EMBO MEMBER'S REVIEW

The role of U5 snRNP in pre-mRNA splicing

Andrew J. Newman

MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, UK

e-mail: newman@mrc-lmb.cam.ac.uk

The current model for the function of the U5 small nuclear ribonucleoprotein particle (snRNP) in the spliceosome proposes that U5 carries binding sites for the 5' and 3' exons, allowing the spliceosome to 'tether' the 5' exon intermediate produced by the first catalytic step and align it with the 3' exon for the second step. Functional analysis of U5 snRNA in *cis*-spliceosomes has provided support for this model, and data from nematode and trypanosome splicing systems suggest that U5 or a U5-like snRNA performs a similar role in *trans*-splicing.

Introduction

Introns are removed from nuclear mRNA precursors via a two-step transesterification pathway in complex ribonucleoprotein particles called spliceosomes. In the first step the 2'-hydroxyl of an adenosine near the 3' end of the intron attacks the 5' splice site, producing the 5' exon and lariat intron–3' exon intermediates. In the second step the 3'-hydroxyl of the 5' exon intermediate attacks the 3' splice site to give the spliced mRNA and lariat intron products of splicing. The spliceosomal small nuclear ribonucleoprotein particles (snRNPs) are major structural and functional components of the splicing machinery. The small nuclear RNA components (U1, U2, U4, U5 and U6 snRNAs) of the snRNPs play diverse roles in intron recognition and splice site definition and may be intimately involved in spliceosomal catalysis (reviewed in Moore *et al.*, 1993; Madhani and Guthrie, 1994; Nilsen, 1994; Ares and Weiser, 1995; Krämer, 1995).

Only three of the five spliceosomal snRNAs—U2, U5 and U6—are thought to contribute functionally during the two transesterification reactions of splicing. Base-pairing between phylogenetically conserved motifs in U2 and U6 snRNAs is proposed to form a discontinuous U2–U6 helix at the core of the catalytically active spliceosome (Madhani and Guthrie, 1992; Sun and Manley, 1995; Field and Friesen, 1996). Adjacent sequences in U2 and U6 snRNAs contact the intron branchpoint sequence and the 5' splice site respectively, and this network provides a structural basis for the attack of the branchpoint adenosine residue at the 5' splice site in the first catalytic step. Functional analysis has shown that mutations at several of the nucleotides in the proposed U2–U6 helix block the first or second catalytic step of splicing, suggesting that these residues of U2 and U6 snRNAs could play central roles in the catalysis of both transesterification reactions (for

review see Moore *et al.*, 1993; Madhani and Guthrie, 1994; Nilsen, 1994).

A model for U5 snRNP function

The role played by U5 snRNA in the spliceosome is gradually becoming clearer with a convergence of genetic and biochemical data from several systems. Phylogenetic comparisons (Frank *et al.*, 1994) have revealed that U5 snRNA carries an invariant sequence of nine nucleotides displayed in an 11 nucleotide loop. Biochemical and genetic data from mammalian and yeast systems have been incorporated into a model for U5 snRNP function in which the U5 loop interacts with exon sequences at the 5' and 3' splice sites (Newman and Norman, 1992; Wyatt *et al.*, 1992; Cortes *et al.*, 1993; Sontheimer and Steitz, 1993; Newman *et al.*, 1995). The data show that contacts between U5 and the 5' exon are established in the pre-mRNA and persist through both catalytic steps. The U5 loop can thereby anchor or 'tether' the 5' exon splicing intermediate produced by the first transesterification, and then assist in the alignment of 5' and 3' exons for the second catalytic step (Newman and Norman, 1992; Sontheimer and Steitz, 1993; Figure 1).

In this view the U5 loop sequence would be the spliceosomal counterpart of the exon-binding loop of subdomain ID3 in Group II autocatalytic introns. These catalytic RNAs resemble nuclear pre-mRNA introns in terms of reaction pathway—both systems produce similar lariat intermediates and products—and stereochemical specificity (reviewed in Michel and Ferat, 1995; Pyle, 1996). However, in the autocatalytic system, exon-ID3 loop interactions involve base-pairing of complementary sequences, whereas the U5 exon-binding loop must be endowed with affinity for all exons, regardless of sequence. The presence in the U5 snRNA loop of four contiguous uridine residues, known to be promiscuous base-pairing partners, may facilitate its exon-tethering functions.

Whilst the evidence for interactions between U5 snRNP and exon sequences at 5' and 3' splice sites is compelling, the model outlined above leaves numerous questions about U5 function unanswered. How is U5 accurately positioned to interact with exon sequences at 5' splice sites in the absence of reliable exon sequence conservation? Given that the U5 snRNA terminal loop makes contact with the 5' exon before the first catalytic step, does the loop sequence itself play a crucial role in catalysis of 5' splice site cleavage? What factors and interactions are necessary to orient U5 and its tethered exon sequences precisely with respect to putative catalytic sequences in the U2–U6 'core' of the active spliceosome? What roles do the protein components of U5 snRNP play in these activities?

Fig. 1. Cartoon of interactions between the loop1 sequence of U5 snRNA and the 5' and 3' exons in the first and second catalytic steps of splicing. The U5 loop contacts the 5' exon in the pre-mRNA and this interaction is maintained through both catalytic steps. Contact between the 3' exon and the U5 loop occurs only after the first catalytic step and is proposed to align the 5' and 3' exons for the second step.

Functional analysis of U5 snRNA in *cis*-spliceosomes

The role of U5 snRNP in the spliceosome has been difficult to investigate directly because of the lack of an *in vitro* depletion–reconstitution system for U5. Recently, however, a technique has been developed for specific ablation of U5 snRNPs in yeast splicing extracts (O'Keefe *et al.*, 1996). In the absence of functional U5 snRNPs, spliceosome assembly is blocked and no splicing occurs. Splicing activity can be restored, however, by addition of U5 snRNA made *in vitro*, which allows the reconstitution of functional U5 snRNPs. Using this system it has been shown that spliceosomes containing U5 snRNPs lacking the invariant U5 loop sequence can carry out the first catalytic step of splicing—5' splice site cleavage and lariat formation—efficiently and accurately. The second catalytic step, however, is severely inhibited in the absence of the U5 snRNA loop. These findings are consistent with the exon tethering and alignment model for U5 snRNP function and they show that the invariant U5 snRNA loop cannot be an essential component of the spliceosomal active site, at least for the first catalytic step. Presumably the interaction of U5 snRNP with exon sequences at the 5' splice site before the first catalytic step serves to establish tethering early, so precluding possible loss of

the 5' exon splicing intermediate from the spliceosomal active site.

The roles of U5 snRNP proteins

Although second catalytic step activity in the yeast U5 snRNP reconstitution system is strongly dependent on the presence of a U5 snRNA exon-binding loop, it is nevertheless surprisingly tolerant of mutations in the phylogenetically invariant loop motif (O'Keefe *et al.*, 1996). This underscores the view that U5 loop–exon interactions can accommodate changes in base-pairing (or mis-pairing) without compromising exon tethering and alignment functions. This strongly suggests that U5 snRNP protein(s) may play a role in modulating or stabilizing RNA–RNA contacts between the U5 snRNA terminal loop and exon sequences. The integral U5 snRNP protein PRP8 of budding yeast (homologous to the human p220 factor) is an excellent candidate for this role, since it has been shown to make extensive contacts with substrate sequences at the 5' and 3' splice sites, including those contacted by the U5 snRNA loop (Wyatt *et al.*, 1992; Teigelkamp *et al.*, 1995; Umen and Guthrie, 1995). PRP8 is extraordinarily conserved in size and sequence across the phylogenetic spectrum, suggesting that it may be of critical importance to spliceosome function (Hodges *et al.*, 1995). In addition to a potential role in exon tethering via the U5 snRNA loop sequence, PRP8/p220 has been implicated in the recognition of both 5' and 3' splice sites (Reyes *et al.*, 1996; Umen and Guthrie, 1995, 1996) and is closely associated with the intron branch site in active spliceosomes (MacMillan *et al.*, 1994). In fact p220 is so far the only spliceosome component that has been shown to contact the invariant GU dinucleotide at the 5' splice site in active spliceosomes (Reyes *et al.*, 1996). This raises the intriguing possibility that PRP8/p220 might contribute functional groups to the catalytic centre of the spliceosome in addition to assisting in precise positioning of the U5 snRNA terminal loop at the 5' splice site. On this U5 positioning question, crosslinking data from the mammalian system hint at the possibility that U1 snRNP—which has long been known to interact with the 5' splice site early in spliceosome assembly—could also play a role in positioning the U5 snRNA loop in readiness to receive the 5' exon intermediate once catalysis begins (Ast and Weiner, 1997).

Biochemical analysis of purified mammalian U5 snRNP and U4/U6·U5 tri-snRNP particles has shown that they contain a 200 kDa ATP-binding protein which displays RNA-dependent ATPase activity (Laggerbauer *et al.*, 1996). Molecular cloning revealed that this protein includes two DEXH domains characteristic of a family of putative RNA helicases (Lauber *et al.*, 1996). A closely homologous 246 kDa protein (encoded by the essential gene *SNU246*) is found in yeast, and is likewise a component of the U4/U6·U5 tri-snRNP particle. Antibodies specific for the mammalian 200 kDa factor block the second step of splicing *in vitro*, demonstrating that it is an essential spliceosome component. The presence of motifs characteristic of RNA helicases suggests that these factors may be involved in one or more of the conformational changes in the network of RNA–RNA interactions in the spliceosome (Lauber *et al.*, 1996). Intriguingly an

allele of *SNU246* has been isolated independently using a genetic screen based on synthetic lethality with a mutation near the 5' end of U2 snRNA, close to a region which is proposed to form a catalytically critical helix with U6 snRNA (Xu *et al.*, 1996). Splicing reactions containing the synthetic lethal mutant protein accumulate an abnormal complex containing U2 and U6 snRNAs but lacking U5 snRNA. These findings give us a glimpse of the intricate mechanisms which are likely to underlie formation of the spliceosome's active core and to orchestrate its interactions with U5 snRNP.

Involvement of U5 snRNP in *trans*-splicing and AT-AC intron splicing

In *trans*-splicing in trypanosomes and nematodes the 5' terminal exon or spliced leader sequence (SL) is derived from a *trans*-splicing specific snRNP (the SL RNP). SL addition occurs via a two-step transesterification pathway analogous to *cis*-splicing, and involves U2, U4 and U6 snRNP co-factors. It has long been thought that U5 snRNP might not be required for *trans*-splicing, particularly because no U5 homologue had been demonstrated in trypanosomes. These organisms splice pre-mRNAs by *trans*-splicing, but apparently lack conventional *cis*-splicing. However, it is now clear that trypanosomes do indeed contain a U5-like small RNA (SLA2 RNA) associated with the SL RNA (Dungan *et al.*, 1996). Secondary structure analysis suggests that SLA2 forms a stem-loop structure with an 11 nucleotide loop sequence characteristic of U5. Like U5, SLA2 forms a complex with U4/U6. Crucially, crosslinking data show that the SLA2 loop interacts with the 5' splice site region in the SL RNA and with the free 5' exon-splicing intermediate in precisely the same way that the conventional U5 snRNA loop interacts with 5' exon sequences in *cis*-splicing (Dungan *et al.*, 1996). The identification of SLA2 as the trypanosomal *trans*-spliceosomal U5 homologue has been strengthened by the finding that trypanosomes contain a 277 kDa protein which is extensively homologous to PRP8/p220 and is found in a stable snRNP particle together with SLA2 RNA (Lücke *et al.*, 1997).

Direct analysis of affinity-purified *trans*-spliceosomes from *Ascaris* splicing extracts has shown that U5 snRNP is also an integral component of *trans*-spliceosomes in nematodes (Maroney *et al.*, 1996). Indeed, in *Ascaris* extracts U5 is found in the form of a tetra-snRNP (containing U4, U5, U6 and SL RNAs) which may be the *trans*-spliceosomal counterpart of the U4/U6/U5 tri-snRNP that participates in conventional *cis*-spliceosome assembly. Once again crosslinking experiments revealed that the U5 snRNA loop makes contacts with the 5' splice site in the SL RNA and with the free SL exon intermediate (Maroney *et al.*, 1996). Together with the trypanosome data these findings establish that the basic exon-interaction functions of U5 snRNP are conserved between *cis*- and *trans*-splicing.

An unusual new type of spliceosome containing several novel snRNPs has recently been shown to be responsible for excision of a minor class of introns in metazoans: AT-AC introns, so-called for their unusual 5' and 3' splice site consensus sequences. AT-AC intron splicing occurs in a complex analogous to conventional GT-AG spliceosomes

and is achieved via a similar two-step transesterification pathway. AT-AC spliceosomes contain distinct analogues of U1, U2, U4 and U6, and the AT-AC analogues of U2 and U6 form a helix strikingly similar to the U2-U6 helix at the heart of the GT-AG spliceosome. Nevertheless, AT-AC spliceosomes contain U5 snRNP, presumably engaged in the same exon-binding role that it plays in conventional *cis*- and *trans*-spliceosomes (reviewed in Tarn and Steitz, 1997). The finding that U5 is the only snRNP common to both AT-AC spliceosomes and the more familiar GT-AG *cis*- and *trans*-spliceosomes confirms its unique importance in pre-mRNA splicing reactions. Clearly it will be very interesting to compare the interactions of U5 with the putative catalytic core snRNPs of these two types of spliceosome.

Exon tethering in Group II autocatalytic introns

Extensive similarities between nuclear pre-mRNA splicing and autocatalytic Group II intron splicing have led to speculation that there may be an evolutionary relationship between these systems. If so, the spliceosomal snRNAs might correspond to specific functional domains from an ancestral Group II-like self-splicing intron (Cavalier-Smith, 1991; Sharp, 1991; Weiner, 1993). The similarities are particularly striking in the case of the Group II intron ID3 stem-loop and its proposed spliceosomal counterpart, U5 snRNA. Hetzer *et al.* (1997) have recently shown that deletion of the ID3 stem-loop from a yeast mitochondrial Group II intron allows the first step of splicing (5' splice site cleavage via lariat formation) to proceed accurately and efficiently but severely inhibits the second catalytic step. This finding echoes the situation in spliceosomes lacking the invariant exon-binding loop of U5 snRNA.

In the Group II system, addition of a small ID3 stem-loop RNA *in trans* to an intron with a deletion of ID3 restores the second catalytic step. *Trans*-activation depends upon complementarity between ID3 loop and 5' exon sequences, consistent with the idea that the role of ID3 is to tether the 5' exon-splicing intermediate and target it to the 3' splice site in the second catalytic step. Intriguingly the terminal stem-loop of human U5 snRNA can substitute for ID3 to mediate the second catalytic step in the *trans*-activation assay, and experiments using compensating base-pair exchanges show that, as with ID3, the U5 loop contacts the 5' exon by base-pairing. These results argue that the ID3 and U5 snRNA stem-loops fulfil similar exon-binding roles in Group II introns and spliceosomes, and they add to a growing list of structural and functional similarities between the two systems. Comparative analysis of exon-binding and catalysis in *cis*- and *trans*-spliceosomes and in autocatalytic introns promises to yield further insights into the workings of these complex splicing machines.

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References

- Ares,M.J. and Weiser,B. (1995) Rearrangement of snRNA structure during assembly and function of the spliceosome. *Prog. Nucleic Acids Res. Mol. Biol.*, **50**, 131–159.
- Ast,G. and Weiner,A.M. (1997) A novel U1/U5 interaction indicates proximity between U1 and U5 snRNAs during an early step of mRNA splicing. *RNA*, **3**, 371–381.
- Cavalier-Smith,T. (1991) Intron phylogeny: a new hypothesis. *Trends Genet.*, **7**, 145–148.
- Cortes,J.J., Sontheimer,E.J., Seiwert,S.D. and Steitz,J.A. (1993) Mutations in the conserved loop of human U5 snRNA generate use of novel cryptic 5' splice sites *in vivo*. *EMBO J.*, **12**, 5181–5189.
- Dungan,J.M., Watkins,K.P. and Agabian,N. (1996) Evidence for the presence of a small U5-like RNA in active *trans*-spliceosomes of *Trypanosoma brucei*. *EMBO J.*, **15**, 4016–4029.
- Field,D.J. and Friesen,J.D. (1996) Functionally redundant interactions between U2 and U6 spliceosomal snRNAs. *Genes Dev.*, **10**, 489–501.
- Frank,D.N., Roiha,H. and Guthrie,C. (1994) Architecture of the U5 small nuclear RNA. *Mol. Cell. Biol.*, **14**, 2180–2190.
- Hetzer,M., Wurzer,G., Schweyen,R.J. and Mueller,M.W. (1997) *Trans*-activation of group II intron splicing by nuclear U5 snRNA. *Nature*, **386**, 417–420.
- Hodges,P.E., Jackson,S.P., Brown,J.D. and Beggs,J.D. (1995) Extraordinary conservation of the PRP8 splicing factor. *Yeast*, **1**, 337–342.
- Krämer,A. (1995) The biochemistry of pre-mRNA splicing. In Lamond,A.I. (ed.), *Pre-mRNA Processing*. R.G. Landes, pp. 35–64.
- Laggerbauer,B., Lauber,J. and Lührmann,R. (1996) Identification of an RNA-dependent ATPase activity in mammalian U5 snRNPs. *Nucleic Acids Res.*, **24**, 868–875.
- Lauber,J., Fabrizio,P., Teigelkamp,S., Lane,W.S., Hartmann,E. and Lührmann,R. (1996) The HeLa 200 kDa U5 snRNP-specific protein and its homologue in *Saccharomyces cerevisiae* are members of the DEXH-box protein family of putative RNA helicases. *EMBO J.*, **15**, 4001–4015.
- Lücke,S., Klöckner,T., Palfi,Z., Boshart,M. and Bindereif,A. (1997) *Trans* mRNA splicing in trypanosomes: cloning and analysis of a PRP8-homologous gene from *Trypanosoma brucei* provides evidence for a U5-analogous RNP. *EMBO J.*, **16**, 4433–4440.
- MacMillan,A.M., Query,C.C., Allerson,C.R., Chen,S., Verdine,G.L. and Sharp,P.A. (1994) Dynamic association of proteins with the pre-mRNA branch region. *Genes Dev.*, **8**, 3008–3020.
- Madhani,H.D. and 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.
- Madhani,H.D. and Guthrie,C. (1994) Dynamic RNA–RNA interactions in the spliceosome. *Annu. Rev. Genet.*, **28**, 1–26.
- Maroney,P.A., Yu,Y.-T., Jankowska,M. and Nilsen,T.W. (1996) Direct analysis of nematode *cis*- and *trans*-spliceosomes: A functional role for U5 snRNA in spliced leader addition *trans*-splicing and the identification of novel Sm snRNPs. *RNA*, **2**, 735–745.
- Michel,F. and Ferat,J.-L. (1995) Structure and activities of group II introns. *Annu. Rev. Biochem.*, **64**, 435–461.
- Moore,J.M., Query,C.C. and Sharp,P.A. (1993) Splicing of precursors to mRNA by the spliceosome. In Gesteland,R.F. and Atkins,J.F. (eds), *The RNA World*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 303–357.
- Newman,A.J. and Norman,C. (1992) U5 snRNA interacts with exon sequences at 5' and 3' splice sites. *Cell*, **68**, 743–754.
- Newman,A.J., Teigelkamp,S. and Beggs,J.D. (1995) snRNA interactions at 5' and 3' splice sites monitored by photoactivated crosslinking in yeast spliceosomes. *RNA*, **1**, 968–980.
- Nilsen,T.W. (1994) RNA–RNA interactions in the spliceosome: Unraveling the ties that bind. *Cell*, **78**, 1–4.
- O'Keefe,R.T., Norman,C. and Newman,A.J. (1996) The invariant U5 snRNA loop 1 sequence is dispensable for the first catalytic step of splicing in yeast. *Cell*, **86**, 679–689.
- Pyle,A.M. (1996) Catalytic reaction mechanisms and structural features of group II intron ribozymes. *Nucleic Acids Mol. Biol.*, **10**, 75–107.
- Reyes,J.L., Kois,P., Konforti,B.B. and Konarska,M.M. (1996) The canonical GU dinucleotide at the 5' splice site is recognized by p220 of the U5 snRNP within the spliceosome. *RNA*, **2**, 213–225.
- Sharp,P.A. (1991) Five easy pieces. *Science*, **252**, 663.
- Sontheimer,E.J. and Steitz,J.A. (1993) The U5 and U6 small nuclear RNAs as active site components of the spliceosome. *Science*, **262**, 1989–1996.
- Sun,J.-S. and Manley,J.L. (1995) A novel U2-U6 snRNA structure is necessary for mammalian mRNA splicing. *Genes Dev.*, **9**, 843–854.
- Tarn,W. and Steitz,J.A. (1997) Pre-mRNA splicing: the discovery of a new spliceosome doubles the challenge. *Trends Biochem. Sci.*, **22**, 132–137.
- Teigelkamp,S., Newman,A.J. and Beggs,J.D. (1995) Extensive interactions of PRP8 protein with the 5' and 3' splice sites during splicing suggest a role in stabilization of exon alignment by U5 snRNA. *EMBO J.*, **14**, 2602–2612.
- Umen,J.G. and Guthrie,C. (1995) A novel role for a U5 snRNP protein in 3' splice site selection. *Genes Dev.*, **9**, 855–868.
- Umen,J.G. and Guthrie,C. (1996) Mutagenesis of the yeast gene *PRP8* reveals domains governing the specificity and fidelity of 3' splice site selection. *Genetics*, **143**, 723–739.
- Weiner,A.M. (1993) mRNA splicing and autocatalytic introns: distant cousins or the products of chemical determinism? *Cell*, **72**, 161–164.
- Wyatt,J.R., Sontheimer,E.J. and Steitz,J.A. (1992) Site-specific cross-linking of mammalian U5 snRNP to the 5' splice site before the first step of pre-mRNA splicing. *Genes Dev.*, **6**, 2542–2553.
- Xu,D., Nouraini,S., Field,D., Tang,S.-J. and Friesen,J.D. (1996) An RNA-dependent ATPase associated with U2/U6 snRNAs in pre-mRNA splicing. *Nature*, **381**, 709–713.

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