unclear how this interaction contributes to activation. While p300/CBP and PCAF could activate HIV-1 transcription by acetylating nucleosomal histones, they also acetylate Tat [7, 8]. Interestingly, p300/CBP and PCAF appear to contribute to HIV-1 activation via alternative mechanisms by acetylating distinct residues of Tat. Acetylation of Lys28 by PCAF has been shown to stimulate interaction of Tat with P-TEFb, whereas acetylation of Lys50 by p300/CBP leads to dissociation of Tat from TAR.

Now, in the March issue of *Molecular Cell*, Mujtaba et al. [9] explain how acetylation of Lys50 of Tat contributes to HIV-1 activation at the structural level. They demonstrate that the bromodomain of PCAF recognizes Lys50-acetylated Tat, but not Lys28-acetylated Tat. In contrast, the bromodomain of CBP recognizes neither Lys28- nor Lys50-acetylated Tat, suggesting that Lys50-acetylated Tat by p300/CBP is recognized by the bromodomain of PCAF (see Figure, panel D). Moreover, they determined the NMR structure of the PCAF bromodomain bound to the Lys50-acetylated Tat peptide. Interestingly, the peptide induces local conformational alterations around its binding sites on the PCAF bromodomain, producing a hydrophobic cavity that accommodates the Lys50-acetylated Tat peptide. Moreover, TAR RNA does not significantly affect the conformational alterations of the PCAF bromodomain induced by the acetylated Tat, indicating that the affinity of Lys50-acetylated Tat with the PCAF bromodomain is much higher than that with TAR RNA. These data support the previous model that Lys50 acetylation of Tat leads to dissociation of Tat from TAR RNA, contributing to stimulation of transcriptional elongation of HIV-1 [7, 8].

This work has significantly advanced our knowledge of how acetylation contributes to gene activation. Nevertheless, an additional issue must be addressed in the near future. Jones and colleagues demonstrated that binding of cyclin T1 (a subunit of P-TAFb) to Tat significantly increases the affinity and specificity of the interaction between Tat and TAR RNA: the Tat/cyclin T1 complex recognizes sequences in the loop of TAR RNA, while Tat alone does not [10]. Therefore, it is important to demonstrate whether the PCAF bromodomain dissociates Tat from the Tat-P/TAFb complex bound to TAR RNA.

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A Glimpse of the Catalytic Core of a Group II Intron

A paper in a recent issue of *Science* describes the first high-resolution structure of part of the catalytic core of a group II intron that will allow more detailed comparisons between the excision of introns by self-splicing group II introns and by nuclear pre-mRNA introns.

The processes by which self-splicing group II introns and nuclear pre-mRNA introns are excised have long been compared and contrasted. The former occurs by an RNA-only mechanism, and the latter within a large RNP assemblage, the spliceosome. Yet both systems share strikingly similar structural features within their cores. So far, however, no high-resolution structural information has existed. Now, a new structure of core components of a group II intron and new evidence of bound metal ions will allow for more detailed future comparison [1–4].

Group II introns are found in eubacteria and in the organellar genomes of fungi and plants. The best characterized of these, ai5γ, excises itself from pre-mRNA by several related RNA-catalyzed pathways: two *cis* pathways, branching or hydrolysis, and a newly recognized *trans* pathway. The branching pathway is chemically identical to the excision of nuclear pre-mRNA introns by the U2- and U12-dependent spliceosomes, involving two sequential transesterification reactions (Figure 1A, pathway 1): (i) nucleophilic attack by an adenosine 2′OH within the intron (the branch site) at the 5′ splice site phosphate, forming a free exon 1 intermediate and a lariat intron, followed by (ii) attack of the free exon 1 3′OH at the 3′ splice site, resulting in exon-exon ligation and release of the intron. The hydrolysis pathway uses water or hydroxide as the first step nucleophile, followed by a second step chemically identi-

cal to that of the branching pathway, resulting in release of a linear intron along with exon ligation (Figure 1A, pathway 2). Recently, group II introns have been reported also to excise as circles, by a more complex *trans* pathway [5]. All three pathways have been observed both in vitro and in vivo. Both branching and hydrolytic pathways have been extensively characterized biochemically. Although these pathways are distinct, mutations within the core (in particular D5) have similar effects on both.

Typically, group II introns consist of six conserved domains of secondary structure, D1–D6 (Figure 1A), of which D1 and D5 are necessary for activity. Much work argues that D5 is a component of the catalytic core, binding both to D1 and to D6. D5 contains the most conserved sequences of the intron. Because of the parallel chemistry with nuclear pre-mRNA splicing, D5 has been extensively compared with core components of the snRNAs, in particular U2/U6 helix Ib and the adjacent intramolecular U6 helix [6].

A New General Strategy for Designing Crystallizable RNAs

Most promising for the future of RNA structural biology, Doudna and coworkers [1] have taken advantage of a specific RNA-RNA interaction module in order to design derivatives of interesting RNAs, which are then much more amenable to crystallization than the natural sequences. By placing a GAAA tetraloop and its 11 nt tetraloop receptor [7] at differing sites within RNAs, they have created potential intermolecular interactions that generate a lattice-like network (i.e., “crystal contacts”). This approach is reminiscent of using a well-characterized RNA binding protein (e.g., U1A protein), and engineering into the RNA of interest a site for its binding to help induce crystal formation. But the new approach may be much more advantageous (and perhaps more aesthetically pleasing), as it avoids the requirement of proteins at all and establishes the major crystal contacts by design. The authors have already shown that this approach yielded numerous crystal forms of constructs representing group II introns and hepatitis delta virus ribozyme [8]. In the case of group II domains 5 and 6 (Figure 1B), this was particularly facile, as D5 contained a natural GAAA tetraloop, and the receptor was taken from elsewhere in the group II intron; but many different tetraloop/tetraloop receptors are available [9]. Judicial placement of these crystallization modules—along with careful biochemical testing of the effects of such placement—will undoubtedly contribute significantly to the diversity of RNA structures in the near future.

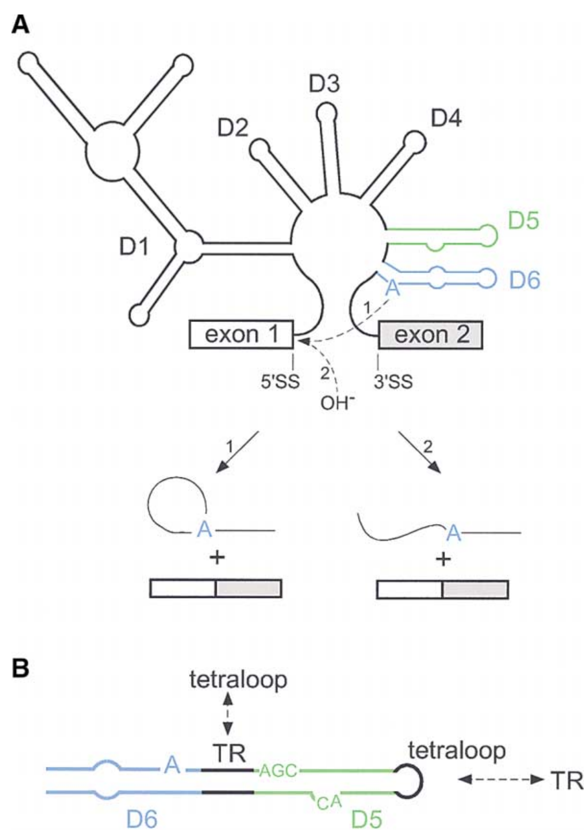

Figure 1. Group II Intron Secondary Structure, Catalytic Reactions, and Crystallization Construct

(A) Schematic representation of the ai5γ group II intron from yeast mitochondrion. The six domains are labeled D1–D6. D5 (green) is an essential component of the catalytic core; D6 (blue) contains the branch site adenosine. Exons are represented by boxes. Dotted lines and the arrowhead represent different pathways leading to 5′ exon cleavage: (1) branching, in which the 2′OH of the adenosine shown in D6 is the nucleophile, and (2) hydrolysis, initiated by water or hydroxide; a third pathway, not shown, leads to circle formation. (B) Crystallization construct of [1], in which a tetraloop receptor (TR) specific for the D5 tetraloop has been placed at the base of D5, and extended coaxially with the D6 helix. Binding of the TR to the tetraloop of a different molecule establishes designer crystallization contacts.

The Structure of Domain 5

Two of the most interesting sequence features of D5 are its AGC triad that is essential for catalysis ([10] and references therein) and its bulged AC dinucleotide. In the structure D5 is an A form continuous coaxial helix, and the bulged AC is accommodated by extrahelical base flipping, rather than helical intercalation (see [11] for other recent examples of base flipping.) The flipping of this bulged dinucleotide is especially relevant, as it provides part of a platform for metal ion binding. Such bound metal ions are required for both folding and catalysis. Moreover, D5 was originally proposed to serve as a metal binding platform as part of a two-metal ion mechanism of splicing catalysis, applicable to both group II introns and the spliceosome [12]. Indeed, metal ion coordination with the leaving group is required for both steps of splicing, in both systems [13, 14]. Thus, defining the structures that bind to and position metal ions will lead to further understanding of the active sites of these enzymes and possibly clues as to their evolutionary relationships.

Three main sites of metal ion binding in D5 have been observed, collectively by metal ion cleavage experiments [2], thiophillic metal rescue of phosphorothioate replacements [3], and within the crystal structure [1]: (i) at the AC bulge, observed in the crystal and by metal cleavage experiments, but not rescuable by metal re-

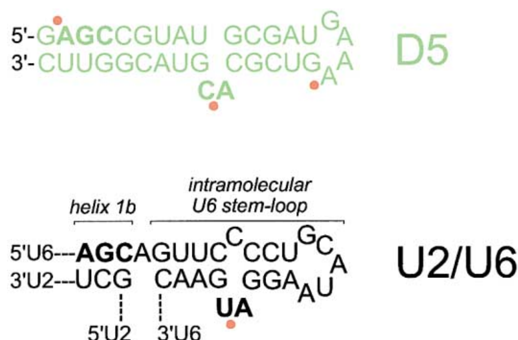

Figure 2. Comparison of D5 and U2/U6 snRNAs

The highly conserved AGC triads and the dinucleotide bulges are set in bold. Red circles represent binding sites of divalent metal ions, demonstrated by metal cleavage, thiophillic metal replacement experiments, and/or crystallography [1–4]. Similar secondary structure is also formed by U12/U6atac snRNAs in the U12-dependent minor spliceosome (not shown).

placement experiments; (ii) at the AGC triad, observed by thiophillic metal rescue but not in the crystal, implying that binding of the metal may be weak, at least in the absence of other intron components; and (iii) at the tetraloop, detected both by cleavage experiments and in the crystal. One of these, the metal ion at the AC bulge, contributes significantly to comparison with the U2/U6 snRNA structure of the spliceosome (Figure 2). The U2/U6 interaction forms a secondary structure similar to D5, with a similarly placed AGC triad and bulged AU dinucleotide. Recent metal ion replacement experiments demonstrated that metal coordination at this AU bulge was required for spliceosome function [4]. Thus, both D5 and U2/U6 structures coordinate essential metal ions at a bulged dinucleotide, similarly spaced from essential AGC triads. While further investigation will be needed to determine whether these two metal binding domains play analogous functional roles during splicing, collectively these data argue more strongly that these two structures play parallel roles in metal ion binding within the cores of group II introns and the spliceosome.

The Arrangement of the Branch Site in Domain 6

Three elements of the D6 branch site structure are unexpected and therefore provocative: a double-nucleotide bulge, base flipping of these same two nucleotides, and metal ion binding. While biochemical experiments favor a side-by-side arrangement of D5 and D6 for catalysis [15], by its design, the crystallized D56 molecule is arranged as coaxially stacked helices; thus, these contacts were not observed. (Something to anticipate in the future!) Most striking, however, is the observation that the branch site region of D6 contains two adjacent bulged bases, whereas traditional secondary structure schemes have modeled this region as a single bulged adenosine. Adjacent to these nucleotides is another bound metal, which also correlates with the metal cleavage experiments [2]. The role of this metal ion and the contribution of the double bulge are not yet clear, but these observations will undoubtedly stimulate new experiments. Because the crystallized RNA participates in the hydrolysis reaction exclusively, the functional significance of the double-bulged structure relative to branching is unclear. It is possible that this structure also reflects that of RNAs that participate in branching; alternatively, this structure may represent the conformation active for hydrolysis and be distinct from structures active in branching. Either way, future comparisons with structures active for branching and with spliceosomal constituents will be fascinating and enlightening.

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