 94:11798–11802

Ares M Jr, Grate L, Pauling MH (1999) A handful of intron-containing genes produces the lion's share of yeast mRNA. RNA 5:113–1139

Bartels C, Urlaub H, Lührmann R et al (2003) Mutagenesis suggests several roles of Snu114p in pre-mRNA splicing. J Biol Chem 278:28324–28334

Bell M, Schreiner S, Damainov A et al (2002) p110, a novel human U6 snRNP protein and U4/U6 snRNP recycling factor. EMBO J 21:2724–2735

Bellare P, Small EC, Huang X et al (2008) A role for ubiquitin in the spliceosome assembly pathway. Nat Struct Mol Biol 15:444–451

Bon E, Casaregola S, Blandin G et al (2003) Molecular evolution of eukaryotic genomes: hemiascomycetous yeast spliceosomal introns. NAR 31:1121–1135

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

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

Brody E, Abelson J (1985) The "Spliceosome": Yeast pre-messenger RNA associates with a 40S complex in a splicing-dependent reaction. Science 228:963–967

Brow DA, Guthrie C (1988) Spliceosomal RNA U6 is remarkably conserved from yeast to mammals. Nature 334:213–218

Brow DA, Guthrie C (1989) Splicing a spliceosomal RNA. Nature 337:14–15

Brow DA (2002) Allosteric cascade of spliceosomal activation. Annu Rev Genet 36:333–360

Brys A, Schwer B (1996) Requirement for Slu7 in yeast pre-mRNA splicing is dictated by the distance between the branchpoint and 3′ splice site. RNA 2:707–717

BurgessSM Guthrie C (1993) A mechanism to enhance mRNA splicing fidelity: the RNA-dependent ATPase Prp16 governs usage of a discard pathway for aberrant lariat intermediates. Cell 73:1377–1391

Burset M, Seledtov IA, Solovyev VV (2000) Analysis of canonical and non-canonicle splice sites in mammalian genomes. NAR 28:4364–4375
Cech T (1986) The generality of self-splicing RNA: relationship to nuclear mRNA splicing. Cell 44:207–210
Chan SP, Kao DI, Tsai WY et al (2003) The Prp19p-associated complex in splicesome activation. Science 302:279–282
Chan SP, Cheng SC (2005) The Prp19-associated complex is required for specifying interactions of U5 and U6 with the pre-mRNA during spliceosome activation. J Biol Chem 280:31190–31199
Chen HC, Cheng SC (2012) Functional roles of protein splicing factors. Biosci Rep 32:345–359
Chen HC, Tseng CK, Tsai RT et al (2013) Link of NTR-mediated spliceosome disassembly with DEAH-box ATPases Prp2, Prp16, and Prp22. Mol Cell Biol 33:514–525
Chen JY, Stands L, Staley JP et al (2001) Specific alterations of U1-C protein or U1 small nuclear RNA can eliminate the requirement of Prp28p, an essential DEAD box splicing factor. Mol Cell 7:227–232
Cheng SC, Abelson J (1987) Spliceosome assembly in yeast. Genes Dev 1:1014–1027
Chua K, Reed R (1999) The RNA splicing factor hSlu7 is required for correct 3′ splice site choice. Nature 402:207–210
Collemare J, van der Burget A, de Wit PJ (2013) At the origin of spliceosomal introns: is multiplication of intron-like elements the main mechanism of intron gain in fungi? Commun. Integr. Biol. 6(2):e23147
Company M, Arenas J, Abelson J (1991) Requirement of the RNA helicase-like Prp22 for release of messenger RNA from spliceosomes. Nature 349:487–493
Coulombe-Huntington J, Majewski J (2007) Characterization of intron loss events in mammals. Genome Res 17:23–32
Crawford DJ, Hoskins AA, Friedman LJ et al (2013) Single-molecule colocalization FRET evidence that spliceosome activation precedes stable approach of 5′ splice site and branch site. PNAS 110:6783–6788
Deutsch M, Long M (1999) Intron-exon structures of eukaryotic model organisms. NAR 27:3219–3228
Dlakic M, Mushegian A (2011) Prp8, the pivotal protein of the spliceosomal catalytic center, evolved from a retroelement-encoded reverse transcriptase. RNA 17:799–808
Dix I, Russell CS, O'Keefe RT et al (1998) Protein–RNA interactions in the U5 snRNP of *Saccharomyces ceravisiae*. RNA 4:1675–1686
Fabrizio P, Abelson J (1992) Thiophosphates in yeast U6 snRNA specifically affect pre-mRNA splicing in vitro. NAR. 20:3659–3664
Fabrizio P, Laggerbauer B, Lauber J et al (1997) An evolutionarily conserved U5 snRNP-specific protein is a GTP-binding factor closely related to the ribosomal translocase EF-2. EMBO 16:4092–4106
Fabrizio P, Dannenberg J, Dube P et al (2009) The evolutionarily conserved core design of the catalytic activation step of the yeast spliceosome. Mol Cell 36:593–608
Farlow A, Meduri E, Schlötterer C (2011) DNA double-strand break repair and the evolution of intron density. Trends Genet 27:1–6
Fica SM, Tuttle N, Novak T et al (2013) RNA catalyses nuclear pre-mRNA splicing. Nature 503:229–234
Fink GR (1987) Pseudogenes in yeast? Cell 49:5–6
Fortner DM, Troy RG, Brow DA (1994) A stem/loop in U6 RNA defines a conformational switch required for pre-mRNA splicing. Genes Dev 8:221–233
Fourmann JB, Schmitzová J, Christian H et al (2013) Dissection of the factor requirements for spliceosome disassembly and the elucidation of its dissociation products using a purified splicing system. Genes Dev 27:413–428
Fromont-Racine M, Mayes AE, Brunet-Simon A et al (2000) Genome-wide protein interaction screens reveal functional networks involving Sm-like proteins. Yeast 17:95–110

Gordon PM, Piccirilli JA (2001) Metal ion coordination by the AGC triad in domain 5 contributes to group II intron catalysis. Nat Struct Biol 8:893–898

Gozani O, Feld R, Reed R (1996) Evidence that sequence-independent binding of highly conserved U2 snRNP proteins upstream of the branch site is required for assembly of spliceosomal complex A. Genes Dev 10:233–243

Gozani O, Potashkin J, Reed R (1998) A potential role for U2AF-SAP 155 interactions in recruiting U2 snRNP to the branch site. Mol Cell Biol 18:4752–4760

Grabowski PJ, Seiler SR, Sharp PA (1985) A multicomponent complex is involved in the splicing of messenger RNA precursors. Cell 42:345–353

Guthrie C, Patterson B (1988) Spliceosomal snRNAs. Annu Rev Genet 22:387–419

Hahn D, Kudla G, Tollervey D et al (2012) Brr2p-mediated conformational rearrangements in the spliceosome during activation and substrate repositioning. Genes Dev 26:2408–2421

Hilliker AK, Staley JP (2004) Multiple functions for the invariant AGC triad of U6 snRNA. RNA 10:921–928

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

Hopfield JJ (1974) Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. PNAS 71:4135–4139

Hoskins AA, Friedman LJ, Gallagher SS et al (2011) Ordered and dynamic assembly of single spliceosomes. Science 331:1289–1295

Jackson SP, Lossky M, Beggs JD (1988) Cloning of the RNA8 gene of *Saccharomyces cerevisiae*, detection of the RNA8 protein, and demonstration that it is essential for nuclear pre-mRNA splicing. Mol Cell Biol 8:1067–1075

James SA, Turner W, Schwer B (2002) How Slu7 and Prp18 cooperate in the second step of yeast pre-mRNA splicing. RNA 8:1068–1077

Jurica MS, Moore MJ (2003) Pre-mRNA splicing: awash in a sea of proteins. Mol Cell 12:5–14

Käufer NF, Potashkin J (2000) Analysis of the splicing machinery in fission yeast: a comparison with budding yeast and mammals. NAR 28:3003–3010

Kistler AL, Guthrie C (2001) Deletion of MUD2, the yeast homolog of U2AF65, can bypass the requirement for Sub2, an essential spliceosomal ATPase. Genes Dev 15:42–49

Konarska MM, Grabowski PJ, Padgett RA et al (1985) Characterization of the branch site in lariat RNAs produced by splicing of mRNA precursors. Nature 313:552–557

Koodathingal P, Novak T, Piccirilli JA et al (2013) The DEAH-box ATPase Prp16 and Prp43 cooperate to proofread 5′ splice site cleavage during pre-mRNA splicing. Mol Cell 39:385–395

Kuhn AN, Li Z, Brow DA (1999) Splicing factor Prp8 governs U4/U6 RNA unwinding during activation of the spliceosome. Mol Cell 3:65–75

Kuhn AN, Reichl EM, Brow DA (2002) Distinct domains of splicing factor Prp8 mediate different aspects of spliceosome activation. PNAS 99:9145–9149

Kwan SS, Brow DA (2005) The N- and C-terminal RNA recognition motifs of splicing factor Prp24 have distinct functions in U6 binding. RNA 11:808–820

Lardelli RM, Thompson JX, Yates JR et al (2010) Release of SF3 from the intron branchpoint activates the first catalytic step of pre-mRNA splicing. RNA 16:516–528

Lee C, Jaladat Y, Mohammadi A et al (2010) Metal binding and substrate positioning by evolutionarily invariant U6 sequences in catalytically active protein-free snRNAs. RNA 16:2226–2238

Legrain P, Séraphin B, Robash M (1985) Early commitment of yeast pre-mRNA to the spliceosome pathway. Mol Cell Biol 8:3755–3760

Li Z, Brow D (1996) A spontaneous duplication in U6 spliceosomal RNA uncouples the early and late functions of the ACAGA element in vivo. RNA 2:879–894

Liu HL, Cheng SC (2012) The interaction of Prp2 with a defined region of the intron is required for the first splicing reaction. Mol Cell Biol 32:5056–5066

Madhani HD, Guthrie C (1992) A novel base-pairing interaction between U2 and U6 snRNAs suggests a mechanism for the catalytic activation of the spliceosome. Cell 71:803–817

Maeder C, Kutach AK, Guthrie C (2009) ATP-dependent unwinding of U4/U6 snRNAs by the Brr2 helicase requires the C terminus of Prp8. Nat Struct Mol Biol 16:42–48
Maschhloff K, Padgett R (1993) The stereochemical course of the first step of pre-mRNA splicing. NAR. 21:5456–5462
Mayas RM, Maita H, Staley JP (2006) Exon ligation is proofread by the DExD/H-box ATPase Prp22p. Nat Struct Mol Biol 13:482–490
Mayes AE, Verdone L, Legrain P et al (1999) Characterization of Sm-like proteins in yeast and their association with U6 snRNA. EMBO J 18:4321–4331
McGrail JC, Krause A, O'Keefe RT (2009) The RNA binding protein Cwc2 interacts directly with the U6 snRNA to link the nineteen complex to the spliceosome during pre-mRNA splicing. NAR 37:4205–4217
Mefford MA, Staley JP (2009) Evidence that U2/U6 helix I promotes both catalytic steps of pre-mRNA splicing and rearranges in between these steps. RNA 15:1386–1397
Mekouar M, Blanc-Lenfle I, Ozanne C et al (2010) Detection and analysis of alternative splicing in Yarrowia lipolytica reveal structural constraints facilitating nonsense-mediated decay of intron-retaining transcripts. Genome Biol 11:R65. doi:10.1186/gb-2010-11-6-r65
Mitrovich QM, Guthrie C (2007) Evolution of small nuclear RNAs in *S. cerevisiae*, *C. albicans*, and other hemiascomycetous yeasts. RNA 13:2066–2080
Moore MJ, Sharp PA (1993) Evidence for two active sites in the spliceosome provided by stereochemistry of pre-mRNA splicing. Nature 365:364–368
Mozaffari-Jovin S, Santos KF, Hsiao HH et al (2012) The Prp8 RNase H-like domain inhibits Brr2-mediated U4/U6 snRNA unwinding by blocking Brr2 loading onto the U4 snRNA. Genes Dev 26:2422–2434
Mozaffari-Jovin S, Wandersleben T, Santos KF et al (2013) Inhibition of RNA helicase Brr2 by the C-terminal tail of the spliceosomal protein Prp8. Science 341:80–84
Neuvéglise C, Marck C, Gaillardin C (2011) The intronome of budding yeasts. C R Biol 34:662–670
Newman AJ, Teigelkamp S, Beggs JD (1995) snRNA interactions at 5′ and 3′ splice sites monitored by photoactivated crosslinking in yeast spliceosomes. RNA 1:968–980
Nielsen KH, Staley JP (2012) Spliceosome activation: U4 is the path, stem I is the goal, and Prp8 is the keeper. Let's cheer for the ATPase Brr2! Genes Dev 26:2461–2467
Ninio J (1975) Kinetic amplification of enzyme discrimination. Biochimie 57:587–595
O'Day CL, Dalbadie-McFarland G, Abelson J (1996) The *Saccharomyces cerevisiae* Prp5 protein has RNA-dependent ATPase activity with specificity for U2 small nuclear RNA. J Biol Chem 271:33261–33267
Ohi MD, Vander Kooi CW, Rosenberg JA et al (2003) Structural insights into the U-box, a domain associated with multi-ubiquitination. Nat Struct Biol 10:250–255
Ohrt T, Odenwälder P, Dannenberg J et al (2013) Molecular dissection of step 2 catalysis of yeast pre-mRNA splicing investigated in a purified system. RNA 19:902–915
Oubridge C, Ito N, Evans PR et al (1994) Crystal structure at 1.92 Å resolution of the RNA-binding domain of the U1A spliceosomal protein complexed with an RNA hairpin. Nature 372:432–438
Padgett RA, Konarska MM et al (1984) Lariat RNAs as intermediates and products in the splicing of messenger RNA precursors. Science 225:898–903
Parker R, Siliciano PG, Guthrie C (1987) Recognition of the TACTAAC box during mRNA splicing in yeast involves base pairing to the U2-like snRNA. Cell 49:229–239
Patterson B, Guthrie C (1987) An essential yeast snRNA with a U5-like domain is required for splicing in vivo. Cell 49:613–624
Peebles CL, Zhang M, Perlman PS et al (1995) Catalytically critical nucleotides in domain 5 of a group II intron. PNAS 92:4422–4426
Pena V, Rozov A, Fabrizio P et al (2008) Structure and function of an RNAse H domain at the heart of the splicesome. EMBO J 27:2929–2940
Puig O, Gottschalk A, Fabrizio P et al (1999) Interaction of the U1 snRNP with nonconserved intronic sequences affects 5′ splice site selection. Genes Dev 13:569–580

Puig O, Bragado-Nilsson E, Koski T et al (2007) The U1 snRNP-associated factor Luc7p affects 5′ splice site selection in yeast and human. NAR 35:5874–5885
Rader SD, Guthrie C (2002) A conserved Lsm-interaction motif in Prp24 required for efficient U4/U6 di-snRNP formation. RNA 8:1378–1392
Raghunathan PL, Guthrie C (1998a) A spliceosomal recycling factor that reanneals U4 and U6 small nuclear ribonucleoprotein particles. Science 29:857–860
Raghunathan PL, Guthrie C (1998b) RNA unwinding in U4/U6 snRNPs requires ATP hydrolysis and the DEIH-box splicing factor Brr2. Curr Biol 8:847–855
Rhode BM, Hartmuth K, Westhof E et al (2006) Proximity of conserved U6 and U2 snRNA elements to the 5′ splice site region in activated spliceosomes. EMBO J 25:2475–2486
Rymond BC, Rosbash M (1985) Cleavage of 5′ splice site and lariat formation are independent of 3′ splice site in yeast mRNA splicing. Nature 317:735–737
Sakharkar K, Chow V, Kangueane P (2004) Distribution of exons and introns in the human genome. In Silico Biol 4:387–393
Schellenberg MJ, Wu T, Ritchie DB et al (2013) A conformational switch in Prp8 mediates metal ion coordination that promotes pre-mRNA exon ligation. Nat Struct Mol Biol 20:728–724
Schwer B, Guthrie C (1991) Prp16 is an RNA-dependent ATPase that interacts transiently with the spliceisome. Nature 349:494–499
Schwer B, Guthrie C (1992) A conformational rearrangement in the spliceosome is dependent on Prp16 and ATP hydrolysis. J EMBO 11:5033–5039
Schwer B, Gross CH (1998) Prp22, a DExD-box RNA helicase, plays two distinct roles in yeast pre-mRNA splicing. EMBO J 17:2086–2094
Schwer B (2008) A conformational rearrangement in the spliceosome sets the stage for Prp22-dependent mRNA release. Mol Cell 30:743–754
Schwer B, Chang J, Shuman S (2013) Structure-function analysis of the 5′ end of yeast U1 snRNA highlights genetic interactions with the Msl5•Mud2 branchpoint-binding complex and other spliceosome assembly factors. NAR 41:7485–7500
Semlow DR, Staley JP (2012) Staying on message: Ensuring fidelity in pre-mRNA splicing. Trends Biochem Sci 37:263–273
Shannon KW, Guthrie C (1991) Suppressors of a U4 snRNA mutation define a novel U6 snRNP protein with RNA-binding motifs. Genes Dev 5:773–785
Shuster EO, Guthrie C (1988) Two conserved domains of yeast U2 snRNA are separated by 945 nonessential nucleotides. Cell 55:41–48
Shuster EO, Guthrie C (1990) Human U2 snRNA can function in pre-mRNA splicing in yeast. Nature 345:270–273
Sigel RK, Vaidya A, Pyle AM (2000) Metal ion binding sites in a group II intron core. Nat Struct Biol 7:1111–1116
Siliciano PG, Guthrie C (1988) 5′ splice site selection in yeast: genetic alterations in base-pairing with U1 reveal additional requirements. Genes Dev 2:1258–1267
Small EC, Leggett SR, Winans AA et al (2006) The EF-G-like GTPase Snu114p regulates spliceosome dynamics mediated by Brr2p, a DExD/H box ATPase. Mol Cell 23:389–399
Song EJ, Werner SL, Neubauer J et al (2010) The Prp19 complex and the $Usp4^{Sart3}$ deubiquitinating enzyme control reversible ubiquitination at the spliceosome. Genes Dev 24:1434–1447
Sontheimer EJ, Steitz JA (1993) The U5 and U6 small nuclear RNAs as active site components of the spliceosome. Science 262:1989–1996
Soochin C, Suk-Won J, Cohen A et al (2004) A phylogeny of *Caenorhabditis* reveals frequent loss of introns during nematode evolution. Genome Res 14:1207–1220
Staley JP, Guthrie C (1999) An RNA switch at the 5′ splice site requires ATP and the DEAD box protein Prp28p. Mol Cell 3:55–64
Steitz TA, Steitz JA (1993) A general two-metal-ion mechanism for catalytic RNA. PNAS 90:6498–6502

Stevens SW, Barta I, Ge HY et al (2001) Biochemical and genetic analyses of the U5, U6, and U4/U6•U5 small nucler ribonucleoproteins from *Saccharomyces cerevisiea*. RNA 7:1543–1553

Stevens SW, Ryan DE, Ge HY et al (2002) Composition and functional characterization of the yeast spliceosomal penta-snRNP. Mol Cell 9:31–44

Spingola M, Grate L, Haussler D et al (1999) Genome-wide bioinformatics and molecular analysis of introns in *Saccharomyces cerevisiae*. RNA 5:221–234

Surowy CS, van Santen VL, Scheib-Wixted SM et al (1989) Direct, sequence specific binding of the human U1-70 k ribonucleoprotein antigen protein to loop I of U1 small nuclear RNA. Mol Cell Biol 9:4179–4186

Tanaka N, Aronova A, Schwer B (2007) Ntr1 activates the Prp43 helicase to trigger release of lariat-intron from the spliceosome. Genes Dev 21:2312–2325

Tsai RT, Fu RH, Yeh FL et al (2005) Spliceosome disassembly catalyzed by Prp43 and its associated components Ntr1 and Ntr2. Genes Dev 19:2991–3003

Tsai RT, Tseng CK, Lee PJ et al (2007) Dynamic interactions of Ntr1-Ntr2 with Prp43 and with U5 govern the recruitment of Prp43 to mediate spliceosome disassembly. J Mol Cell Biol 27:8027–8037

Tseng CK, Liu HL, Cheng SC (2011) DEAH-box ATPase Prp16 has dual roles in remodeling of the spliceosome in catalytic steps. RNA 17:145–154

Valadkhan S, Manley J (2001) Splicing-related catalysis by protein-free snRNAs. Nature 413:701–707

Van der Veen R, Arnberg A, van der Horst G et al (1986) Excised group II introns in yeast mitochondria are lariats and can be formed by self-splicing in vitro. Cell 44:225–234

Venter JC, Adams MD, Myers EW et al (2001) The sequence of the human genome. Science 291:1304–1351

Vidovic I, Nottrott S, Hartmuth K et al (2000) Crystal structure of the spliceosomal 15.5kD protein bound to a U4 snRNA fragment. Mol Cell 6:1331–1342

Vinogradov AE (2001) Intron length and codon usage. J Mol Evol 52:2–5

Wang Q, Zhang L, Lynn B et al (2008) A BBP-Mud2p heterodimer mediates branchpoint recognition and influences splicing substrate abundance in budding yeast. NAR 36:2787–2798

Warkocki Z, Odenwälder P, Schmitzová J et al (2009) Reconstitution of both steps of *Saccharomyces cerevisiae* splicing with purified spliceosomal components. Nat Struct Mol Biol 16:1237–1243

Weber S, Aebi M (1988) In vitro splicing of mRNA precursors: 5′ cleavage site can be predicted from the interaction between the 5′ splice site region and the 5′ terminus of U1 snRNA. NAR 16:471–486

Wiest DK, O'Day CL, Abelson J (1996) In vitro studies of the Prp9•Prp11•Prp21 complex indicate a pathway for U2 small nuclear ribonucleoprotein activation. J Biol Chem 271:33268–33276

Wood V, Gwilliam R, Rajandream MA et al (2002) The genome sequence of *Schizosaccharomyces pombe*. Nature 415:871–880

Wu S, Romfo CM, Nilsen TW et al (1999) Functional recognition of the 3′ splice site AG by the splicing factor U2AF35. Nature 402:832–835

Xu D, Nouraini S, Field D et al (1996) An RNA-dependent ATPase associated with U2/U6 snRNAs in pre-mRNA splicing. Nature 381:709–713

Xu YZ, Query CC (2007) Competition between the ATPase Prp5 and branch region U2 snRNA pairing modulates the fidelity of spliceosome assembly. Mol Cell 28:838–849

Yang F, Wang X, Zhang ZM et al (2013) Splicing proofreading at 5′ splice sites by ATPase Prp28p. NAR 41:4660–4670

Yean SL, Wuenschell G, Termini J et al (2000) Metal-ion coordination by U6 small nuclear RNA contributes to catalysis in the spliceosome. Nature 408:881–884

Zhu T, Niu DK (2013) Mechanisms of intron loss and gain in the fission yeast *Schizosaccharomyces*. PLoS ONE 8:e61683

http://www.springer.com/978-3-319-05686-9

Fungal RNA Biology
Sesma, A.; von der Haar, T. (Eds.)
2014, IX, 395 p. 56 illus., 37 illus. in color., Hardcover
ISBN: 978-3-319-05686-9