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Structural Insights into RNA Splicing

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Summary of recent advances

Intron splicing is a fundamental biological process whereby non-coding sequences are removed from precursor RNAs. Recent work has provided new insights into the structural features and reaction mechanisms of two introns that catalyze their own splicing from precursor RNA: the group I and II introns. In addition, there is an increasing amount of structural information on the spliceosome, which is a ribonucleoprotein machine that catalyzes nuclear pre-mRNA splicing in eukaryotes. Here, we compare structures and catalytic mechanisms of self-splicing RNAs and we discuss the possible implications for spliceosomal reaction mechanisms.

Introduction

The discovery that most eukaryotic genes were interrupted by large, non-coding segments called “introns” fundamentally altered basic understanding of genetic organization and resulted in the 1993 Nobel Prize in Medicine for Philip Sharp and Richard Roberts. This work, done largely during the 1970s, was a watershed event for the field of gene expression. Introns were found to be transcribed along with the coding regions, but were removed or “spliced out” before messages were translated by the ribosome. Subsequently, it was found that many genes have several introns and that alternative splicing leads to the production of multiple protein isoforms from one gene, thus greatly expanding the proteome and leading to the complexity found in higher eukaryotes. The excision of nuclear-encoded introns from pre-mRNAs is performed by a massive ribonucleoprotein complex called the spliceosome.

Additional classes of introns were subsequently found in both eukaryotes and prokaryotes. Group I introns were the first self-splicing ribozymes to be discovered [1] and, together with the discovery of pre-tRNA cleavage by RNase P [2], they provided the first examples of RNA catalysis. Several years later, self-splicing by the phylogenetically unrelated group II introns was reported [3]. Group II introns have a highly complex RNA structure and they possess an unusually diverse repertoire of chemical reactivity, including catalysis of 2'-5' bond formation [4] and the ability to retrotranspose into DNA with the help of intron-encoded proteins. Analysis of secondary structure revealed that group II introns have six structural domains [5].

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Domain V (DV) is the most phylogenetically conserved region in group II introns and it consists of a 30-34 nt stem loop structure. The lower helix of DV contains a highly conserved “catalytic triad” of nucleotides, which are located exactly five base pairs away from a two-nucleotide bulge in the helix. This secondary structural arrangement is strikingly similar to that of U6 spliceosomal RNA [6,7] (Figure 1), which is believed to play a central role in catalysis during nuclear splicing. This fact, combined with other similarities, such as lariat formation during splicing, has led to the hypothesis that group II introns share a common ancestor with nuclear introns and the eukaryotic spliceosome [8]. This evolutionary connection, along with the elaborate tertiary structure of group II introns and their role as common retrotransposons, has made the crystal structure of an intact group II intron a major goal.

A class of small group II introns facilitate structural studies

Group II introns can be classified into three main structural groupings based on RNA secondary structure: IIA, IIB, and the more recently discovered IIC introns [9,10]. Phylogenetic analysis of intron-encoded reverse transcriptases (RTs) has revealed that the IIC introns are the most basal lineage of group II introns, indicating that they are most similar to the primordial group II intron [11]. Furthermore, the secondary structure of IIC introns is more simplified than that of IIA and IIB introns.

Group IIC introns are particularly attractive targets for crystallization because of their small size and apparent structural stability (Figure 2). Attempts to crystallize the larger IIA and IIB introns have not yet been successful. The first group II intron to be successfully crystallized and studied using x-ray diffraction methods was a IIC intron from the halophilic and alkaliphilic eubacterium *Oceanobacillus iheyensis* (*O. iheyensis*) [12], which is an extremophilic organism isolated from seabed mud at a depth of 1 km off the coast of Japan [13].

The intron was purified in a native state immediately after it had gone through both steps of splicing, thereby ensuring that the intron RNA was homogeneously folded and represented a catalytically active form of the molecule. This type of procedure is unusual, as free RNAs are typically subjected to structural characterization after they have been purified on denaturing polyacrylamide gels. It is notable, however, that the tRNA samples used for early crystallographic studies of RNA were isolated directly from natural sources, in the folded state. Thus, the group II intron purification represents a “back-to-basics” approach for obtaining folded RNA.

Like other group IIC introns, the *O. iheyensis* intron catalyzes self-splicing *in vitro* by a mechanism that differs from that of the well-studied IIA and IIB introns [10,14]. The nucleophile during the first step of group IIC splicing is a water molecule, whereas group IIA and B introns utilize the 2'-OH of a bulged adenosine in domain VI. As a result, group IIC introns are released as linear molecules, while group IIA and B introns are usually released as a branched lariat species (hydrolytic splicing is an important alternative pathway for splicing by IIB introns [15]). Group IIC introns may require interactions with their encoded maturase in order to activate domain VI for participation in splicing, as lariat reaction products are observed for IIC intron splicing in the presence of this cognate protein [16]. All known IIC introns have a conserved adenosine in domain VI [9] suggesting that lariat formation is the dominant pathway *in vivo*. These observations underscore the importance future of crystallographic investigations on the group II intron holoenzyme, which contains both RNA and protein components.

Tertiary architecture of the group II intron

The crystal structure of the *O. iheyensis* intron reveals an elaborate topology and tertiary interaction network that is fully consistent with decades of biochemical and genetic studies on

the aI5 γ group IIB intron from *Saccharomyces cerevisiae* [4], the *Pylaiella littoralis* group IIB intron [17] and the *Lactococcus lactis* group IIA intron [18,19]. Although interactions such as β - β' [5] and μ - μ' [20] are absent in IIC introns, and a few interactions such as ω - ω' [12] and a modified ζ - ζ' interaction [21] are present only in IIC introns, almost every other important structural element is shared among the classes. The close parity between the crystal structure and prior work on distantly related classes of introns suggests that core architectural features of the *O. iheyensis* intron are characteristic of group II introns in general and that the various intron families share a common tertiary structure.

Consistent with numerous studies of group II intron folding [4], Domain I forms a hollow scaffold that dictates the overall intron shape and provides a series of receptors for the binding of other intron domains. Several key interactions play a major role in establishing the architectural fold of DI: of central importance is the five-way junction, which collapses into a compact and elaborate tertiary structural element composed of interdigitated loops and helices. This novel substructure anchors the five structural helices of DI and projects them into orientations that permit additional tertiary interactions among the DI helices and with other core elements. The most notable of these tertiary interactions is the z-anchor, which welds DI helices IC and I(ii) in a side-by-side arrangement through an RNA interaction which is reminiscent of the J8/7 region in group I introns [22]. Nucleotides of the z-anchor motif represent an “inside-out” form of RNA, in which the phosphate backbone is in the center and the bases are splayed out in an alternating pattern that forms interactions with two adjacent strands on either strand. The architectural form of this motif is reminiscent of “Pauling DNA”, in which Linus Pauling proposed that DNA might form multi-stranded structures in which phosphates were in the center, rather than on the outside of the array.

Docked within the large cavity created by domain I, DV is the central core of the intron (Figure 3). It is largely shielded from solvent, as anticipated from hydroxyl radical footprinting studies on the IIB intron aI5 γ [23]. A network of tertiary interactions anchor DV and rigidify the core, including the ζ - ζ' interaction that orients the terminal loop region of DV [12]. While ζ - ζ' is a typical tetraloop-receptor motif in Group IIA and B introns [9,24], in the group IIC intron, it adopts an entirely novel architecture that is dominated by a single base-stack between the tetraloop and its receptor interface [21]. The bulge and lower helix of DV, which contain the catalytic groups, are docked with a network of interactions involving the z-anchor motif and κ - κ' . At this location within the intron, several highly conserved structural elements converge to form a cohesive unit that supports splicing. For example, nucleotides involving the DV bulge and the junction between domains II and III, called J2/3, form a triple helix with three conserved base pairs of DV (the region known as the DV “catalytic triad”, Fig. 1). In addition, G5 at the 5' end of the intron stacks with DV bulge base A376. Like most of the base-stacks observed in this structure, the existence of these interactions is confirmed by photochemical crosslinking data [19,25].

The only solvent-exposed portion of DV is the region where DVI is anticipated to lie adjacent. Crosslinking experiments have shown that DVI should lie parallel, approximately side-by-side with DV [26]. But in the *O. iheyensis* intron structure, density for DVI is lacking. This instability of DVI is not anticipated for aI5 γ and other group II introns that undergo splicing through lariat formation, and it is not anticipated for the *O. iheyensis* intron when it is bound to its cognate maturase.

Metal ions in the active sites in the group I and II introns

Previous mutagenesis and chemical interference data has strongly suggested that the active site of group II introns is composed of the bulge and catalytic triad of DV [27-30], and both of these regions have been implicated in the binding of catalytic metal ions [31-33]. The crystal

structure reveals that the triad and bulge are proximal and that they jointly coordinate two divalent metal ions that are separated by a distance of $\sim 4\text{\AA}$ (Figure 4A). This type of metal ion arrangement is common in the active sites of proteins that make or break phosphodiester linkages, such as DNA and RNA polymerases [34] and is consistent with previous biochemical data for the $\alpha 5\gamma$ intron [35]. Divalent metal ions in the group II core coordinate directly to the scissile phosphate of nucleic acid fragments that mimic ligated exons [36], suggesting that these metals are involved in the catalytic mechanism. The positions of the metals relative to the exon junction allow them to be tentatively assigned as M1 and M2, using the spatial designation established for group I introns and other enzymes [37]. Importantly, there are additional divalent metal ions within the core, including a magnesium ion that binds to the C289 residue of J2/3 and the bulge nucleotides [36]. This ion, like the one observed in the exon-binding platform [21], helps link active site moieties together and may play an important role in modulating the electrostatic environment of the core [38].

Despite the interesting locations of these metals, it is important to interpret their significance conservatively, as the group II intron structure does not reflect the approach to the transition-state; rather, it represents the post-catalytic state of the intron that follows exon ligation and precedes exon release. Many more structural states have been obtained for group I introns [22,37,39-42], and in that case it is easier to interpret the role of metal ions in the reaction. For example, the crystal structure of a group I intron from *Azoarcus* has revealed the existence of two divalent catalytic metal ions in the core of the ribozyme [37]. This construct was crystallized in a state immediately preceding the second step of splicing [22], and it reflects the approach to the transition-state with an attacking nucleophile. In this case, the distance between the two metal ions is $4\text{\AA}$ and these are coordinated to the scissile phosphate and the attacking nucleophile. Subsequently, this same group I intron was also crystallized with a ligated exon substrate [42], in a construct analogous to that studied for the group II intron [36]. In this case, the group I intron crystals contains molecules in two states immediately preceding and following the second step of splicing. In the “post-second step” state, the active site adopts a more relaxed structural conformation in which the location of the scissile phosphate has shifted away from the catalytic metal ions. This finding underscores the potential problems with determining chemical mechanism from a ribozyme bound to product in the ground state: the active site may have undergone some reorganization. That said, a comparison of the group I and II active sites in coordination with ligated exons reveals some strong similarities (Fig. 4), and suggests an interesting example of convergent evolution.

The catalytic mechanism of the spliceosome

U6 snRNA of the spliceosome is structurally similar to DV [8] and its bulge region has been shown to contain a metal binding site that is critical for splicing activity [43]. Mutagenesis studies have underscored the importance of both the U6 triad [44] and bulge [45,46] for catalysis. These facts, combined with the obvious structural and sequence similarities, support the notion that the spliceosome is a ribozyme and that it uses a mechanism similar to that of group II introns. However, recent crystallographic evidence has raised some questions as to whether the active site is composed only of RNA, protein, or both. Three research groups simultaneously determined the crystal structure of the core domain of the Prp8 protein from human and yeast [47-49]. Prp8 is a spliceosomal protein that is consistently found within the active site of the spliceosome [50]. Structural analysis shows that the Prp8 core domain contains an RNase H-like fold, with a binding pocket containing conserved aspartate residues that are potentially important for metal binding. However, the structures do not reveal any bound metal ions at these positions and it is possible that the protein only serves to bind RNA in a structural role. Further experiments should answer these questions. However, one should be open to the possibility that the spliceosome might utilize both RNA and protein components in the splicing mechanism. One scenario could be that there are two active sites in close proximity to each

other with one composed of RNA and one composed of protein. Each active site could be responsible for the different steps of splicing.

There is ample precedent for a protein enzyme taking over a reaction that was previously the exclusive role of a ribozyme. This has occurred in the case of RNase P in human mitochondria. RNase P has long been considered to be a ribozyme with a catalytic RNA component bound to protein cofactors. However, in human mitochondria, RNase P is now known to be a complex of three proteins, with no RNA component [51]. This complex readily catalyzes the cleavage of precursor-tRNA to form the mature form.

Conclusions

Recent crystal structures of self-splicing introns have revealed an astonishing diversity of RNA tertiary architectural form. Structures of the group I and group II introns suggest that two-metal-ion mechanisms may be ubiquitous for intron splicing. However, much remains to be understood, especially in terms of conformational changes that occur during catalysis by group II introns and the architecture of the spliceosome. In addition, the group II intron structure is of the linear form and therefore does not reveal how the 2'-OH of the bulged adenosine is activated to form the 2'-5' lariat bond in the first step of splicing. Nuclear introns in eukaryotes also form this type of circular RNA in a reaction catalyzed by the spliceosome.

As yet, there are no high resolution structures of large spliceosomal components such as the snRNPs (small nuclear ribonucleoproteins). Therefore, the molecular details of splice site recognition and catalysis by this macromolecular machine remain obscure. Hopefully, additional structural studies will address these issues and provide more information on the evolutionary relationships among splicing systems.

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Figure 1.

A comparison of the secondary structures of the U6 snRNA from the spliceosome (left) and domain V from the *O. iheyensis* group II intron (right). The catalytic triad (boxed) is located at the base of the stem loop structures, with the two-nucleotide bulge spaced five base pairs away. The ACAGGA box (red) may form base triple interactions with the triad of the U6 snRNA, analogous to that observed between J2/3 and domain V.

Figure 2. RNA secondary structures of the IIA, IIB, and IIC intron classes. The IIC class is smaller and more compact, especially in domain I. Tertiary interactions are indicated with Greek letters and domains with Roman numerals.

Figure 3.
Overall tertiary structure of the group II intron. Domain V (red) forms the catalytic core with the rest of the domains encapsulating this active site.

Figure 4.

Comparison of the group I and group II active sites with bound exons. (a) In the group II intron, putatively catalytic metal ions (M1 and M2) are within inner shell coordination distance of the scissile phosphate at the exon junction. Note that other metal ions, omitted for clarity, have also been identified within the intron core (reference). (b) The group I structure represents a fully active intron with ~70% of the pre-ligated form (transparent) and ~30% of the ligated (solid). The scissile phosphate of the ligated exons is not within coordination distance (~4.5-6 Å) of the metal ions. In the pre-ligated state, the distance changes to ~2.5 Å, which is appropriate for coordination. The group II structure is analogous to the ligated, product form of the group I intron.