Intrinsic metal binding by a spliceosomal RNA

Saba Valadkhan and James L. Manley

A conserved stem-loop in the spliceosomal U6 snRNA is thought to play an important role in splicing of mRNA precursors. Recent work describes structural features of this stem-loop and highlights the significance of a previously noted catalytically important metal ion.

Almost all genes in eukaryotes contain non-coding regions that give rise to introns in the newly transcribed pre-messenger RNAs. These bits of sequence must be removed before the mRNAs can function as templates for protein synthesis. The spliceosome, a gigantic ribonucleoprotein machine made up of more than 70 proteins and five RNA molecules[1,2], catalyzes the removal of introns through two consecutive transesterification reactions. Although the exact make-up of the spliceosome active site is not known, two of the RNA components of the spliceosome (the U2 and U6 small nuclear RNAs or snRNAs), have long been considered the main candidates[1–3] to constitute this domain. Now the first structural study of a fragment of U6 snRNA[4], reported in a recent issue of *Nature Structural Biology*, provides a glimpse into the folding of a key region of U6 and, significantly, provides structural evidence supporting its role in catalysis.

Of the five spliceosomal snRNAs, U6 and U2 are the strongest candidates for the active site components of the spliceosome. Mutational analysis has shown the presence of key regions in U6 — the so-called ACAGAGA box and AGC triad (Fig. 1) — where mutations or modifications in the bases or RNA backbone block splicing activity. Additionally, crosslinking and genetic suppression studies show that both U2 and U6 bind *via* base pairing to the regions of the pre-mRNA involved in the first step of splicing[1]. Furthermore, U2 and U6 interact with each other through an extensive base-pairing network that provides a scaffold for positioning the reactants of the splicing reaction in close proximity[1]. Finally, the base paired complex of U2 and U6 was recently shown to catalyze a reaction closely related to the first step of splicing in the absence of proteins[5].

The possibility of an RNA active site in the spliceosome is further strengthened by similarities between the spliceosome and self-splicing group II introns. Group II introns are ribozymes found in organelles of certain fungi, plants and protists that catalyze their own removal from transcripts[6]. A highly conserved and catalytically crucial region of group II introns, domain V, has many sequence and structural similarities to a conserved stem-loop in U6 snRNA: both contain a catalytically-important AGC sequence at the 5′ end, a GNRA-type loop[7,8] capping the stem-loop, and a one- or two-nucleotide asymmetric internal loop on the 3′ side (Fig. 1*a*). Additionally, phosphorothioate substitution studies in group II introns and the spliceosome have demonstrated the functional importance of phosphate oxygens located in almost identical locations in domain V and the U6 stem-loop[9–12] (Fig. 1*a*).

The spliceosome and group II introns also share the same splicing chemistry, with catalysis proceeding *via* two transesterification reactions, and both maintain a strict requirement for divalent cations. Biochemical and structural studies in both domain V and the U6 stem-loop have shown specific metal binding by the 3′ loop[13–15]. The NMR structural data of Huppler *et al.*[4] establishes the ability of the protein-free RNA stem-loop

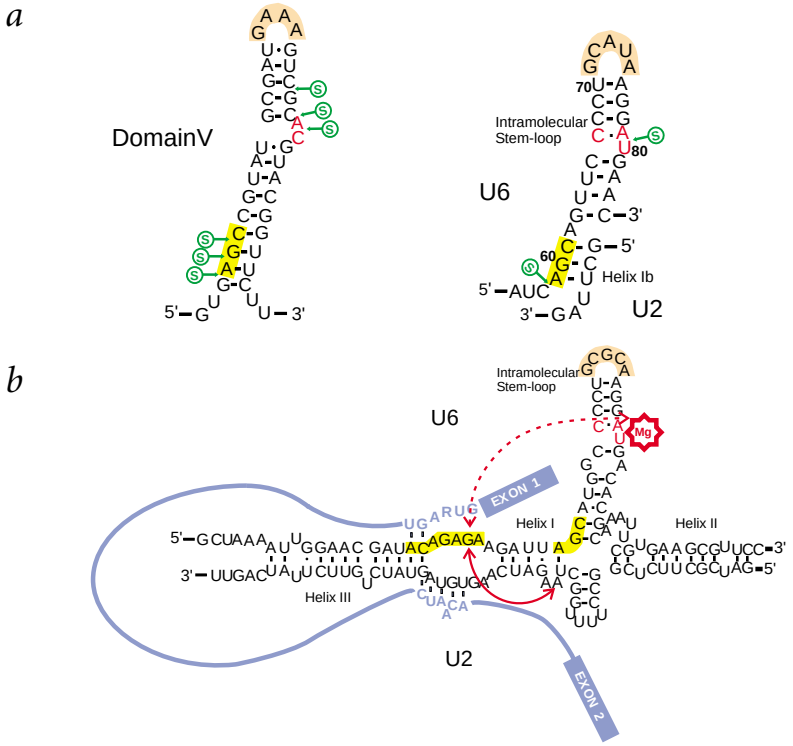

Fig. 1 ***a***, The base paired structure of the U6 intramolecular stem-loop in yeast (right) and domain V of the group II intron aI5γ (left). The yellow highlighted regions mark the invariant AGC triad. The orange highlighted regions are the GNRA-type loops. The asymmetric internal loop of U6 and the two-nucleotide bulge of Domain V are shown in red. Sites of phosphorothioate interference are indicated with green symbols. ***b***, The base pairing interactions between the central domains of human U2 and U6, and an mRNA precursor. The genetically-determined helices I, II and III are shown. The highlighted regions mark the invariant ACAGAGA and AGC domains of U6 and the GNRA-type loop capping the intramolecular stem-loop. The internal loop is shown in red, and the location of the bound metal in the intramolecular stem-loop of U6 is indicated. Red solid arrows connect residues involved in a UV-induced crosslink in a protein-free complex of human U2 and U6. Red dotted line suggests a possible interaction between catalytically important regions of U6.

domain of U6 to bind a divalent cation in the internal loop with the same stereospecificity observed in the intact spliceosome. Since this metal ion has been shown to be important for catalysis in the spliceosome[13], this study provides additional support for the competence of the spliceosomal snRNAs to form the active site of the spliceosome.

The stereospecificity of the metal binding, to the *S*p and not the *R*p phosphate oxygen located 5′ to the second nucleotide of the loop (U80), results from the specific geometry of the loop. It is interesting that this metal binding specificity is lost upon changing the pH: a more acidic pH promotes the formation of a protonated A•C base pair, which is incompatible with metal binding. The possibility that a change in local pH could modulate binding of a crucial metal ion may have important functional implications. For example, interaction with a protein/RNA that perturbs the pK_a of the adenine might lead to arrest of the splicing reaction, or alternatively, another component of the spliceosome might prevent the adenine from being protonated, thereby keeping the U6 stem-loop functionally intact. In addition, the non-canonical A•C base pair might serve as a recognition motif for an interaction, by exposing N4 of C79 in the major groove. However, while the structure of Huppler *et al.*[4] suggests an important role for this ionizable interaction, it is not essential for splicing. Mutations that convert adenine to any of the three other nucleotides, and thereby result in a canonical base pair or other types of mismatch in this region, are well tolerated, both in yeast and in mammalian systems *in vitro* and *in vivo*[16–19]. Additionally, it is not clear if the ability to form this non-canonical A•C pair is conserved in all U6 snRNAs (see, for example, ref. 20).

The demonstration of a GNRA-like fold for the pentaloop capping the U6 stem-loop suggests that this loop is involved in an important interaction in the spliceosome. GNRA loops are known to mediate interactions in many ribozymes[21–24], and intriguingly, the GNRA loop capping the domain V stem-loop in group II introns is involved in a functionally important tertiary interaction with a 'receptor' domain elsewhere in the intron. This interaction appears to help position domain V in the active site of the intron[23], while other interactions (as an example, see ref. 25) further assemble the active site components around it. It is plausible that the GNRA-like motif capping the U6 stem-loop is also involved in an interaction with an RNA/protein receptor, and that this interaction would help position the stem-loop, and ultimately the catalytically important metal ion it binds, at the active site of the spliceosome.

Two other regions of U6 that are thought to take part in the formation of the active site, the ACAGAGA box and AGC triad (Fig. 1*b*), are positioned close to each other in the active spliceosome, as determined by genetic suppression data in yeast[26] and by UV crosslinking in an *in vitro*-assembled base paired complex of human U2 and U6[27], which also showed that the formation of this functionally crucial tertiary fold is an intrinsic property of these two RNA molecules. If the Mg^{2+} bound to the internal loop of the U6 stem-loop is indeed part of the active site of the spliceosome, then it should be brought into close proximity to the ACAGAGA/AGC sequences, and an interaction mediated by the GNRA-like loop of U6 might be one of the means by which this positioning occurs. It will therefore be of great interest to determine the structure of a larger fragment of U6 that also includes the catalytically important AGC and ACAGAGA domains.

Another significant conclusion from the U6 stem-loop structure reported by Huppler *et al.*[4] is that the nucleotides of the internal loop, including the unpaired U, are not bulged but rather stacked within the helix. This result is satisfying because it offers an explanation for results from a mutational study in human cells that had questioned the significance of the internal loop. While deletion or mutation of this U residue was shown to be incompatible with splicing, mutations that incorporated the U into the helix by insertion of an A across the helix were well tolerated[19], likely reflecting a stacked conformation similar to that seen in the NMR structure for this nucleotide within the active spliceosome.

In recent work by Zhang and Doudna[15], the crystal structure of domain V of group II introns, along with the adjacent domain VI (which contains a region similar to the U2 branch site helix), was determined. Similar to the U6 structure of Huppler *et al.*[4], the structure of domain V contains a two-nucleotide unpaired region. However, unlike U6, the unpaired nucleotides of domain V are not stacked within the helix. In both structures metal binding was observed[4,15]. Both *S*p and *R*p oxygen phosphates in the asymmetric loop are catalytically crucial in both systems[9–13], but, unlike the case in U6, the block to splicing due to thio substitution of either *S*p oxygen in the domain V loop could not be rescued by Mn^{2+} (ref. 14) Whether or not this reflects a difference in the function of the loop in catalysis in the two systems awaits further studies.

According to the 'RNA World' hypothesis, RNA molecules were the first forms of life on planet Earth, not only functioning as a reservoir of information, but also performing all the enzymatic reactions necessary to maintain life. Gradually, with the increasing complexity of the living systems, they relinquished their enzymatic roles to proteins, although some RNA molecules persisted in their catalytic roles up to today as molecular fossils. With the discovery that the ribosome is one such remnant from the RNA world, the accumulating evidence for the catalytic competence of the spliceosomal snRNAs makes a serious case for the spliceosome to be the next in the line for becoming a legitimate ribozyme.

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Structure and catalytic mechanism of the *E. coli* chemotaxis phosphatase CheZ

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The PDB accession code for the structure of CheZ–CheY-BeF_3^- was incorrectly reported in the 'Coordinates' section of this paper. The correct PDB accession code is 1KMI. We apologize for any inconvenience this may have caused.

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A mistake occurred during the production of this News and Views report. The observation stated at the bottom of the second column on page 499 was incorrectly referenced to ref. 14. The correct reference for this observation is ref. 12, and the correct sentence is printed as follows: "… the block to splicing due to thio substitution of either *S*p oxygen in the domain V loop could not be rescued by Mn^{2+} (ref. 12)." We apologize for any inconvenience this may have caused.