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Review

Splicing of a rare class of introns by the U12-dependent spliceosome

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Abstract

Pre-mRNA splicing is catalyzed by two unique spliceosomes, designated U2- or U12-dependent. In contrast to the well-characterized U2-dependent spliceosome, much remains to be learned about the less abundant U12-type spliceosome. This review focuses on recent advances in elucidating the structure and function of the minor U12-dependent spliceosome. Interesting similarities and differences between the U12- and U2-dependent spliceosomes are also highlighted.

Keywords: pre-mRNA splicing; U11/U12 snRNP; U12-dependent spliceosome.

Introduction

Most eukaryotic genes are transcribed into precursor mRNAs (pre-mRNAs) that must undergo splicing, an essential step of gene expression. During pre-mRNA splicing, non-coding sequences (introns) are removed from the pre-mRNA and coding sequences (exons) are ligated together to form mRNA. Splicing is catalyzed by a dynamic, multi-megaDalton ribonucleoprotein (RNP) enzyme termed the spliceosome. To date, two spliceosomes of unique composition have been characterized. The U2-dependent (major) spliceosome is found in all eukaryotes and catalyzes the removal of U2-type introns, the most commonly encountered class of introns (Moore et al., 1993; Burge et al., 1999). The less abundant U12-dependent (minor) spliceosome, on the other hand, splices the rare U12-type class of pre-mRNA introns (reviewed by Tarn and Steitz, 1997; Burge et al., 1999; Wu and Krainer, 1999; Patel and Steitz, 2003). U12-type introns are not found in a number of organisms, including yeast and nematodes, and thus, unlike the major spliceosome, the U12-dependent spliceosome is present in only a subset of eukaryotes (Burge et al., 1998). The U2- and U12-dependent spliceosomes coexist in eukaryotic cells and pre-mRNAs containing both types of introns serve as substrates for both splicing machineries.

U2- versus U12-type introns

U2- and U12-type introns are delineated by short, conserved sequences at the 5′ splice site, 3′ splice site and branch site (Figure 1A) and their mean lengths are very similar (4130 vs. 3600 nucleotides, respectively) (Levine and Durbin, 2001). Different splice site and branch site consensus sequences are found in U2- versus U12-type introns (Figure 1A) (reviewed by Burge et al., 1999). U2-type introns of higher eukaryotes possess the sequence AG/GURAGU at the 5′ splice site, CURACU at the branch site and YAG/G at the 3′ splice site (where '/' denotes an exon-intron boundary, A is the branchpoint adenosine, R is a purine and Y a pyrimidine). A polypyrimidine tract is located between the branch site and 3′ splice site, and

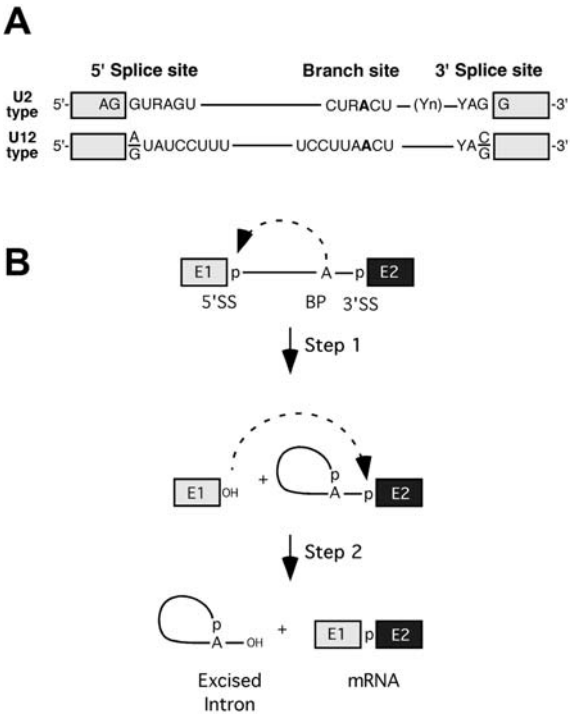

Figure 1 U2- and U12-type pre-mRNA introns and their conserved two-step splicing mechanism.
(A) Consensus sequences at the 5′ and 3′ splice sites and branch site of U2-type and U12-type pre-mRNA introns. The conserved sequences of mammalian U2-dependent introns and U12-type introns from diverse organisms are shown, where Y=pyrimidine and R=purine. Exons (shaded boxes), introns (lettering or a solid line), the branch adenosine (bold lettering), and the polypyrimidine tract (Yn) are indicated. (B) Two-step mechanism of pre-mRNA splicing. The exons (E1, E2) and the intron are depicted by boxes and solid lines, respectively. Dashed arrows indicate a nucleophilic attack and the branch site adenosine is indicated by the letter A. The phosphate groups (p) at the 5′ and 3′ splice sites are also shown.

the vast majority of U2-type introns have GT and AG dinucleotides at their 5′ and 3′ ends, respectively. By comparison, consensus sequences delineating the U12-type 5′ splice site –A(or G)UAUCCUUU– and branch site –UCCUUAACU– are longer and more tightly constrained. U12-type 3′ splice sites are typically denoted by YAC/ or YAG/. However, in contrast to U2-type introns, there is no strict 3′ splice site sequence requirement for U12-type introns; various other dinucleotides can serve as U12-type 3′ splice sites (Dietrich et al., 2001a; Levine and Durbin, 2001; Hastings et al., 2005). In addition, the average distance between the U12-type branch site and 3′ splice site (i.e., 12–15 nucleotides) appears to be shorter and more highly constrained than that observed with U2-type introns (i.e., 18–40 nucleotides) (Hall and Padgett, 1994; Dietrich et al., 2001a; Levine and Durbin, 2001) and, similar to U2-type introns present in yeast, U12-type introns lack a polypyrimidine tract.

U12-type introns were discovered only very recently (Jackson, 1991; Hall and Padgett, 1994) and the first set of U12-type introns identified contained the dinucleotides AT and AC at their 5′ and 3′ ends, respectively; for this reason they were originally referred to as ATAC introns. Subsequent studies, however, revealed that some U2-type introns also end in AT-AC and conversely that a large number of U12-type introns have the dinucleotides GT and AG at their 5′ and 3′ ends, respectively (Dietrich et al., 1997; Wu and Krainer, 1997). Thus, the nature of the 5′ splice site and branch site sequences are the main determinants for distinguishing between U2- and U12-type introns, and they also subsequently dictate which type of spliceosome will assemble on a particular intron. Although U12-type introns represent less than 1% of all introns present in human cells (Levine and Durbin, 2001), they are found in genes carrying out essential cellular functions (e.g., DNA replication and repair, transcription, RNA processing and translation) and their removal can be a rate limiting step in the splicing of pre-mRNAs containing both U2- and U12-type introns (Patel et al., 2002).

U2-type and U12-type introns appear to be removed by an identical two-step mechanism that involves two consecutive transesterification reactions (Figure 1B) (reviewed by Moore et al., 1993; Patel and Steitz, 2003). In the first step, the 2′-OH group of the branchpoint adenosine of the intron carries out a nucleophilic attack on the 5′ splice site, resulting in cleavage of the pre-mRNA at this site. Concomitantly, the 5′ end of the intron is ligated via a 2′–5′ phosphodiester bond to the branchpoint adenosine, generating a lariat-like structure (Figure 1B). In the second step, the 3′-OH group of the 5′ exon carries out a nucleophilic attack on the 3′ splice site. Upon cleavage of the latter, the 5′ and 3′ exons are ligated together (forming the mRNA), and the intron is released.

Assembly of the U2- and U12-dependent spliceosomes

A large number of *trans*-acting factors interact with the pre-mRNA to form the spliceosome. Within this RNP complex the reactive groups of the pre-mRNA (i.e., the branch adenosine and 5′ splice site or subsequently the 3′-OH of the excised 5′ exon and the 3′ splice site) are spatially positioned for catalysis. The U2-dependent spliceosome is formed by the interaction of the U1, U2, U5, and U4/U6 small nuclear ribonucleoproteins (snRNPs) and numerous non-snRNP proteins with the pre-mRNA. The U12-dependent spliceosome, in contrast, consists of the U11, U12, U5, and U4atac/U6atac snRNPs and an unknown number of non-snRNP proteins (Hall and Padgett, 1996; Tarn and Steitz, 1996a,b). Thus, of the main spliceosomal subunits (i.e., the snRNPs), only the U5 snRNP is common to both spliceosomes.

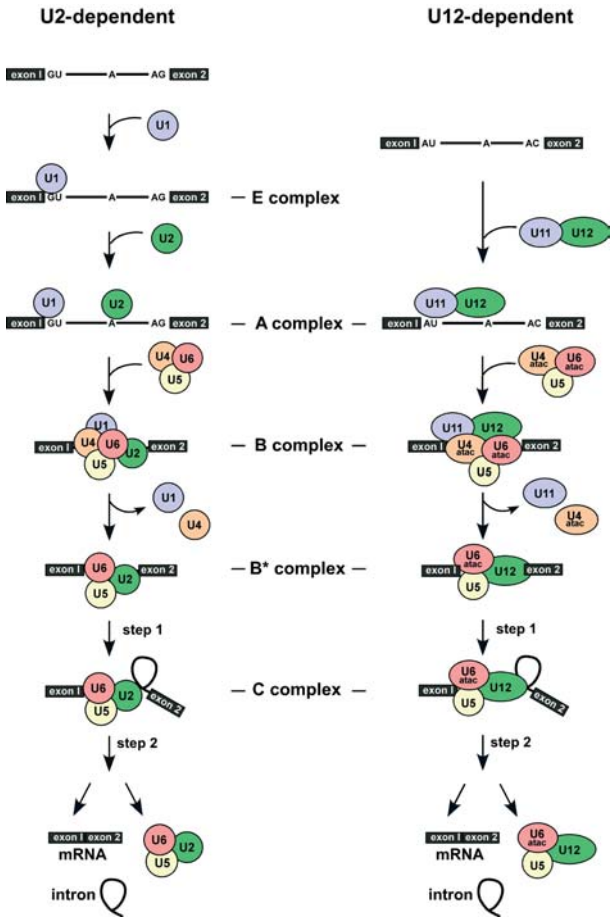

Figure 2 Stepwise assembly of the U2- and U12-dependent spliceosomes.
For simplicity, snRNPs (indicated by circles or ellipses), but not non-snRNP proteins, are shown. Exons are indicated by boxes and the intron, with the exception of the branch adenosine and the first and last dinucleotides, is depicted by a line.

Both the major and minor spliceosomal snRNPs interact stepwise, in a highly ordered manner with the pre-mRNA (Figure 2). During its assembly and catalytic activity a complex, highly dynamic RNA-RNA network involving pre-mRNA-snRNA and snRNA-snRNA interactions is formed within the spliceosome (Figure 3) (reviewed by Nilsen, 1998). Several distinct spliceosomal complexes denoted E, A, B, B* and C can be distinguished biochemically in higher eukaryotes (Figure 2) (reviewed by Reed and Palandjian, 1997). Assembly of the U2-dependent spliceosome is initiated by the interaction of U1 with the 5′ splice site, leading to the formation of the E complex. This interaction involves base pairing between the U1 snRNA and the 5′ splice site and is also facilitated by U1 snRNP proteins, namely U1-70K

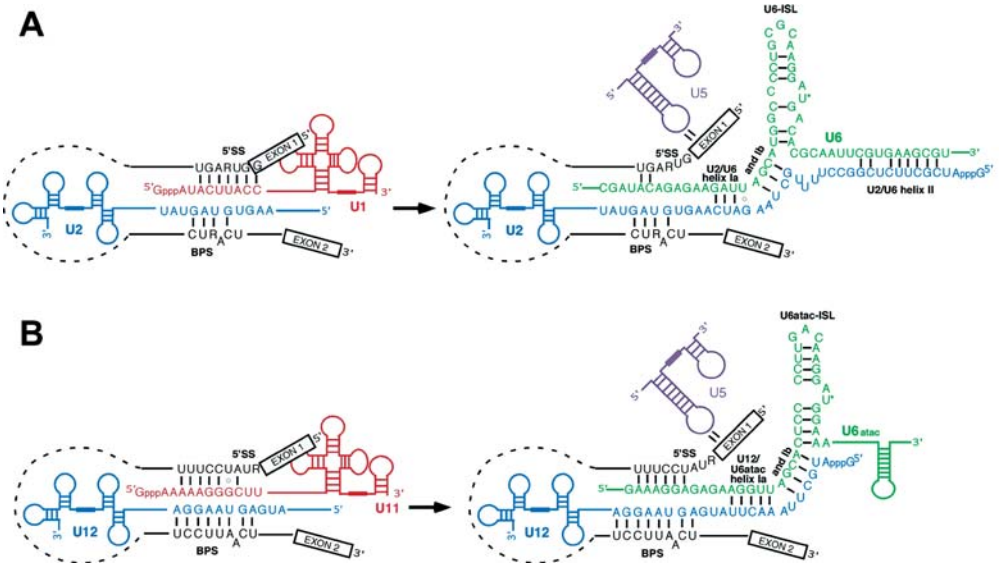

Figure 3 Network of RNA interactions in the major (A) and minor (B) spliceosomes.
Interactions observed in the spliceosomal A complex (left) and the subsequently formed activated B* complex (right) are shown (adapted from Tarn and Steitz, 1996a). Conserved nucleotides found at the 5′ splice site and branch site in mammals are indicated, as well as additional intron (black line) and exon (open boxes) sequences. Nucleotide sequences and structural models of the major and minor snRNAs are from human. U2/U6 helix III (Sun and Manley, 1995) and the interaction between the U12 snRNA and the 5′ exon in the minor spliceosomal A complex (Frilander and Meng, 2005) are not shown. An asterisk indicates the U residue in the U6 snRNA (or U6atac) implicated in metal ion binding.

and U1-C (Figures 3 and 7) (reviewed by Reed, 1996; Will and Lührmann, 1997). In the following step, the U2 snRNP interacts stably with the branch site to generate the A complex (also referred to as the pre-spliceosome). Within this complex, the U2 snRNA base pairs with the branch site and proteins of the U2 snRNP, namely components of the heteromeric SF3a and SF3b complexes, contact the pre-mRNA near or at the branch site, thereby stabilizing the interaction of the U2 snRNP with the pre-mRNA (Reed, 1996; Will and Lührmann, 1997).

After A complex formation, the U5 and U4/U6 snRNPs, in the form of a pre-formed U4/U6.U5 tri-snRNP complex, associate generating the B complex. In a subsequent step, major structural rearrangements occur that lead to the catalytically activated B* complex. During this step the U4/U6 base-pairing interaction is disrupted. U6 then base pairs with the 5′ splice site, displacing the U1 snRNA, and also engages in base pairing with the U2 snRNA, forming several short snRNA-snRNA duplexes termed helix I, II and III (Figure 3). A region of U6 also forms an internal stem-loop (U6-ISL) that is implicated in the catalysis of splicing (see below). In addition to the formation of novel U6 interactions, the U5 snRNA interacts with exon nucleotides at the 5′ splice site and at a later stage also with exon nucleotides near the 3′ splice site. As a consequence of these rearrangements, the U1 and U4 snRNPs are destabilized or dissociate from the spliceosome. Subsequently, the first catalytic step of splicing occurs and the C complex is generated. After the second catalytic step, the spliceosome dissociates, releasing the mRNA and excised intron, as well as the snRNPs, which are recycled to take part in new rounds of splicing.

Despite the fact that spliceosomes are comprised predominantly of protein and that catalytic activation of the spliceosome is absolutely dependent on the presence/activity of numerous spliceosomal proteins, the catalysis of pre-mRNA splicing appears to be RNA-based, with the U2 and U6 snRNAs (or U12 and U6atac in the case of the minor spliceosome) playing central roles (reviewed by Collins and Guthrie, 2000). For example, several intermolecular structures formed by the pre-mRNA and the U2, U5 and U6 snRNAs exhibit similarities with intramolecular structures formed by self-splicing group II introns (Nilsen, 1998). That the spliceosome is an RNA enzyme (i.e., a ribozyme), with an active site or sites comprised of RNA, is further supported by several studies. For example, it has been demonstrated that the first step of splicing is mediated by a metal ion (Sontheimer et al., 1997), and a nucleotide in the U6-ISL, whose structure mimics that of a catalytically active structure (i.e., domain 5) in self-splicing group II introns (Sashital et al., 2004), has been shown to bind a divalent metal ion required for the first step of splicing (Yean et al., 2000). Perhaps the most convincing evidence is the ability of an RNA complex, consisting solely of fragments of U6, U2 and a pre-mRNA branchpoint RNA, which can form base-pairing interactions identical to those observed in the spliceosome, to catalyze a transesterification reaction analogous, but not identical, to the first step of splicing (Valadkhan and Manley, 2001, 2003).

Assembly of the U12-dependent spliceosome is similar to that of the U2-dependent spliceosome, with a major difference occurring at the earliest step (Figure 2). The U11, U12 and U4atac/U6atac snRNPs are functional analogs of the U1, U2 and U4/U6 snRNPs (Hall and Padgett, 1996; Tarn and Steitz, 1996a,b; Kolossova and Padgett, 1997; Yu and Steitz, 1997; Incorvaia and Padgett, 1998). Prior to their association with the pre-mRNA, the U11 and U12 snRNPs form a highly stable di-snRNP that

binds cooperatively to the 5′ splice site and branch site during the first step of minor spliceosome assembly (Wassarman and Steitz, 1992; Frilander and Steitz, 1999). Thus, in contrast to the major spliceosome, the earliest assembly step involves formation of the A complex. In a subsequent step, the U4atac/U6atac and U5 snRNPs, in the form of a pre-formed tri-snRNP complex, interact giving rise to the B complex (Tarn and Steitz, 1996a,b). Activation of the minor spliceosome also appears to involve the loss or destabilization of the U11 and U4atac snRNPs, and thus they are not directly involved in catalysis. Indeed, the main role of the U4 or U4atac snRNA is thought to be that of a chaperone, sequestering the U6 or U6atac snRNA via extensive base-pairing interactions prior to catalytic activation of the spliceosome (Guthrie and Patterson, 1988; Tarn and Steitz, 1996a). The remaining steps appear to mirror those of the major spliceosome, with the appearance of a C-like complex at the time of the first transesterification reaction and subsequent dissociation of the minor spliceosome after the second transesterification.

Within the minor spliceosome, an analogous RNA-RNA network is formed (Figure 3). Initially the U11 and U12 snRNAs base pair with the 5′ splice site and branch site, respectively (Figure 3B; Hall and Padgett, 1996; Kolossova and Padgett, 1997; Yu and Steitz, 1997). Recent crosslinking studies have revealed that, at the time of A complex formation, the 5′ end of the U12 snRNA also transiently contacts exon nucleotides just upstream of the 5′ splice site (Frilander and Meng, 2005); this interaction is lost when the U4atac/U6atac.U5 tri-snRNP joins the spliceosome. Thus, U12 can simultaneously interact with the branch site and nucleotides of the pre-mRNA near the 5′ splice site, at the earliest stages of minor spliceosome assembly. Upon integration of the minor U4atac/U6atac.U5 tri-snRNP, the U4atac and U6atac base-pairing interaction is disrupted. The U6atac then interacts with the 5′ splice site (displacing U11) and also with the 5′ end of the U12 snRNA, whereas U5 interacts with exon nucleotides near the 5′ splice site and, at a subsequent stage, with exon nucleotides near the 3′ splice site (Figure 3B; Tarn and Steitz, 1996a,b; Yu and Steitz, 1997; Incorvaia and Padgett, 1998; Frilander and Steitz, 2001). As the 5′ end of U12 is truncated (relative to U2), a U6atac/U12 helix II cannot be formed (compare Figure 3A and B). This difference aside, the intermolecular structures formed by the pre-mRNA and the U12, U5 and U6atac snRNAs appear to be similar to those formed by U2, U5 and U6 in the major spliceosome. Likewise, the U6atac forms an intramolecular stem-loop structure similar to that of U6-ISL. The formation of an analogous RNA network in the catalytically activated minor spliceosome further strengthens the idea that pre-mRNA splicing is catalyzed by RNA. Significantly, the U6atac-ISL can be functionally replaced by U6-ISL or a catalytically active, group II intron D5 sequence (Shukla and Padgett, 2001, 2002), consistent with the idea that catalysis of U12-dependent splicing proceeds by an identical RNA-based mechanism. Thus, mechanistic differences between U2- and U12-dependent splicing appear to be limited to early intron recognition events, rather than to catalysis.

The minor snRNAs

In addition to containing multiple proteins (see below), the minor snRNPs are comprised of an snRNA molecule, or two in the case of the U4atac/U6atac di-snRNP. The primary sequences and most probable secondary structures of both the major and minor human spliceosomal snRNAs are shown in Figure 4. Similar to U4 and U6, the U4atac and U6atac snRNAs base pair extensively with one another within the U4atac/U6atac di-snRNP and also in the U4atac/U6atac.U5 tri-snRNP complex (Tarn and Steitz, 1996a). As described in detail above, upon engaging in base-pairing interactions with the pre-mRNA and/or other snRNAs within the spliceosome, several of these snRNAs undergo major structural rearrangements. Although the minor U4atac and U6atac snRNAs are functional analogs of U4 and U6, respectively, they share only limited sequence homology (ca. 40%; Tarn and Steitz, 1996a). Furthermore, the sequence of U11 in comparison with U1, as well as U12 compared with U2, are completely unrelated. Despite this fact, the minor U11, U12, U4atac and U6atac snRNAs can be folded into structures similar to U1, U2, U4 and U6, respectively (Montzka and Steitz, 1988; Tarn and Steitz, 1996a).

Characteristic features of all spliceosomal snRNAs are their small size, metabolic stability, and high degree of conservation of their sequence and secondary structure (Guthrie and Patterson, 1988). A comparison of snRNAs between kingdoms, however, indicates that the major snRNAs are much more highly conserved than U11, U12, U4atac and U6atac snRNA (Shukla and Padgett, 1999). The spliceosomal snRNAs contain multiple internal nucleotide modifications (e.g., pseudouridines and 2′O-methylations), several of which contribute to snRNA function during splicing (Yu et al., 1998; Dönmez et al., 2004). However, compared with the major snRNAs, the U11, U12, U4atac and U6atac snRNAs contain notably fewer internal nucleotide modifications (Massenet and Branlant, 1999). The U11, U12, U4atac snRNAs (like their major counterparts), as well as the U5 snRNA, also possess a 2,7,7-trimethylguanosine (m_3G) 5′ cap structure. These snRNAs contain a conserved single-stranded region—the so-called Sm site—with the consensus $PuAU_{4-6}GPu$ (where Pu is a purine) that is normally flanked by two hairpins and serves as the binding site of the Sm proteins (reviewed by Will and Lührmann, 2001). In contrast, U6atac (like U6) possesses a monomethyl phosphate cap and lacks an Sm site (Tarn and Steitz, 1996a).

Protein composition of the minor snRNPs and minor spliceosome

The protein compositions of the major snRNPs, as well as of the major spliceosome, are well characterized. Indeed, mass spectrometry analyses of purified U2-type spliceosomes have identified more than 200 spliceosome-associated proteins (Hartmuth et al., 2002; Jurica et al., 2002; Makarov et al., 2002; Rappsilber et al., 2002; Zhou et al., 2002; Makarova et al., 2004). Due to the more recent discovery of the U12-dependent spliceosome and the fact that the minor snRNPs are ~1/100th as abun-

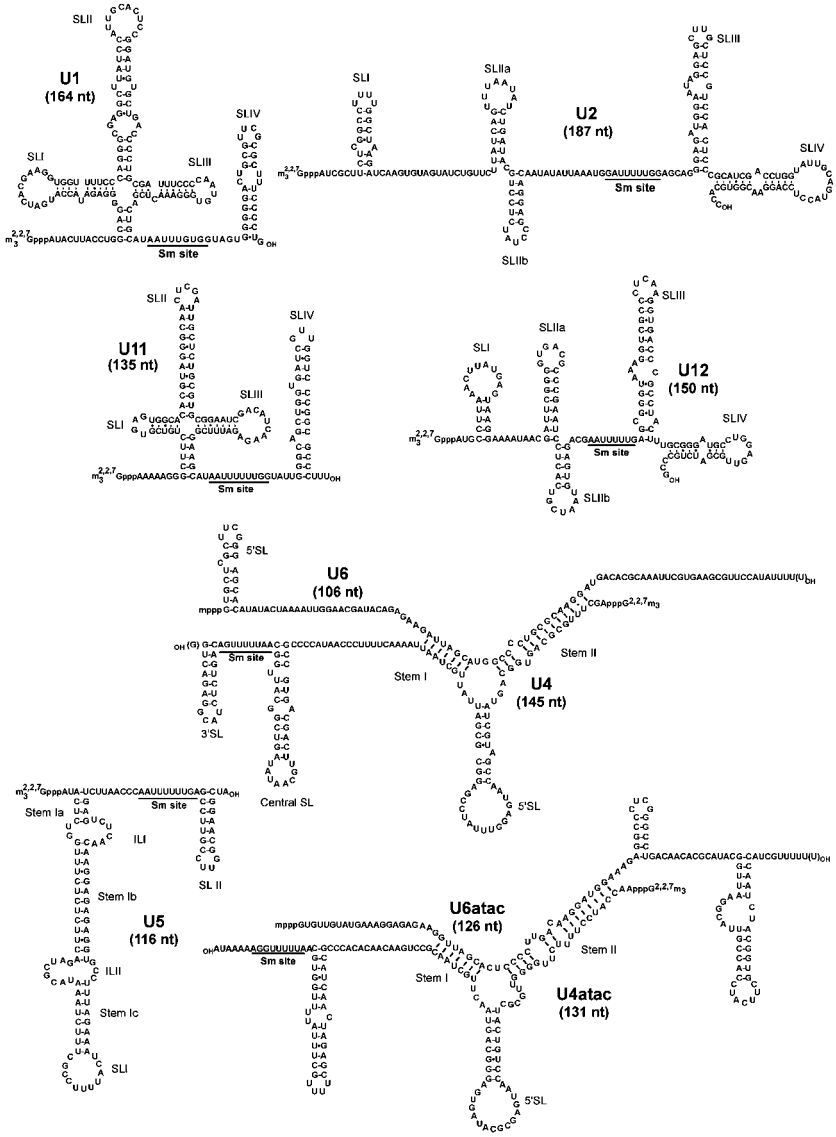

Figure 4 Sequence and secondary structure models of the human major and minor spliceosomal snRNAs (Guthrie and Patterson, 1988; Wassarman and Steitz, 1992; Padgett and Shukla, 2002).
The conserved sequences of the Sm site are underlined, and various stems and stem-loop (SL) structures are indicated. This Figure was kindly provided by Heike Benecke.

dant in the cell as their major counterparts (Yu et al., 1999), comparatively little is known about the protein composition of the minor spliceosome. Most of what we know about proteins present in the minor spliceosome has come from the characterization of the human U11/U12 di-snRNP and U4atac/U6atac.U5 tri-snRNP. These studies have revealed that many, but not all, snRNP-associated proteins are shared by both spliceosomes, consistent with the apparently conserved catalytic mechanisms of both spliceosomes.

The U11/U12 di-snRNP

In Hela nuclear extracts, the human U11 and U12 snRNAs are present not only as 18S U11/U12 di-snRNP particles, but also as 12S U11 and 15S U12 mono-particles (Wassarman and Steitz, 1992). The protein composition of affinity-purified human 18S U11/U12 and 12S U11 snRNPs was recently determined by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (Will et al., 2004). These studies revealed that the U11/U12 di-snRNP not only shares several proteins with the major U2 snRNP, but also contains unique proteins not found in the U2-type spliceosome (Figure 5). Consistent with the presence of a highly conserved Sm binding-site in the U11 and U12 snRNAs, 18S U11/U12 di-snRNPs contain the seven Sm proteins E, F, G, D1, D2, D3, and B/B′, which associate with all snRNAs possessing an Sm site. In addition, they contain all seven subunits of the 17S U2-associated, essential splicing factor SF3b (SF3b155, SF3b145, SF3b130, SF3b49, SF3b14a/p14, SF3b14b and SF3b10)—including the branch adenosine binding protein p14 (Figure 5; Will et al., 1999, 2001, 2004). SF3b facilitates the interaction of U2 with the branch site and contributes to branch site recognition in U2-type introns (Gozani et al., 1996). The presence of this protein complex in both 18S U11/U12 di-snRNPs and 17S U2 snRNPs indicates that many aspects of

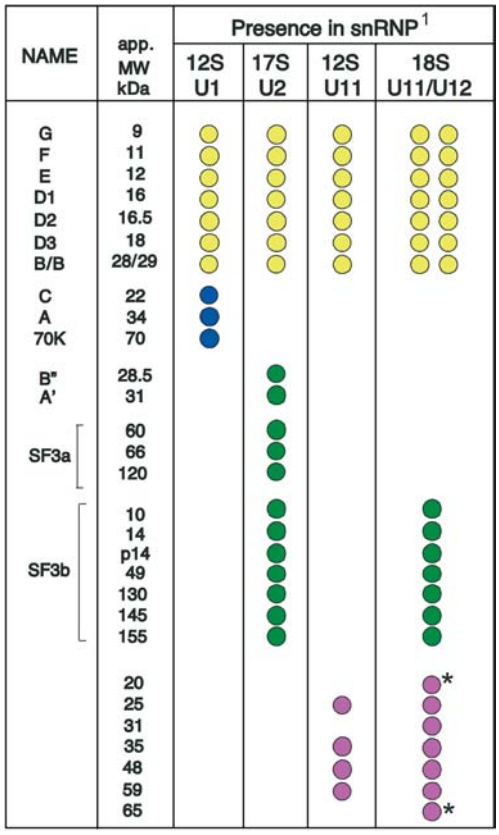

Figure 5 Protein composition of the human 12S U11 and 18S U11/U12 snRNPs versus 12S U1 and 17S U2 snRNPs.
The presence of a given protein in an snRNP is indicated by a colored circle.
[1]Several newly identified U2 proteins (e.g., hPrp5, SR140, CHERP, hPrp43, SPF45 and SPF30) that appear to be present in substoichiometric amounts in purified 17S U2 snRNPs are not shown (Will et al., 2002). Mass spectrometry also revealed the presence of the proteins hPrp43, Urp and YB-1 in purified human 18S U11/U12 snRNPs (Will et al., 2004). *U11/U12-specific protein for which an apparent homolog could be identified by BLAST searches in the fruit fly *D. melanogaster*.

branch site recognition are conserved in both spliceosomes. However, the U2-associated splicing factor SF3a, which also promotes the U2/branch site interaction, was not detected in purified 18S U11/U12 snRNPs. Likewise, neither 18S U11/U12 di-snRNPs nor 12S U11 snRNPs contain U1-specific proteins (Figure 5), including U1-70K and U1-C, which have been shown to facilitate the binding of the U1 snRNP to the U2-type 5′ splice site (reviewed by Will and Lührmann, 1997). The apparent lack of these U1-associated proteins in minor snRNPs suggests that there are significant differences in 5′ splice site recognition in the minor versus major spliceosome.

Significantly, U11/U12 snRNPs contain seven novel proteins with apparent molecular masses of 20, 25, 31, 35, 48, 59 and 65 kDa, which are not found in major snRNPs or the major spliceosome (Figure 5; Will et al., 2004). Four of the U11/U12-specific proteins (25K, 35K, 48K and 59K) are also associated with 12S U11 monoparticles and thus likely contribute to U12-type 5′ splice site recognition. Interestingly, several U11/U12-specific proteins (65K, 35K and 20K) share general structural features with proteins found in the U1 and U2 snRNPs (U1-A/U2-B′, U1-70K and U1-C, respectively), suggesting that they are functional analogs. However, as only moderate levels of sequence homology are observed between these minor and major proteins, and this homology is limited to their shared structural motifs, the significance of this observation is presently unclear. The identification of novel U11 proteins, coupled with the apparent absence of U1-specific proteins, indicates that protein-protein and protein-RNA interactions contributing to 5′ splice site recognition are not conserved between both spliceosomes. Likewise, it further suggests that one or more proteins involved in the molecular bridge connecting the 5′ and 3′ ends of the intron during the early stages of intron recognition are different in U12- and U2-dependent spliceosomes (see below).

Database searches revealed that all seven of the novel U11/U12-associated proteins are only found in organisms known to contain U12-type introns. Furthermore, most of them are evolutionarily highly conserved, suggesting that they carry out important cellular functions. Indeed, RNAi-mediated knockdowns of the 59K, 35K, 25K and 20K proteins, demonstrated that these U11/U12 proteins are essential for cell viability, consistent with the idea that they play key roles in U12-dependent splicing (Will et al., 2004). However, the precise role of these and other proteins in U12-dependent splicing remains to be established.

Drosophila melanogaster possesses a divergent U11 snRNP

Surprisingly, database searches failed to reveal *Drosophila melanogaster* homologs of proteins associated with human 12S U11 mono-particles; in contrast, putative orthologs of the human 65K and 20K proteins, which are found in U11/U12 but not in U11 snRNPs, could be detected (Figure 5). Consistent with this observation, the recently identified *Drosophila* U11, unlike other *Drosophila* snRNAs of the U12-type spliceosome, is highly divergent (Schneider et al., 2004). Both its sequence and length differ significantly from vertebrate and plant U11 snRNAs. Nonetheless, the 5′ end of the *Drosophila* U11 snRNA can potentially engage in base-pairing interactions with U12-type 5′ splice sites. A comparison of U11 snRNAs that were identified from vertebrates, plants and insects, further suggests that an evolutionarily divergent U11 snRNP may be unique to *Drosophila* and not characteristic of insects in general (Schneider et al., 2004). Thus, the *Drosophila* U11 snRNP appears to have diverged significantly from vertebrate and plant U11 snRNPs, suggesting that U12-type 5′ splice site recognition in fruit flies might involve unique protein-protein and protein-pre-mRNA interactions. Consistent with this idea, a comparison of 5′ splice sites in *Drosophila* versus humans revealed that an A-rich region is located just downstream of U12-type 5′ splice sites in *Drosophila*, but is absent from human U12-type 5′ splice sites (Schneider et al., 2004). Although experimental evidence is currently lacking, this region may serve as a binding site for proteins contributing to U12-type 5′ splice site recognition in flies.

Architecture of the U11/U12 di-snRNP

Little is currently known about intermolecular interactions in the U11/U12 di-snRNP, including which proteins directly contact the U11 and U12 snRNAs. The Sm proteins form a heptameric ring, containing one copy of each Sm protein in the order G-E-F-D2-D1-B-D3, which binds the Sm site of an snRNA (reviewed by Will and Lührmann, 2001). Given the presence of the Sm proteins and two Sm sites in the U11/U12 di-snRNP, two Sm ring-like structures are expected to be present. More recently, the 3D architecture of the human 18S U11/U12 snRNP was determined at a resolution of 13-10 Å using single particle electron cryomicroscopy (Golas et al., 2005). The 3D reconstruction revealed that it is a 260-Å-long globular particle, with multiple protuberances and a width of 150–170 Å (Figure 6). Components of SF3b, including the HEAT repeats of SF3b155 and the RRMs of SF3b49 and SF3b14a/p14, could be localized within the di-snRNP. However, the position of other U11/U12-associated proteins, as well as the U11 and U12 snRNAs remains unclear. The 3D structure of purified SF3b has also been determined by electron cryomicroscopy (Golas et al., 2003). These studies revealed that SF3b14a/p14, which directly contacts the branch adenosine in the spliceosome, is located in a central cavity of the SF3b complex. A comparison of the conformation of isolated SF3b with that of SF3b in the U11/U12 di-snRNP indicates that SF3b is in a more open conformation in the latter complex (Golas et al., 2005). This suggests that SF3b undergoes a conformational change that makes SF3b14a/p14 more accessible for intermolecular interactions at the time of its interaction with the U12 snRNP. As the U11/U12 di-snRNP is the major subunit of the U12-dependent spliceosomal A complex, information about the 3D structure of this snRNP also provides an initial glimpse into the molecular architecture of this spliceosomal complex.

The U4atac/U6atac.U5 tri-snRNP complex

To date, the minor tri-snRNP complex has not been affinity-purified and thus a comprehensive analysis of its protein composition has not been performed. However, immunoprecipitation and *in vitro* binding studies indicate that most proteins associated with the major U4/U6.U5 tri-snRNP, including U4/U6 proteins, are also present in the U4atac/U6atac.U5 tri-snRNP and minor spliceosome (Luo et al., 1999; Nottrott et al., 1999; Schneider et al., 2002). Thus, in contrast to the U11/U12 snRNP, which contains unique proteins not found in the major spliceosome, U4atac/U6atac.U5 tri-snRNP proteins appear to be identical to those of the major tri-snRNP and likely carry out conserved or even identical functions in both spliceosomes. Furthermore, the higher order structure of the major and minor tri-snRNP complexes is also likely to be very similar. Within the U4/U6 snRNP, the U4/U6-15.5K protein binds to a region of the 5′ stem-loop (SL) of the U4 snRNA (Nottrott et al., 1999) and serves as a platform for the subsequent association of the U4/U6-61K protein, as well as the U4/U6-90K, -60K and -20K proteins, which form a trimeric complex (Horowitz et al., 1997; Nottrott et al., 1999, 2002; Teigelkamp et al., 1998). The 61K also plays a key role in association of the U4/U6 snRNP with U5 to form the tri-snRNP complex, interacting with the U5-102K protein (Makarov et al., 2000; Makarova et al., 2002; Schaffert et al., 2004). Significantly, the U4/U6-15.5K protein also binds the equivalent 5′ stem-loop of the U4atac snRNA (Nottrott et al., 1999). It, therefore, can also act as a platform for the binding of the U4/U6-61K, -90K, -60K and -20K proteins to the U4atac/U6atac snRNP, which in turn associates with the U5 snRNP via the 61K protein (Schneider et al., 2002).

The similar, if not identical, protein composition of the major and minor tri-snRNP complex raises questions as to what distinguishes these complexes during the formation of the spliceosomal B complex. That is, hybrid spliceosomes containing U1 and U2 and the U4atac/U6atac.U5 tri-snRNP or alternatively U11/U12 and the U4/U6.U5 tri-snRNP have, to date, not been observed. Recent studies have demonstrated that a mutant form of U4 snRNA designed to base pair with U6atac can functionally replace the U4atac within the U12-dependent spliceosome (Shukla and Padgett, 2004). Thus, the main specificity determinant is likely to be the U6atac snRNA (or U6 in the case of the major spliceosome), which engages in analogous but unique base-pairing interactions with the U12-type 5′ splice site and the U12 snRNA upon minor tri-snRNP association with the U12-type spliceosomal A complex.

Non-snRNP proteins and intron/exon bridging interactions

Major spliceosomes contain a multitude of non-snRNP proteins (reviewed by Jurica and Moore, 2003). Whether this class of proteins is also largely shared by both spliceosomes is presently not clear and awaits the isolation and characterization of U12-dependent spliceosomal complexes. Members of the SR protein family, which are characterized by one or more RRMs and a C-terminal domain rich in arginine-serine dipeptides, have been shown to function in both U2- and U12-dependent splicing (Wu and Krainer, 1998; Hastings and Krainer, 2001). In the major spliceosome, SR proteins play important roles in splice site selection and facilitate snRNP-pre-mRNA interactions (reviewed by Manley and Tacke, 1996; Sanford et al., 2003). SR proteins also bind to so-called exonic splicing enhancer (ESE) sequences, short regulatory elements found in many pre-mRNAs that enhance splicing of adjacent introns, and have been shown to stimulate ESE-dependent splicing of both U2- and U12-type introns (Wu and Krainer, 1998; Hastings and Krainer, 2001).

SR proteins are also involved in protein-protein interactions that span the intron (so-called intron bridging) thereby connecting the 5′ splice site and the branch site. In the U2-dependent spliceosomal E complex, SR proteins appear to bridge the U1 snRNP at the 5′ splice site, and SF1/mBBP and U2AF which bind to the branch site and the polypyrimidine tract (Figure 7A) (reviewed by Reed, 1996). In the subsequently formed A complex, the U2 snRNP associates with the branch site, SF1/mBBP is displaced and a new set of interactions that juxtapose

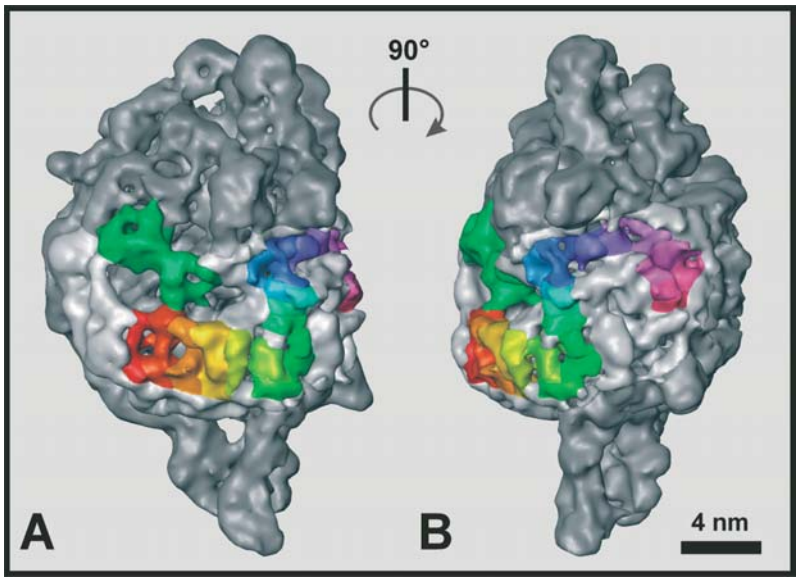

Figure 6 Three-dimensional structure of the human 18S U11/U12 snRNP.
(A, B) 3D surface representations of the human 18S U11/U12 di-snRNP obtained by electron cryomicroscopy are shown (Golas et al., 2005). The view in (B) has been rotated clockwise 90 degrees relative to that in (A). SF3b densities are indicated by silver with the two RRMs of SF3b49 highlighted green and the ladder-like SF3b155 HEAT repeats rainbow-colored. Although p14 could be localized in the U11/U12 snRNP, it is not visible in these views. The remaining densities (corresponding to U11 and additional U12 components) are colored dark gray. This Figure was kindly provided by Monika Golas.

the reactive groups of the pre-mRNA is formed. In the major spliceosome, the DEAD-box protein Prp5 appears to be involved in bridging the U1 and U2 snRNPs (Xu et al., 2004). Due to the concomitant binding of U11 and U12 as a di-snRNP, interactions bridging the 5′ splice site and branch site within the minor pre-spliceosome must initially be mediated by U11/U12 proteins and likely do not involve SR proteins (although SR proteins could potentially facilitate the interaction of U11/U12 snRNPs with the 5′ splice site and branch site; see Figure 7C). As unique proteins are associated with the U11 snRNP, and Prp5 was not detected in the U11/U12 di-snRNP, intron bridging interactions at the early stages of spliceosome assembly appear to be different in the major versus minor spliceosomes (Will et al., 2004).

In some cases, spliceosomal complexes initially form across an exon (for detailed discussions see Berget, 1995; Reed, 1996). In the major spliceosome, these so-called exon bridging interactions also involve SR proteins, which span components bound to the polypyrimidine tract and 3′ splice site (e.g., U2AF), and the U1 snRNP bound to the downstream 5′ splice site (Figure 7B). Binding of the U1 snRNP to a U2-type 5′ splice site can enhance splicing not only of an upstream U2-type intron but also of an upstream U12-type intron, indicating that components of both spliceosomes can interact with each other (Wu and Krainer, 1996). However, it is presently not known whether the binding of the U11 snRNP to a U12-type 5′ splice site can stimulate the splicing of an upstream U2-type intron. Introduction of an ESE into an exon can also stimulate the splicing of both an upstream U2- or U12-type intron (Wu and Krainer, 1998; Dietrich et al., 2001b). However, in contrast to the situation with U2-type introns, the ESE-mediated stimulation of splicing of an upstream U12-type intron does not require the presence of the U1 snRNP, suggesting that the molecular mechanism of splicing stimulation by ESEs may in some cases differ in the minor versus major spliceosome (Wu and Krainer, 1998).

Alternative splicing of U12-type pre-mRNAs

The proteomic complexity of many higher eukaryotes is achieved in part by alternative splicing events (reviewed by Graveley, 2001). During alternative splicing, different combinations of splice site pairs within a single pre-mRNA are utilized, expanding the number of unique mRNAs (and thus proteins) that are generated by a single gene. Bioinformatic approaches indicate that U12-type introns can be alternatively spliced, but additionally suggest that the frequency of alternative splicing is significantly lower than that observed with U2-type introns (Levine and Durbin, 2001). A number of alternative splicing patterns are observed with U2-type introns, including—among others—the alternative use of 5′ splice sites or 3′ splice sites present in a neighboring intron (resulting in so-called intron inclusion or exon skipping) (reviewed by Black, 2003). However, as U12-type 5′ and 3′ splice sites are not compatible with U2-type splice sites, alternative splicing patterns combining splice sites from neighboring U2- and U12-type introns have not been observed. Rather, alternative splicing of U12-type introns appears to predominantly involve the use of alternative U12-type 5′ or 3′ splice sites within the same intron, with alternative usage of U12-type 3′ splice sites most frequently observed (Levine and Durbin, 2001). An unusual example of alternative splicing involving a U12-type intron is found in the *Drosophila melanogaster prospero* gene. In the *prospero* pre-mRNA, U2-type splice sites are nested within a larger intron containing U12-type splice sites (generating a so-called twintron), and splicing by the U12-dependent spliceosome (as compared to the U2-dependent spliceosome) generates a larger isoform of the Prospero protein, a neuronal transcription factor

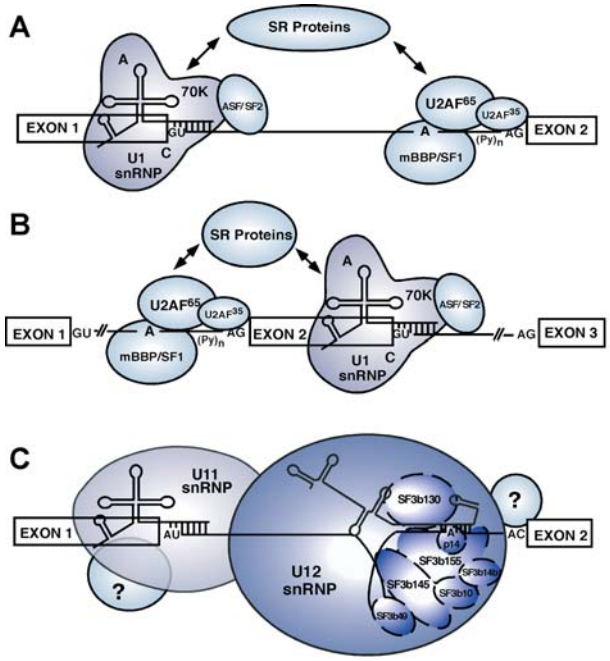

Figure 7 Intron and exon bridging interactions in the spliceosome.
Schematic depiction of interactions in the major spliceosomal E complex between factors bound at the 5′ splice site with those bound at the branch site/3′ splice site either across an intron (A) or across an exon (B), as proposed by the exon definition model (Berget et al., 1995). The ability of SR proteins to bridge the 5′ and 3′ splice sites (both across an intron or an exon) is indicated by arrows. SR proteins such as ASF/SF2, as well as the U1-70K and U1-C proteins, facilitate the interaction of the U1 snRNP with the 5′ splice site. The proteins mBBP/SF1, U2AF65 and U2AF35 bind the branchpoint adenosine, the polypyrimidine tract and the 3′ splice site AG dinucleotide, respectively (Wu et al., 1999 and references therein). (C) Intron bridging in the minor spliceosomal A complex. Due to the concomitant binding of the U11/U12 di-snRNP, proteins of this snRNP complex appear to be involved in intron bridging. However, the precise nature of this bridge is presently not known. Likewise, those proteins facilitating the U11/5′ splice site interaction or mediating 3′ splice site recognition (indicated by circles with a question mark) are presently not known. In the major spliceosome, subunits of SF3b (dashed ellipses in the U12 snRNP) contact the pre-mRNA at or near the branch site (Gozani et al., 1996); experimental evidence for analogous interactions in the minor spliceosome is currently lacking. The U1, U11, and U12 snRNAs are shown schematically within their respective snRNPs. Boxes indicate exon sequences; solid line, intron sequences; A, branchpoint adenosine; (Py)n, the polypyrimidine tract; AG or AC, the conserved dinucleotide at the 3′ splice site.

(Scamborova et al., 2004). The use of the U12- versus U2-type splice sites is developmentally regulated and competition between the major and minor spliceosomes for their respective splice sites is mediated by a purine-rich intronic enhancer element (Scamborova et al., 2004).

Evolutionary relationship of the major and minor spliceosomes

The presence of two distinct spliceosomes in some eukaryotes has led to intriguing questions regarding their origin and evolutionary relationship (for detailed discussions see Burge et al., 1998, 1999; Lynch and Richardson, 2002). U12-dependent introns have been identified in plants and animals, indicating that the U2- and U12-dependent spliceosomes have co-existed for at least a billion years. On the other hand, there are no U12-introns in lower eukaryotes, such as yeast and the nematode *C. elegans*. A careful analysis of U12-type introns in different gene families and their presence/absence in different organisms suggested that over time U12-type introns have slowly been converted into U2-type introns (Burge et al., 1998). This idea is additionally consistent with the fact that U2-type splice sites are much more degenerate than U12-type ones, making it easier to convert a U12-type site into a U2-type site, rather than *vice versa*. It has thus been proposed that all eukaryotes previously contained U12-type introns but, due to their conversion to U2-type introns, this class of intron, as well as the splicing machinery responsible for their removal, was completely lost in some organisms (Burge et al., 1998).

Three models for the potential evolutionary relationship of the major and minor spliceosomes have been proposed, namely the fission-fusion, codivergence and parasitic invasion models (Burge et al., 1998). The first two models propose that both spliceosomes are derived from a common ancestor and thus have a homologous origin. In the latter, one of the spliceosomes pre-existed in a eukaryotic progenitor and the other was generated by the parasitic invasion of an organism that introduced a new class of pre-mRNA introns into the genome of this eukaryotic progenitor. The observation that most snRNP-associated proteins are common to both spliceosomal complexes and that the U5 snRNP is shared by both machineries argues for a homologous origin (i.e., common ancestry) of both spliceosomes. On the other hand, the identification of unique U11/U12-associated proteins, together with the lack of sequence similarity between the U11 and U12 versus U1 and U2 snRNAs, respectively, and the limited sequence homology between the U4atac and U6atac versus U4 and U6 snRNAs, favors a non-homologous origin of the snRNAs and some, but not all, of the snRNP proteins. Additional characterization of the components of the U12-dependent spliceosome may shed more light on these intriguing questions.

Conclusions and perspectives

Although much has been learned about the structure and function of the U12-dependent spliceosome since its discovery 9 years ago, a number of important questions remain. Mechanistic differences between U2- and U12-dependent pre-mRNA splicing appear to be primarily limited to splice site recognition and pairing events. At present, essentially nothing is known about protein-protein and protein-RNA interactions contributing to splice site recognition and pairing in the minor spliceosome. Thus, a primary goal remains to precisely identify those proteins contacting the 5′ splice site, branch site and 3′ splice site of U12-type introns, and to elucidate protein-protein interactions within the U11/U12 di-snRNP, as well as between U11/U12 proteins and other spliceosomal proteins. Relative to the well-characterized U2-dependent spliceosome, there is a paucity of information about the protein composition and architecture

of the minor spliceosome. The isolation of native U12-dependent spliceosomal complexes and the subsequent characterization of their composition and higher order structure are additional future goals, which should shed more light on the inner workings of the U12-dependent splicing machinery.

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