Group II Intron Self-splicing

ALTERNATIVE REACTION CONDITIONS YIELD NOVEL PRODUCTS*

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Reaction parameters were modified to enhance the in vitro reaction rate and to reveal partial and novel reactions of the group II intron 5g of the mitochondrial gene from Saccharomyces cerevisiae encoding cytochrome c oxidase subunit I. Ose alteration yields separarate 3'- and 3'-exons plus linear excised intron as the main products. A linear reaction intermediate, containing intron and 3'-exon, and products resulting from cleavages at two unexpected sites were identified. Spliced exon "reopening," a novel reaction between excised intron and spliced exons, appears responsible for separate 5'- and 3'-exon products.

Group II introns are found in structural genes of fungal and plant mitochondrial DNA (mtDNA) and in structural and tRNA genes of chloroplast DNA (cpDNA) (Keller and Michel, 1985). Several laboratories have reported that group II introns of yeast mtDNA self-splice in vitro and yield intron lariat and accurately spliced exons (Peebles et al., 1986; Schmelzer and Schwyen, 1986; van der Veen, 1985). The pathway for splicing nuclear pre-mRNAs involves a linear reaction intermediate analogous to the lariat intermediate of nuclear introns (reviewed by Cech and Bass, 1986). Recently, the predicted lariat intermediate of the 3'-exon. Single-stranded DNA from this construct is the complement of message.

EXPERIMENTAL PROCEDURES

Plasmids and Constructions—Plasmids pKM2 and pJD1 have already been described (Peebles et al., 1986). RNA transcribed from pKM2 was used in the experiments shown in Fig. 1, but in all other experiments with full-length RNA, pJD1 was used. A diagram of pKM2 has been published (Peebles et al., 1986). pJD1 contains the same insert cloned behind the T7 (rather than SP6) promoter. Single-stranded DNA of pseudophage pJD-ME(mp18) was used in the S1 protection experiment; it was constructed by subcloning the insert from pJD-ME(pUC9) into M13 mp18. pJD-ME(pUC9) was constructed by isolating the insert from pJD1 and digesting it completely with EcoRI and partially with Sau3AI. The products were ligated into pUC9 that was cleaved with BamHI and EcoRI. The resulting clones were screened by size and restriction mapped to identify pJD-ME(pUC9). The insert was removed from this construct with EcoRI and HindIII and ligated into M13 mp18 cut with EcoRI and HindIII. pJD-ME(mp18), therefore, is a recombinant plasmid which contains 155 nucleotides of the 5'-exon, all of the intron, and 184 nucleotides of the 3'-exon. Single-stranded DNA from this construct is the complement of message.

Enzyme Reagents—Restriction enzymes were purchased from Bethesda Research Laboratories, New England Biolabs, Boehringer Mannheim, and International Biotechnologies Inc. Phase T4 DNA ligase was purchased from New England Biolabs. SP6 RNA polymerase was purchased from Promega Biotech, and T7 RNA polymerase was purchased from United States Biochemical Corp. Ribonuclease T1 and S1 nuclease were purchased from Boehringer Mannheim. Phase T4 RNA ligase was purchased from Du Pont-New England Nuclear. Reverse transcriptase was purchased from Seikagaku America Inc.

Transcription and Purification of RNA—RNA was transcribed and purified as described in Peebles et al. (1986). The RNA was labeled by incorporation of [α-32P]UTP (600 Ci/mmol, purchased from Du Pont-New England Nuclear) during transcription. A standard transcription reaction contained 30 μCi of labeled nucleotide triphosphate in 100 μl of reaction buffer containing 0.4 mM unlabeled UTP. The plasmids used to synthesize RNA were linearized in the downstream polylinker with HindIII.

Self-splicing Reactions—RNA was diluted in water and mixed with...
an equal volume of buffer stock solution to achieve a final concentration of 40 mM Tris-HCl, 100 mM MgCl₂, and 0.1% sodium dodecyl sulfate, pH 7.6. Control experiments showed that sodium dodecyl sulfate (up to 1%) had no effect on the reactivity of the RNA, so it was generally included. When salts of monovalent cations were included in the reactions, they were present in a final concentration of 500 mM. Reactions were incubated at 45 °C and stopped by addition of an equal volume of gel loading buffer; products were analyzed on 4% polyacrylamide gels containing 8 M urea.

**Nomenclature of RNA Molecules**—The precursor RNA is referred to as TXT (transcript). IVS-LAR is excised intron in the form of a lariat. IVS-BL is broken intron lariat or a linear excised intron depending on the reaction condition. IVS-E3 is a linear 2/3' molecular containing the intron plus the 3'-exon. E5-E3 is spliced exons. E5 and E3 are the free 5' and 3'-exons, respectively. E5s is related to E5 but lacks 25 nucleotides at its 5'-end (relative to E5).

**Ribonuclease T1 Digestion of RNA**—One µl (5000 cpm) of each RNA sample was mixed with 9 µl of RNAse digestion buffer (10 mM Tris-HCl, 5 mM EDTA, and 300 mM NaCl, pH 7.5). Seven units of ribonuclease T1 in 1 µl was added, and the mixture was incubated at 30 °C for 1 h. An equal volume of gel loading buffer was added to the reaction, and the products were fractionated on a 20% acrylamide, 8 M urea gel. An oligo(dT) ladder (purchased from Bethesda Research Laboratories) was 5'-end-labeled and used as a size standard.

**Other Methods**—Northern blots were done as described in Peebles et al. (1986) using the same oligonucleotide probes. The nuclease S1 protection experiment was carried out as described by Maniatis et al. (1982). Phage T4 RNA ligase was used to label 3'-ends of RNA molecules as described by England et al. (1980). Primer extension experiments were carried out as described by Peebles et al. (1986); oligonucleotides used were a 1-mer complementary to nucleotides 172-190 of the intron and a 19-mer complementary to positions 55-73 of the 3'-exon.

**RESULTS**

**New Reaction Conditions**—The search for modified reaction conditions involved further variations of the published experiments (Peebles et al., 1986). The splicing reaction requires at least 5 mM MgCl₂ and proceeds at similar reaction rates throughout the range of 10-100 mM. We found that spermidine is neither stimulatory nor essential at 100 mM MgCl₂ (Fig. 1A, lanes 4 and 5). It appears that at 10 mM MgCl₂, spermidine serves primarily as a counterion for the RNA (compare lanes 2 and 3 with lane 4). We next investigated the reactivity of the transcript at higher concentrations of MgCl₂. The products and extent of reaction are essentially the same between 100 mM and 1.5 mM MgCl₂; MgCl₂ at 2 M or above inhibits splicing completely. Other salts of magnesium give essentially the same results (not shown).

In reactions with either 10 mM Mg(OAc)₂ or MgCl₂, various salts of monovalent cations have no effect at low concentration and inhibit splicing when present at concentrations of 50 mM or higher (Peebles et al., 1986). Initial experiments examining salt effects in the presence of 100 mM MgCl₂ showed that the RNA remains reactive in the presence of LiCl, NH₄Cl, NaCl, and KCl at 500 mM (Fig. 1B). Added LiCl does not alter the array of products relative to reactions containing 100 mM MgCl₂ (compare Fig. 1A, lane 5, with Fig. 1B, lane 1). Inclusion of each of the other three salts, however, results in the appearance of some new products in addition to IVS-LAR, IVS-BL, and E5-E3 (lanes 2-4). The most extreme alteration of the pattern of products was obtained with added KCl (lane 4) where two major new products are apparent; it will be shown below that they are separate 3'-exon (E3) and 5'-exon (E5). The other major products are IVS-LAR and material comigrating with IVS-BL that will be shown to be linear excised intron. In this reaction little E5-E3 accumulates. Products obtained in the presence of added NH₄Cl or NaCl (lanes 2 and 3, respectively) are the same as with added KCl, but with these salts there is more E5-E3 and IVS-LAR. Since NH₄Cl, NaCl, and KCl yield the same set of products, though in varying proportions, they can be used interchangeably for most purposes. Because KCl yields the most extreme deviation from the standard pattern of products, it was used in most of the following experiments.

Unexpectedly, the presence of high salt concentrations speeds the rate of reaction. In 100 mM MgCl₂, the reaction is not significantly different from that obtained with 10 mM MgCl₂ plus 2 mM spermidine (compare Fig. 1A, lanes 3 and 4). The reaction in the presence of 500 mM KCl is more rapid, with products evident after only 5 min of incubation and over 90% conversion of transcript into products after 1 h of incubation (Fig. 2A). Two additional products are resolved in this experiment; as will be shown below, one denoted IVS-E3 contains the intron and 3'-exon and the one denoted E5a is 5'-exon lacking about 25 nucleotides from its 5'-end.

While anion effects were not investigated in detail, (NH₄)₂SO₄ was found to have a strikingly different effect on the reaction than does NH₄Cl. The rate of the self-splicing reaction is increased by the addition of 500 mM (NH₄)₂SO₄ without yielding any prominent new products (Fig. 2B). The major products comigrate with IVS-LAR and E5-E3, indicating that the main effect of this salt is to enhance the rate of the splicing reaction. Minor amounts of E5, E3, and IVS-E3 are sometimes present, but they are much more prominent in KCl-containing reactions.

**Characterization of Products of Salt-containing Reactions**—Portions of the precursor sequence present in the various products were identified by probing RNA immobilized on filters with intron- or exon-specific oligonucleotides. The data...
(not shown) reveal that products E5-E3, E5, and E5s contain 5'-exon sequences; IVS-E3, E5-E3, and E3 contain 3'-exon sequences; and IVS-LAR, IVS-E3, and IVS-BL contain intron sequences. The size of each product was determined (not shown). The products identified in Fig. 2A as IVS-LAR, IVS-BL, and E5-E3 comigrate with the previously characterized products of standard reactions. Product IVS-E3 is about 300 nucleotides smaller than the transcript consistent with the removal of E5. Product E3 is measured as 316 nucleotides, while bands E5 and E5s are 294 and 269 nucleotides, respectively. From the DNA sequence of the plasmid used here, the 5'-exon is 297 nucleotides and the 3'-exon, 319 nucleotides. Next, products containing the 3'-end of the RNA precursor were identified. Transcripts were 3'-end-labeled and incubated either in 100 mM MgCl₂ for 1 h or in 100 mM MgCl₂ plus 500 mM NaCl for 10 min (Fig. 3A). The unincubated sample (lane 1) shows that most of the radioactivity is present in full-length transcripts. After incubation with 100 mM MgCl₂, products IVS-E3, E5-E3, and E3 are radioactive, while labeled IVS-LAR, IVS-BL, E5, and E5s are not present (lane 2). A similar result was obtained with the products of a NaCl-containing reaction (lane 3), although, there, more of the radioactivity appears in E3. Thus, products IVS-E3, E5-E3, and E3 contain 3'-exon sequences including the extreme 3'-end of the transcript. In a separate experiment, unlabeled RNA was reacted in 100 mM MgCl₂ with or without added NaCl; those products were 3'-end-labeled and analyzed (Fig. 3A, lanes 4 and 5). Each one of the products was labeled, indicating that each has a 3'-hydroxyl end.

The analysis of E3 was completed by locating its 5'-end by primer extension. A 3'-exon-specific oligonucleotide was annealed to purified E5 and extended using reverse transcriptase. The products of that reaction were fractionated beside a sequencing ladder obtained using the same oligonucleotide and an M13 DNA clone as template. The major and largest product of extension on the E3 RNA was 73 nucleotides long (Fig. 3D). That termination site coincides with the first nucleotide of the 3'-exon, indicating that the 5'-end of E3 results from cleavage at the 3'-splice junction.

Both E5 and E5s contain sequences near the 3'-end of the 5'-exon, since the oligonucleotide probe that anneals to them is complementary to 19 nucleotides adjacent to the exon-intron junction. Their 3'-ends were identified by an S1 endonuclease protection experiment. Radioactive E5 and E5s were purified and annealed to M13 virion DNA containing the complement of the transcript extending from the MboI site within the 5'-exon through the EcoRI site in the 3'-exon (cf. Fig. 1 of Peebles et al., 1986). The hybrid was treated with S1 endonuclease, and the protected RNA was fractionated on an appropriately calibrated sequencing gel. The DNA strand contains the 3'-terminal 155 nucleotides of the 5'-exon and protected up to 155 nucleotides of both E5 and E5s (Fig. 3C, lanes 1 and 2). This shows that E5 and E5s share the same 3'-end and within the limits of this method result from cleavage at or very near the 5'-splice junction. Product E5s, therefore, lacks about 25 nucleotides from its 5'-end. This appears to result from cleavage of the precursor RNA since the transcripts do not contain this 5'-end before incubation (see Fig. 3D, lane 2). The sequence near that cleavage site resembles the 3'-exon-intron boundary.

The 5'-ends of IVS-E3 and IVS-BL from KCl-containing reactions were determined by extension of an intron-specific oligonucleotide with reverse transcriptase. Both the experimental samples and the IVS-LAR control yield the same 190-191-nucleotide extension product (Fig. 3D, lanes 1, 3, and 4). The strong stop to reverse transcription is at the position of the first nucleotide of the intron. This result suggests that IVS-E3 and IVS-BL result from cleavage at the 5'-splice junction.

We have already established that linear excised intron migrates like broken lariat (Peebles et al., 1986) and that ribonuclease T1 fragment patterns are suitable to distinguish branched from unbranched intron forms (Perlman et al., 1987). To learn which intron-containing products of KCl-containing reactions are branched, samples of all four intron-containing molecules were digested with ribonuclease T1 and...
the products fractionated on a sequencing gel. As shown in Fig. 4, the branched oligonucleotide that is diagnostic of the intron lariat is present in IVS-LAR (lane 1) and absent from the other species (lanes 2-4). Similarly, the single 11-nucleotide fragment that contains the intron branch site is absent from IVS-LAR but present in IVS-BL (compare lanes 1 and 4). These data show that both IVS-E3 and IVS-BL from KCl-containing reactions are not branched at the site previously defined, while IVS-LAR is branched there. The same analysis was carried out using intron-containing RNAs from (NH₄)₂SO₄ reactions (Fig. 4, lanes 5–8). Again, IVS-LAR is branched (lane 5) while the other two intron products are essentially unbranched (lanes 7 and 8).

Four observations indicate that neither IVS-E3 nor IVS-BL is branched at a novel site. First, with the exceptions indicated by the arrows in Fig. 4 and explained above, IVS-LAR yields the same overall pattern of T1 fragments as does IVS-LAR (compare lanes 1 with 4 and 5 and 6). Second, no T1 fragments are obtained from IVS-E3 that are not also present in the pattern from (unbranched) precursor RNA. Third, IVS-E3 and IVS-BL from KCl-containing reactions are unaffected by debranching enzyme; and finally IVS-E3 contains no strong stop to primer extension before the 5′-end of the intron sequence (not shown).

We conclude that IVS-E3 results from cleavage (probably hydrolysis) at the 5′-splice junction without associated branch formation. In reactions with 500 mM KCl, linear intron accounts for at least two-thirds of the excised intron; the rest is branched and migrates as IVS-LAR (see Fig. 2A). There is no indication that IVS-LAR “debranches” under these conditions, so we infer that linear excised intron is a primary product derived from IVS-E3. It appears that KCl and, to a lesser extent, some other salts tested decrease the efficiency of branch formation relative to reactions containing (NH₄)₂SO₄. Or said differently, KCl increases the efficiency of an alternative nucleophile (probably water) relative to that of the 2′-OH of the branch AMP residue. The overall rates of precursor utilization, however, are similar for these salts.

**IVS-E3 Is Part of a Reaction Intermediate**—To determine whether IVS-E3 is an intermediate or side product of the KCl-stimulated reaction, it was isolated and further incubated. In preliminary experiments it was found that fragments of the transcript can contaminate preparations of IVS-E3 and complicate this analysis. This difficulty was minimized by preparing IVS-E3 from transcript that had been carefully purified, incubating it for an hour in (NH₄)₂SO₄-containing reaction buffer, and isolating the unreacted IVS-E3. This purification strategy was based on observations that contaminants are much more reactive in (NH₄)₂SO₄ than is IVS-E3 and that their products are smaller than IVS-E3. Purified IVS-E3 is shown to be slightly reactive in (NH₄)₂SO₄-containing buffer and more reactive in the presence of KCl in Fig. 5, lanes 2 and 3, respectively. No E5 was obtained in those reactions; instead a product slightly larger than IVS-BL and IVS-E3 was obtained. In one experiment, when the KCl concentration was increased from 0.5 M NaCl and the products subsequently 3′-end-labeled and fractionation (Fig. 3).
another a few nucleotides smaller than E5 (called E3-290) were observed. The small amount of IVS-LAR in lane 2 probably results from fragments of precursor not completely eliminated by the isolation procedure. A primer extension experiment using E3-290 as template and the oligonucleotide complementary to the 3'-exon as primer confirmed that it contains part of E3 but lacks 29 nucleotides from the 5'-end of E3 (not shown). It probably results from cleavage after a sequence there identical to the last 6 nucleotides of the 5'-exon (5'-AUUUUC). The additional material migrating like IVS-BL is the other product of that cleavage. Since E3-290 is not evident in the array of products obtained from full-length transcript RNA (cf. Fig. 2), purified IVS-E3 carries out a reaction resembling the first step of splicing that does not occur with full-length precursor or when the other products are present.

We then attempted to reconstitute the original reaction. Incubation of labeled IVS-E3 in the presence of a mixture of unlabeled products from a KCl-containing reaction of tran-
script yields E3 and IVS-BL but little E3-290 (Fig. 5, lane 4). Therefore, conversion of IVS-E3 to linear excised intron and separate 3'-exon requires the participation in trans of some other component of the initial reaction. The addition of isolated E5 to IVS-E3 is sufficient to promote the release of E3 (Fig. 5, lane 5). Therefore, IVS-E3 plus E5 is a bipartite intermediate of the KCl-stimulated reaction that can yield separate E5 and E3 plus linear intron.

We next analyzed this reaction of IVS-E3 and E5 in the presence of 500 mM NH₄Cl (Fig. 5, lane 6). As expected, both linear intron and separate 3'-exon were obtained. Some spliced product, E5-E3, accumulated as well, suggesting the reconstitution of a splicing intermediate. Since we have shown above that IVS-E3 is not branched and because very little IVS-LAR is present using this sample of IVS-E3, it is clear that spliced exon formation can proceed without prior intron branch formation. This result agrees with the findings of Jacquier and Rosbash (1986) using different RNA species.

Excised Intron Reopens Spliced Exons—The experiments described above demonstrate that the reaction of IVS-E3 with E5 can yield spliced exons, but the relative absence of E5-E3 from KCl-containing reactions (e.g. Fig. 2A) remained unexplained without assuming that added KCl uncouples 3'-exon release from exon ligation. It seemed possible that some of the separate exons could result from the “reopening” of spliced exons. To test this, E5-E3 was isolated and incubated either with or without an excess of unlabeled precursor under various conditions (Fig. 6A). In samples lacking added precursor, E5-E3 is unreactive (Fig. 6A, lanes 1 and 2). It remained relatively stable when incubated together with precursor in (NH₄)₄SO₄ (Fig. 6A, lane 3). However, when E5-E3 was incubated with precursor in KCl, E5 and E3 appeared with the concomitant disappearance of E5-E3 (Fig. 6A, lane 4). This reaction requires added precursor and occurs with increasing rate as more unlabeled precursor is added (Fig. 6B). The reaction is progressive with about 50% reopening of E5-E3 in 2 h (Fig. 6C). Excised intron forms were purified
FIG. 6. Spliced exons are reopened by intron RNA. A, exon reopening requires KCl and an active RNA. Labeled E5-E3 (8 nM) was incubated at 45°C for 1 h in (NH₄)₂SO₄ buffer (lane 1), KCl buffer (lane 2), (NH₄)₂SO₄ buffer plus an excess (250 nM) of unlabeled precursor RNA (lane 3), or KCl buffer plus 250 nM unlabeled precursor (lane 4). Each sample was in a total volume of 8 μl. B, exon reopening is a bimolecular reaction. Labeled E5-E3 (8 nM) was incubated in a total volume of 8 μl/sample at 45°C for 1 h with increasing amounts of added unlabeled precursor. The sample of lane 2 contained about 0.5 nM unlabeled precursor RNA, and those of lanes 3-6 contained 2.5, 25, 50, and 250 nM precursor, respectively. Lane 1 contains E5-E3 incubated without unlabeled precursor. C, time course of exon reopening. Seven separate 10-μl reactions containing about 8 nM E5-E3 and about 200 nM unlabeled precursor were incubated for 0, 8, 10, 20, 40, 80, and 120 min, and the products are shown in lanes 1-7, respectively. D, excised intron reopens spliced exons. Purified spliced exon was incubated with IVS-LAR (lanes 1 and 2) or IVS-BL (lanes 3 and 4) in 8 μl of buffer containing KCl for 1 h at 45°C (lanes 2 and 4). Lanes 1 and 3 contain the unincubated mixed reactants. All reactants were adjusted to an initial concentration of 10 nM.

DISCUSSION

Pathway I (Fig. 7) summarizes the sequential transesterification steps for splicing oxy 3 intron 5g of yeast mitochondria (Peebles et al., 1986; van der Veen et al., 1986). The first step involves the release of the 5'‐exon by nucleophilic attack of the phosphodiester bond at the 5'‐splice junction by the 2'‐OH of the branch AMP residue. Jacquier and Rosbash (1986) have suggested that the released 5'‐exon is positioned on the intron prior to and following release. More recently, Jacquier and Michel (1987) have defined two 5'‐exon binding sites within the intron. The other product of that reaction is intron lariat with the 3'‐exon still attached; it appears to be a very minor product first detected by van der Veen et al. (1986) using electron microscopy. It is a more prominent product using mutated precursors (Jacquier and Michel, 1987). The second step is attack by the 3'‐OH of the released 5'‐exon at the 3'‐splice junction to yield spliced exons and excised intron lariat. This pathway predominates under low salt "standard conditions" and is accelerated about 10-fold in the presence of 100 mM MgCl₂ and 500 mM (NH₄)₂SO₄.

About two-thirds of reacting molecules carry out the alternative first reaction of pathway II when 500 mM KCl is provided (Fig. 7). There, the first step is cleavage without transesterification. Since the reaction is not strongly pH‐dependent (data not shown) the nucleophile is probably water rather than hydroxide ion. It is possible that some other
nucleotide attacks the boundary; in that case it would have to yield an unstable adduct with E5 that is ultimately resolved by water (or hydroxide ion) since we have shown that released E5 has a 3'-OH terminus. The alternative, that the 2'-5' branch is unstable in the presence of KCl, is unlikely since purified IVS-LAR does not "debranch" measurably under that condition. It appears that the 5'-splice junction can act as a catalyst for the first step by a KCl-enhanced configuration that differs somewhat from the active structure without added salt or with (NH₄)₂SO₄. In that structure an alternate nucleophile is more effective than is the branch site 2'-hydroxyl.

The products of the first step of pathway II, E5 and linear IVS-E3, were characterized in detail. Purified IVS-E3 is very reactive upon incubation in KCl-containing reaction buffer and cleaves itself at a sequence within the 3'-end of E5 to yield a novel product, E3-290. When IVS-E3 is incubated with purified E5, E3 and linear IVS are obtained while the yield of E3-290 is diminished. Since E3-290 is not obtained in reactions using full-length precursor RNA, it follows that E5 released from precursor RNA probably remains associated with IVS-E3. The reaction intermediate that carries out the second step of this pathway is composed of E5 plus IVS-E3.

Under appropriate reaction conditions those molecules can interact to yield spliced exons. Therefore, the prior formation of a branch is not essential to align E5 for attack on the 3'-boundary. It should be noted, however, that the lariat 2/3 intermediate of pathway I is barely detectable using this precursor while the linear 2/3 molecule of pathway II is prominent at intermediate extents of reaction. Therefore, it is likely that the lariat form is more reactive in the splicing step than is the linear one. In that case, it would follow that the branch contributes to the reaction kinetically but not mechanistically.

The main products of reactions in KCl are separate E5 and E3 and linear excised intron. It is likely that the second step in both pathways involves the attack on the 3'-splice junction by the 3'-OH of the 5'-exon, leading to spliced exons. We showed that excised linear or branched intron promotes spliced exon reopening effectively in the presence of KCl. An exon reopening reaction is slow in the presence of (NH₄)₂SO₄, consistent with the result that spliced exons accumulate. There is an inverse correlation between the rate of exon reopening and the amount of spliced exon obtained under the four reaction conditions reported here (added NaCl, NH₄Cl, KCl, or (NH₄)₂SO₄). If spliced exon remains bound to the intron in KCl then this unexpected reaction can account for the final products obtained in KCl. It remains possible that some E3 released in pathway II results from cleavage at the 3'-splice junction without exon ligation; that boundary, however, is not available for attack in the absence of E5. Unlike the Tetrahymena rRNA gene intron (Inoue et al., 1986), it appears that E3 cannot be released from precursor RNA before E5, since the expected product of such a reaction, E5-IVS, is not observed.

The cleavage reaction of IVS-E3 that yields E3-290 may resemble the first step of splicing. Jacquier and Michel (1987) have shown that 5'-exon release by transesterification requires base pairing between short sequences of the 5'-exon and two exon binding sites within the intron. The part of the 3'-exon at which cleavage to yield E3-290 occurs could pair well with the first exon binding site but does not contain a good complement for the second one. Interestingly, E3-290 is not obtained in reactions of full-length precursor RNA. It appears that E5 interferes with reaction at that site both since the addition of E5 to IVS-E3 lowers the yield of E3-290 (Fig. 5) and no E3-290 is obtained in reactions between excised intron and spliced exons or excised intron and separate exons (Fig. 6).
The spliced exon reopening reaction documented here may also resemble the first reaction step (E5 release) since in both cases the phosphodiester bond following the sequence 5'-AUUUUC is cleaved. Alternatively, it is possible that spliced exon reopening more closely resembles reversal of the last step of splicing. While reversal of splicing is possible it has not been observed for group I introns. It has been suggested that reversal may be prevented by cyclization of the excised intron (Kruger et al., 1982; Zaug et al., 1983); for group II introns that explanation is not attractive since the excised intron lariat has an unblocked 3'-end. More detailed comparisons of the 5'-exon release and spliced exon reopening reactions support the distinction proposed here.1

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REFERENCES

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