RNA (2000), *6*:199–205. Cambridge University Press. Printed in the USA.

Metal ion catalysis during the exon-ligation step of nuclear pre-mRNA splicing: Extending the parallels between the spliceosome and group II introns

PETER M. GORDON,[1] ERIK J. SONTHEIMER,[1,3] and JOSEPH A. PICCIRILLI[1,2]

[1]Department of Biochemistry and Molecular Biology, Howard Hughes Medical Institute, The University of Chicago, Chicago, Illinois 60637, USA
[2]Department of Chemistry, Howard Hughes Medical Institute, The University of Chicago, Chicago, Illinois 60637, USA

ABSTRACT

Mechanistic analyses of nuclear pre-mRNA splicing by the spliceosome and group II intron self-splicing provide insight into both the catalytic strategies of splicing and the evolutionary relationships between the different splicing systems. We previously showed that 3′-sulfur substitution at the 3′ splice site of a nuclear pre-mRNA has no effect on splicing. We now report that 3′-sulfur substitution at the 3′ splice site of a nuclear pre-mRNA causes a switch in metal specificity when the second step of splicing is monitored using a bimolecular exon-ligation assay. This suggests that the spliceosome uses a catalytic metal ion to stabilize the 3′-oxyanion leaving group during the second step of splicing, as shown previously for the first step. The lack of a metal-specificity switch under *cis* splicing conditions indicates that a rate-limiting conformational change between the two steps of splicing may mask the subsequent chemical step and the metal-specificity switch. As the group II intron, a true ribozyme, uses identical catalytic strategies for splicing, our results strengthen the argument that the spliceosome is an RNA catalyst that shares a common molecular ancestor with group II introns.

Keywords: evolution; metal-specificity switch; multipartite assay; ribozyme; sulfur substitution

INTRODUCTION

The removal of introns from pre-mRNA is an essential step in eukaryotic gene expression that is predominantly catalyzed by the spliceosome, a large ribonucleoprotein complex composed of five small nuclear RNAs (snRNAs) and a multitude of proteins. Many of these factors and the dynamic processes of assembly and disassembly that give rise to the spliceosome have been identified and characterized (Burge et al., 1999), but very little is known about the actual catalytic mechanisms employed by the spliceosome (Moore & Sharp, 1993; Sontheimer et al., 1997). Genetic and biochemical data indicate that three of the snRNAs (U2, U5, and U6) are in close proximity to the splice sites during reaction (Nilsen, 1998; Yu et al., 1999), but it is still unclear whether the snRNAs play a direct role in catalysis. Further intrigue surrounding this possibility arose from the discovery of another class of introns, the group II introns (as reviewed by Pyle, 1996). Group II introns are spliced by the same chemical pathway as the nuclear pre-mRNA introns, but do not require the spliceosome or any other protein factor for their removal, because the intron is an RNA catalyst that excises itself in an autocatalytic self-splicing event. The finding that a true ribozyme catalyzes intron excision by the same reaction pathway as the spliceosome and the discovery of additional structural and mechanistic similarities between the two systems have fueled speculation that they evolved from a common molecular ancestor and that the spliceosome is, at heart, an RNA catalyst (Fig. 1; Sharp, 1985; Cech, 1986).

To explore further the possible relationship between these two splicing systems, we previously examined the catalytic mechanisms of the group II intron and the spliceosome by monitoring the effects of 3′-sulfur substitution at the splice sites (Sontheimer et al., 1997,

Reprint requests to: Joseph A. Piccirilli, Department of Biochemistry and Molecular Biology, Howard Hughes Medical Institute, The University of Chicago, 5841 South Maryland Avenue, MC1028, Chicago, Illinois 60637, USA; e-mail: jpicciri@midway.uchicago.edu.
[3]Present address: Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, 4-120 Hogan Hall, 2153 North Campus Drive, Evanston, Illinois 60208-3500, USA.

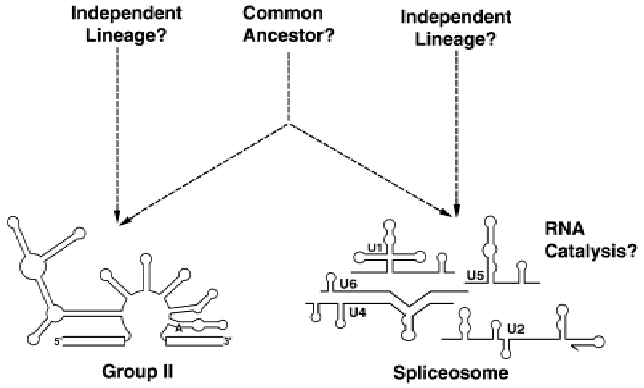

FIGURE 1. Did the spliceosome and group II intron evolve from a common ancestor or from independent lineages? Biochemical similarities between the two splicing machineries include identical reaction pathways, stereochemical requirements (Moore & Sharp, 1993; Padgett et al., 1994), asymmetric responses to 2′-deoxy substitution at the splice sites (Moore & Sharp, 1992; P.M. Gordon, E.J. Sontheimer, J.A. Piccirilli, in prep.), and catalytic mechanisms (Sontheimer et al., 1997, 1999). Additionally, certain elements of secondary and tertiary structure appear somewhat similar (Michel & Ferat, 1995; Nilsen, 1998). However, a lack of sequence similarity and other differences weaken the argument for a common molecular ancestor (Weiner, 1993; Michel & Ferat, 1995) and render the issue controversial. The schematic representations show the group II intron (left), which is derived from the ai5γ intron in the cytochrome oxidase gene of *Saccharomyces cerevisiae*, and the snRNA components of the spliceosome (right).

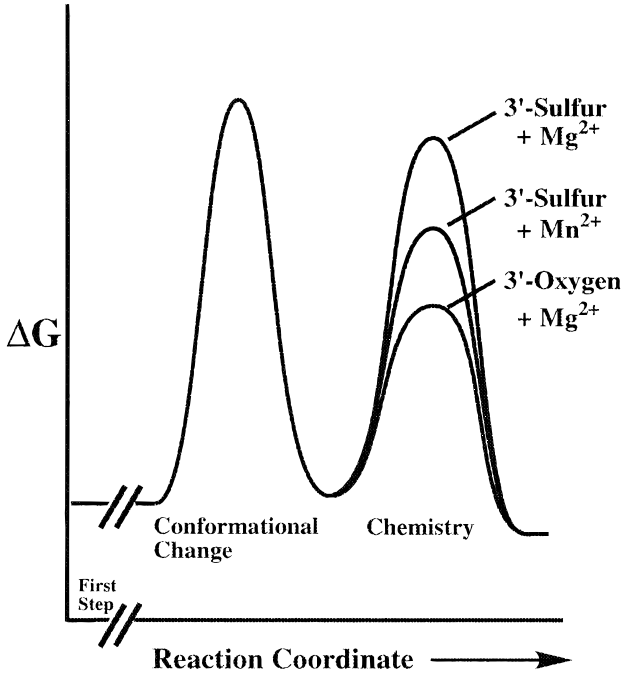

FIGURE 2. A hypothetical free energy diagram illustrating how a metal-specificity switch could be masked during the exon-ligation step of spliceosomal pre-mRNA splicing. The extensive conformational changes that occur within the spliceosome between the two chemical steps of splicing may be rate-limiting rather than the actual chemical step of exon ligation (Umen & Guthrie, 1995; Chua & Reed, 1999). Although a metal ion-leaving group interaction may be important for catalysis (3′-Oxygen + Mg^{2+}), a metal-specificity switch would be masked if disruption of the stabilizing interaction (3′-Sulfur + Mg^{2+}) does not render the chemical step rate-limiting.

1999). For *cis* splicing by the spliceosome, 3′-sulfur substitution at the 5′ splice site shifted the metal ion specificity of splicing from Mg^{2+} to Mn^{2+}, indicating that a metal ion stabilizes the leaving group in the first step of splicing. In contrast, 3′-sulfur substitution at the 3′ splice site had no effect on the metal ion specificity, raising the possibility that the spliceosome does not employ a metal ion to stabilize the 3′-oxyanion leaving group in the second step of splicing. We also obtained precisely the same results for the group II intron: a metal-specificity switch upon 3′-sulfur substitution at the 5′ splice site, indicating metal ion catalysis of the first step of splicing, but no effect from sulfur substitution at the 3′ splice site.

The finding that both the spliceosome and group II intron employ a metal ion to stabilize the leaving group in the first step of splicing bolsters the case for an evolutionary relationship between the two systems (Sontheimer et al., 1997, 1999). However, the similar lack of response by both systems to 3′-sulfur substitution at the 3′ splice site may not necessarily reflect common strategies for catalysis of exon ligation. For example, if the conformational rearrangements that occur within the spliceosome (Umen & Guthrie, 1995; Chua & Reed, 1999) and group II ribozymes (Chanfreau & Jacquier, 1996) are rate limiting rather than the actual chemical step (Fig. 2), then the true effect of sulfur substitution on the exon-ligation step of splicing would be masked. This appears to be the case for the group II intron, because when we isolated the exon-ligation step using a tripartite assay, in which a 3′ splice site oligonucleotide is added in *trans*, a metal-specificity switch was uncovered (Sontheimer et al., 1999). This result underscored the importance of monitoring the chemical step during metal-specificity switch experiments, and demonstrated the possibility that rate-limiting conformational changes also may have masked a metal-specificity switch in our previous experiments on the exon-ligation step of nuclear pre-mRNA splicing by the spliceosome. In this report, we utilize an analogous multipartite assay that monitors the exon-ligation step of nuclear pre-mRNA splicing (Anderson & Moore, 1997) to unmask another metal ion that functions during catalysis by the spliceosome and to extend the parallels with group II introns.

RESULTS AND DISCUSSION

Substitution of the 3′-oxygen leaving group of a scissile phosphodiester with sulfur at splice sites is an effective method for identifying metal ion-leaving group interactions (Piccirilli et al., 1993; Curley et al., 1997; Sontheimer et al., 1997, 1999; Weinstein et al., 1997; Sontheimer, 1999). Divalent metal ions exhibit preferences in coordinating inner-sphere ligands (Sigel et al.,

1997). "Hard" metals such as Mg^{2+} bind with greater affinity to oxygen than sulfur ligands. On the other hand, "softer" metals such as Mn^{2+}, Co^{2+}, or Zn^{2+} accept, and sometimes prefer, sulfur ligands. Accordingly, changes in divalent metal ion specificity that accompany substitution of sulfur for oxygen can be indicative of direct coordination between the metal ion and the substituted atom.

To probe the second step of nuclear pre-mRNA splicing, we used a bimolecular exon-ligation assay, which divides the full-length splicing substrate into two fragments (Fig. 3A; Anderson & Moore, 1997). The 5′ RNA substrate (214 nt) is derived from the adenovirus (AdML) substrate and contains an exon (86 nt), consensus 5′ splice site, branch site, and the 28-nt polypyrimidine tract. Both 3′ RNA substrates used in this report contain the last 5 nt of the intron (GACAG), but have different 3′ exons. The AdML(as) exon (56 nt) is the exact antisense of the AdML exon (Anderson & Moore, 1997) whereas the TNT exon (30 nt) contains splicing enhancer elements that interact with SR proteins and facilitate spliceosome assembly (Ramchatesingh et al., 1995). Incubation of the 5′ RNA substrate under splicing conditions results in cleavage at the 5′ splice site and generation of the lariat intermediate. Subsequent addition of a 3′ RNA substrate results in 3′ splice site cleavage and exon ligation.

To test for leaving group-divalent metal ion interactions during the exon-ligation step of splicing, we used chemical synthesis (Sun et al., 1997) and enzymatic ligation (Moore & Query, 1998) to introduce 3′-thioinosine (AdML(as).I_s; the subscript $_s$ denotes a 3′-*S*-phosphorothiolate linkage, and I corresponds to inosine) at the 3′ splice site. We also prepared ligated control substrates containing guanosine (AdML(as).G) and inosine (AdML(as).I) at the 3′ splice site. Site-specific substitution of inosine for guanosine at the 3′ splice site does not affect the rate or accuracy of pre-mRNA splicing (Tarn, 1996), but does significantly simplify the chemical synthesis of the 3′-*S*-phosphoramidite (Sun et al., 1997).

We tested the three substrates for in vitro splicing in EDTA-pretreated HeLa nuclear extract (Fig. 3A). As expected, with AdML(as).G and AdML(as).I spliced product was readily detectable in 2.0 mM Mg^{2+} or 1.5 mM Mn^{2+} (Fig. 3B, lanes 3, 4, 8, and 9). Co^{2+} (1.5 mM) was unable to support splicing of any substrate tested (Fig. 3B, lanes 5, 10, and 15). In striking contrast to the results obtained under *cis* splicing conditions (Sontheimer et al., 1997), however, 2.0 mM Mg^{2+} was unable to support splicing of the AdML(as).I_s substrate, which contains a 3′-*S*-phosphorothiolate linkage at the cleavage site (Fig. 3B, lane 13). Splicing and ligation were restored in the presence of 1.5 mM Mn^{2+}, a more thiophilic metal than Mg^{2+} (Fig. 3B, lane 14). Sequencing of AdML(as).I_s spliced product demonstrated that the correct 3′ splice site was used in the presence of Mn^{2+} (data not shown). Therefore, the bimolecular exon-ligation assay reveals a metal-specificity switch that is obscured during *cis* splicing.

To ensure that the inhibition of AdML(as).I_s splicing in Mg^{2+} resulted specifically from the 3′-sulfur substitution, we repeated the experiment in the presence of an unmodified and unlabeled TNT 3′ substrate as an internal positive control. In agreement with the results of Figure 3B, HeLa nuclear extract and 2.0 mM Mg^{2+} supported the ligation of the AdML(as).G and AdML(as).I substrates, but not the AdML(as).I_s substrate (Fig. 3C, lanes 3, 7, and 11). The efficiency of splicing with AdML(as).G and AdML(as).I was not influenced appreciably by the addition of an equal amount of TNT substrate (Fig. 3C, lanes 4 and 8). Half of each reaction in Figure 3C was used to assay for TNT spliced product by RT-PCR. Splicing of the TNT substrate was HeLa nuclear extract dependent (Fig. 3D, lanes 2, 4, 7, and 10) and occurred both in the absence (Fig. 3D, lane 3) and presence of the control AdML(as) substrates (Fig. 3D, lanes 6 and 9). Splicing of the TNT substrate was detected in the presence of the modified AdML(as).I_s substrate that did not splice in Mg^{2+} (Fig. 3C,D, lanes 12), indicating that the extract sample used in this reaction was active, and that the inability of the AdML(as).I_s substrate to splice in Mg^{2+} was due to 3′-sulfur substitution.

CONCLUSIONS AND IMPLICATIONS

We previously showed that for the exon-ligation step of self-splicing by the group II intron a metal-specificity switch does not occur during *cis* splicing, but does occur if the 3′ splice site oligonucleotide is supplied in *trans* (Sontheimer et al., 1999). This observation and the possibility that the spliceosome and group II intron may have evolved from a common ancestor led us to explore further the effect of 3′-sulfur substitution on the exon-ligation step of nuclear pre-mRNA splicing. In contrast to the previous results observed during *cis* splicing (Sontheimer et al., 1997), a metal-specificity switch is unmasked during nuclear pre-mRNA splicing when a modified 3′ splice site oligonucleotide is supplied in *trans* to spliceosomes containing lariat intron and 5′ exon. In analogous experiments with the group II intron, 3′-sulfur substitution caused an ~100-fold reduction in the rate of Mg^{2+}-mediated exon ligation, yet had no effect on the rate of *cis* splicing (Sontheimer et al., 1999). Presumably a conformational change that is insensitive to 3′-sulfur substitution at the 3′ splice site limits the rate of exon ligation during *cis* splicing, but when the 3′ splice site oligonucleotide is supplied in *trans*, a step that is sensitive to 3′-sulfur substitution influences the overall reaction rate. Because the bimolecular assays are initiated after spliceosome assembly and 5′ splice site cleavage/lariat formation and because the concentration of 3′ splice site substrate

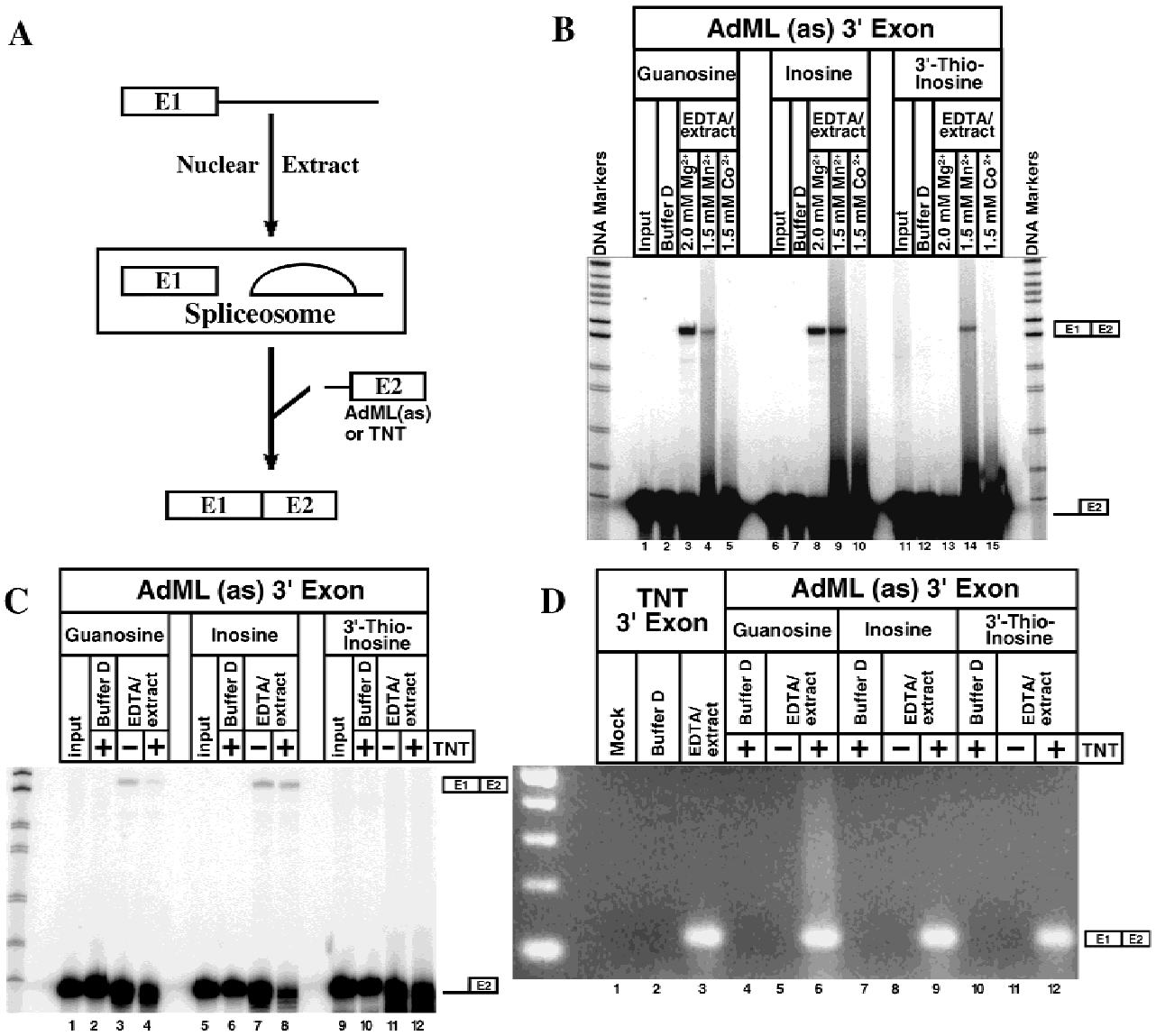

FIGURE 3. **A**: A schematic representation of the bimolecular exon-ligation assay used to isolate the second step of nuclear pre-mRNA splicing by the spliceosome. A transcript corresponding to the 5′ exon and the intron truncated after the polypyrimidine tract are incubated under splicing conditions and allowed to undergo the first step of splicing before the addition of the 3′ splice site substrate, which consists of the last 5 nt of the intron and either the AdML(as) or TNT exon. **B**: 3′-sulfur substitution at the 3′ splice site of the AdML(as) pre-mRNA results in a metal-specificity switch when the exon-ligation step of splicing is isolated. The 5′ substrate was preincubated under splicing conditions for 35 min before the addition of radiolabeled AdML(as).G (lanes 1–5), AdML(as).I (lanes 6–10), or AdML(as).I_S (lanes 11–15). Splicing was then allowed to proceed for an additional 20 min. Lanes 1, 6, and 11 are unspliced RNAs. In lanes 2, 7, and 12, buffer replaced nuclear extract. Reactions contained 2 mM $MgCl_2$ (lanes 3, 8, and 13), 1.5 mM $MnCl_2$ (lanes 4, 9, and 14), or 1.5 mM $CoCl_2$ (lanes 5, 10, and 15) as indicated at the top of each lane. Substrate and spliced product are indicated on the right of the gel. **C**,**D**: The TNT substrate is spliced in the same reaction in which splicing of AdML(as).I_S is inhibited. The 5′ substrate was preincubated under splicing conditions in the presence of 2 mM $MgCl_2$ for 35 min before the addition of radiolabeled AdML(as) substrates, unlabeled TNT splicing substrate, or both substrates. Each reaction was divided after a 30-min incubation. To detect spliced AdML(as) product, one half of each reaction was fractionated by denaturing polyacrylamide gel electrophoresis and subjected to autoradiography, as shown on the left (**C**). With the second half of each sample, RT-PCR was used to amplify TNT spliced product, shown in the agarose gel on the right (**D**). The splicing reactions containing only the TNT substrate (assayed in **D**, lanes 1–3) contained no radiolabeled substrate and, consequently, were not included in **C**.

(~0.1 μM) is well below saturation in these assays (K_m ~ 1–2 μM; Y. Wu, S. Chen, M.J. Moore, pers. comm.), a step involving the 3′ splice site substrate will be rate-limiting. This could be binding of the 3′ splice site substrate, a conformational change, or the chemical step of exon ligation. Although we cannot rule out unequivocally the possibility that the bimolecular exon-ligation assay monitors a binding or conformational step that is sensitive to 3′-sulfur substitution, the observed switch in metal ion specificity is most likely indicative of

a direct interaction between a divalent metal ion and the 3′-oxyanion leaving group in the transition state, as a ground state interaction with a metal ion is expected to be weak (Narlikar et al., 1995). Presumably this metal ion stabilizes the negative charge that accumulates on the 3′-oxyanion leaving group in the transition state (Piccirilli et al., 1993; Narlikar et al., 1995; P.M. Gordon, E.J. Sontheimer, J.A. Piccirilli, in prep.). In conjunction with the previously identified metal ion-leaving group interaction in the first step of splicing (Sontheimer et al., 1997), our results indicate that the spliceosome employs similar strategies for catalyzing both steps of splicing (Fig. 4; Sontheimer et al., 1997), and weakens the argument that the two steps occur in distinct active sites. This work allows for a more detailed understanding of the catalytic mechanisms employed by the spliceosome, and of the possible evolutionary relationship between the spliceosome and group II introns.

Because mechanistic features are among the molecular traits of enzymes expected to be most highly conserved during evolution (Benner & Ellington, 1988), a "mechanistic phylogeny" can be used to evaluate possible evolutionary relationships (Sontheimer et al., 1999). We previously viewed the strikingly similar responses of the spliceosome and group II intron to sulfur substitution as strengthening the case for an evolutionary relationship between the two systems (Sontheimer et al., 1999). Perhaps even more striking, however, is the present observation that both the spliceosome and group II intron undergo a similar *change* in response to sulfur substitution at the 3′ splice site when the assay conditions are switched from intramolecular to intermolecular. Moreover, our work suggests that three mechanistic features of the spliceosome are precisely the same as for the group II intron—metal ion catalysis to stabilize the leaving group during both steps of splicing, and a rate-limiting conformational change that intervenes between the two steps (Fig. 4). Although we cannot rule out the possibility that these mechanistic similarities arose by convergent evolution from two independent lineages, the strong parallels are consistent with evolution from a common ancestor (Fig. 1). Finally, the observation that the spliceosome appears to be using the same catalytic strategies for *both* steps of splicing as a true and unambiguous ribozyme strengthens the argument that the snRNAs may form the catalytic center during nuclear pre-mRNA splicing.

MATERIALS AND METHODS

Oligonucleotide synthesis

Oligoribonucleotides were synthesized on a Millipore solid-phase DNA/RNA synthesizer and deprotected according to standard procedures. Synthesis of AdML(as).I_S, containing a 3′-*S*-phosphorothiolate linkage, required a modified protocol (Sun et al., 1997). All oligoribonucleotides were purified by denaturing polyacrylamide gel electrophoresis. The following oligoribonucleotides were synthesized: AdML(as).G: 5′-GACAGGAUCCAAGAGUACU-3′, AdML(as).I: 5′- GACAIG AUCCAAGAGUACU-3′, AdML(as).I_S: 5′-GACAI_SGAUCCAA GAGUACU-3′).

Plasmids and transcription

The 5′ RNA substrate and the 3′ RNA ligation substrate (AdML(as)+15) were transcribed from PCR products that contained T7 promoters. The PCR products were generated from plasmids HMS106 and HMS81, respectively (a generous gift from S. Chen and M. Moore, Brandeis University). The AdML(as)+15 RNA is a truncated version of the AdML(as)

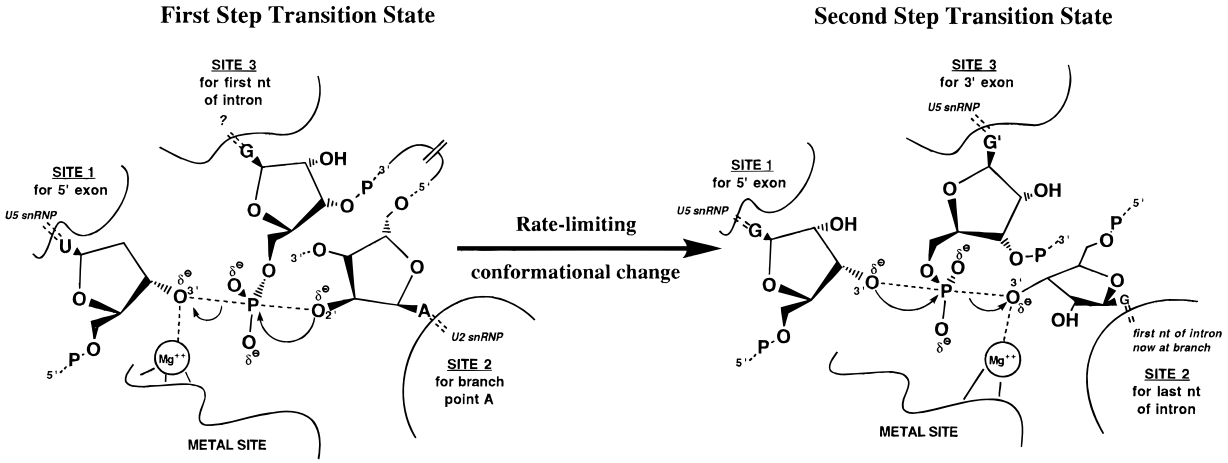

FIGURE 4. A catalytic metal ion is essential for both the first and second steps of pre-mRNA spliceosomal splicing. The lack of a metal-specificity switch under *cis* splicing conditions suggests that a conformational change prior to 3′ splice site cleavage/exon ligation is rate-limiting and, therefore, masks the subsequent chemical step. The 3 nt directly involved in each step of splicing are also depicted within the spliceosomal active sites. The ligands for these catalytic metal ions are currently unknown. Adapted from Steitz and Steitz (1993).

splicing substrate that begins 15 nt downstream of the 3′ splice site. The TNT 3′ splicing substrate (Ramchatesingh et al., 1995; Anderson & Moore, 1997) was transcribed directly from annealed synthetic oligonucleotides containing a T7 promoter. Transcription reactions were performed with a Megascript Kit (Ambion) according to the manufacturer's instructions. G(5′)ppp(5′)G cap was included in the transcription reaction of the 5′ RNA substrate.

Construction of substrate RNAs

The full-length AdML(as) 3′ substrates were constructed by ligating the synthetic oligoribonucleotides that corresponded to the last 5 nt of the intron and 14 nt of the 3′ exon to the AdML(as)+15 RNA with T4 DNA Ligase and a bridging oligonucleotide (Moore & Query, 1998). Before performing ligation reactions, the AdML(as)+15 RNA was dephosphorylated with calf intestinal alkaline phosphatase (Amersham Pharmacia) and subsequently 5′-^{32}P-phosphorylated. Annealing and ligation reactions were performed as described and generally resulted in good yields (Moore & Sharp, 1992). Ligated RNAs were purified by denaturing polyacrylamide gel electrophoresis.

Splicing reactions

HeLa nuclear extract was kindly provided by Shuyan Chen (Brandeis University). Nuclear extract was incubated in 2.0 mM EDTA for 30 min on ice before use in splicing reactions. Splicing reactions were performed at 30 °C and contained 40% nuclear extract, 80 mM KCl, 1 mM ATP, 5 mM creatine phosphate, 1 U μL^{-1} RNA-guard RNase inhibitor (Promega), 20 ng μL^{-1} carrier RNA, and divalent metal ion chlorides (Aldrich, >99.99% pure) as specified in the figure legends. The 5′ splicing substrate (35 nM) was preincubated in the reaction mix for 35 min before the addition of the 3′ splicing substrate (approximately 150 nM). After 20–30 min, RNAs were extracted, precipitated, and fractionated on a denaturing 10% polyacrylamide gel.

RT-PCR

Splicing reactions were performed as described above and RNAs were extracted and precipitated. Primers (AdML(as).3′: 5′-CTGCAGGTCGACGTTGAGG-3′; TNT.3′: 5′-CGTCGTC TTCCTCTTCTTCTTCTTCCTCTT-3′) complementary to the 3′ end of either the AdML(as) or TNT substrate were incubated with the RNA at 95 °C for 3 min and then allowed to anneal during a slow cool to room temperature. Reverse transcription reactions contained 50 mM Tris-HCl (pH 7.9), 50 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 0.04 mM dNTPs, 1 μM primer, and 0.5 U AMV Reverse Transcriptase (Amersham Pharmacia). Reverse transcription reactions were incubated for 45 min at 50 °C. The resulting cDNA was then PCR amplified, and the DNA was fractionated by 2% agarose gel electrophoresis. For sequencing, the band corresponding to spliced product was excised from the gel, and the DNA was extracted from the agarose and cloned with the TOPO TA Cloning Kit (Invitrogen).

ACKNOWLEDGMENTS

We thank Shuyan Chen and Melissa Moore for discussions, unpublished results, and the extremely generous gifts of nuclear extract and plasmids HMS81 and HMS106. We are grateful to Cecilia Cortez for oligonucleotide synthesis. We also thank Jason Schwans and Aiichiro Yoshida for critical reading of the manuscript. J.A.P. is an assistant investigator of the Howard Hughes Medical Institute.

Received September 17, 1999; returned for revision October 18, 1999; revised manuscript received November 4, 1999

REFERENCES

Anderson K, Moore MJ. 1997. Bimolecular exon ligation by the human spliceosome. *Science 276*:1712–1716.

Benner S, Ellington AD. 1988. Interpreting the behavior of enzymes: Purpose or pedigree? *CRC Crit Rev Biochem 23*:369–426.

Burge CB, Tuschl T, Sharp PA. 1999. Splicing of precursors to mRNAs by the spliceosomes. In: Gesteland RF, Cech TR, Atkins JF, eds. *The RNA world*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. pp 525-560.

Cech TR. 1986. The generality of self-splicing RNA: Relationship to nuclear RNA splicing. *Cell 44*:207–210.

Chanfreau G, Jacquier A. 1996. An RNA conformational change between the two chemical steps of group II self-splicing. *EMBO J 15*:3466–3476.

Chua K, Reed R. 1999. Human step II splicing factor hSlu7 functions in restructuring the spliceosome between the catalytic steps of splicing. *Genes & Dev 13*:841–850.

Curley JF, Joyce CM, Piccirilli JA. 1997. Functional evidence that the 3′-5′ exonuclease domain of *Escherichia coli* DNA Polymerase I employs a divalent metal ion in leaving group stabilization. *J Am Chem Soc 119*:12691–12692.

Michel F, Ferat J-L. 1995. Structure and activities of group II introns. *Annu Rev Biochem 64*:435–461.

Moore MJ, Query CC. 1998. Uses of site-specifically modified RNAs constructed by RNA ligation. In: Smith C, ed. *RNA-protein interactions: A practical approach*. London, UK: Oxford University Press. pp 75-108.

Moore MJ, Sharp PA. 1992. Site specific modification of pre-mRNA: The 2′-hydroxyl groups at the splice sites. *Science 256*:992–997.

Moore MJ, Sharp PA. 1993. Evidence for two active sites in the spliceosome provided by stereochemistry of pre-mRNA splicing. *Nature 365*:364–368.

Narlikar GJ, Gopalakrishnan V, McConnell TS, Usman N, Herschlag D. 1995. Use of binding energy by an RNA enzyme for catalysis by positioning and substrate destabilization. *Proc Natl Acad Sci USA 92*:3668–3672.

Nilsen TW. 1998. RNA–RNA interactions in nuclear pre-mRNA splicing. In: Simons RW, Grunberg-Manago M, eds. *RNA structure and function*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. pp 279-307.

Padgett RA, Podar M, Boulanger SC, Perlman PS. 1994. The stereochemical course of group II intron self-splicing. *Science 266*: 1685–1688.

Piccirilli JA, Vyle JS, Caruthers MH, Cech TR. 1993. Metal ion catalysis in the Tetrahymena ribozyme reaction. *Nature 361*:85–88.

Pyle AM. 1996. Catalytic reaction mechanism and structural features of group II intron ribozymes. In: Ekstein F, Lilley DMJ, eds. *Catalytic RNA*. Berlin, Germany: Springer-Verlag. pp 75-107.

Ramchatesingh J, Zahler AM, Neugebauer KM, Roth MB, Cooper TA. 1995. A subset of SR proteins activates splicing of the cardiac troponin T alternative exon by direct interactions with an exonic enhancer. *Mol Cell Biol 15*:4898–4907.

Sharp PA. 1985. On the origin of RNA splicing and introns. *Cell 42*:397–400.

Sigel RKO, Song B, Sigel H. 1997. Stabilities and structures of metal ion complexes of adenosine-5′-*O*-thiomonophosphate ($AMPS^{2-}$)

in comparison with those of its parent nucleotide (AMP^{2-}) in aqueous solution. *J Am Chem Soc 119*:744–755.
Sontheimer EJ. 1999. Bridging sulfur substitutions in the analysis of pre-mRNA splicing. *Methods 18*:29–37.
Sontheimer EJ, Gordon PM, Piccirilli JA. 1999. Metal ion catalysis during group II intron self-splicing: Parallels with the spliceosome. *Genes & Dev 13*:1729–1741.
Sontheimer EJ, Sun S, Piccirilli JA. 1997. Metal ion catalysis during splicing of premessenger RNA. *Nature 388*:801–805.
Steitz TA, Steitz JA. 1993. A general two-metal-ion mechanism for catalytic RNA. *Proc Natl Acad Sci USA 90*:6498–6502.
Sun S, Yoshida A, Piccirilli JA. 1997. Synthesis of 3′-thioribonucleosides and their incorporation into oligoribonucleotides via phosphoramidite chemistry. *RNA 3*:1352–1363.
Tarn WY. 1996. Site-specific substitution of inosine at the terminal positions of a pre-mRNA intron: Implications for the configuration of the terminal base interaction. *Biochimie 78*:1057–1065.
Umen JG, Guthrie C. 1995. The second catalytic step of pre-mRNA splicing. *RNA 1*:869–885.
Weiner AM. 1993. mRNA splicing and autocatalytic introns: Distant cousins or the products of chemical determinism? *Cell 72*:161–164.
Weinstein LB, Jones BCNM, Cosstick R, Cech TR. 1997. A second catalytic metal ion in a group I ribozyme. *Nature 388*:805–808.
Yu YT, Scharl EC, Smith CM, Steitz JA. 1999. The growing world of small nuclear ribonucleoproteins. In: Gesteland RF, Cech TR, Atkins JF, eds. *The RNA world*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. pp 487-524.