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New RNA-mediated reactions by yeast mitochondrial group I introns

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The group I self-splicing reaction is initiated by attack of a guanosine nucleotide at the 5′ splice site of intron-containing precursor RNA. When precursor RNA containing a yeast mitochondrial group I intron is incubated *in vitro* under conditions of self-splicing, guanosine nucleotide attack can also occur at other positions: (i) the 3′ splice site, resulting in formation of a 3′ exon carrying an extra added guanosine nucleotide at its 5′ end; (ii) the first phosphodiester bond in precursor RNA synthesized from the SP6 bacteriophage promoter, leading to substitution of the first 5′-guanosine by a guanosine nucleotide from the reaction mixture; (iii) the first phosphodiester bond in already excised intron RNA, resulting in exchange of the 5′ terminal guanosine nucleotide for a guanosine nucleotide from the reaction mixture. An identical sequence motif (5′-GAA-3′) occurs at the 3′ splice site, the 5′ end of SP6 precursor RNA and at the 5′ end of excised intron RNA. We propose that the aberrant reactions can be explained by base-pairing of the GAA sequence to the Internal Guide Sequence. We suggest that these reactions are mediated by the same catalytic centre of the intron RNA that governs the normal splicing reactions.

Key words: self-splicing/RNA catalysis/group I intron/mitochondria/yeast

Introduction

DNA coding segments are often interrupted by intervening sequences (introns), resulting in split genes. These are widespread in the nuclear genomes of higher eukaryotes, occur in organelle DNAs and examples have also been found in Archaebacteria and in bacteriophage T4. Expression of such genes leads to the synthesis of intron-containing precursor RNAs from which the introns are removed in a process called RNA splicing (Abelson, 1979). Three main themes in the mechanism of RNA splicing can be distinguished (Cech, 1983). (i) Splicing of precursor tRNAs follows conventional rules of catalysis requiring two enzymes: an RNA endonuclease and an ATP-dependent RNA ligase (Peebles *et al.*, 1983; Greer *et al.*, 1983). (ii) A limited group of introns takes care of its own excision in an RNA-mediated reaction called self-splicing. Intramolecular base-pairing folds the intron creating a reactive centre in which phosphodiester bonds can be broken and re-formed between new combinations of nucleotides (Bass and Cech, 1986). Two mechanisms differing in their dependence on a guanosine nucleotide (group I and group II specific) have been described. (iii) The majority of nuclear precursor RNAs are spliced in a complicated process in which many proteins and ribonucleoprotein particles participate. The mechanism of this process is known only in rough outline (Padgett *et al.*, 1986).

Although at first sight self-splicing and nuclear precursor RNA splicing seem to work along different principles, more recent studies suggest that common features may exist (Sharp, 1985;

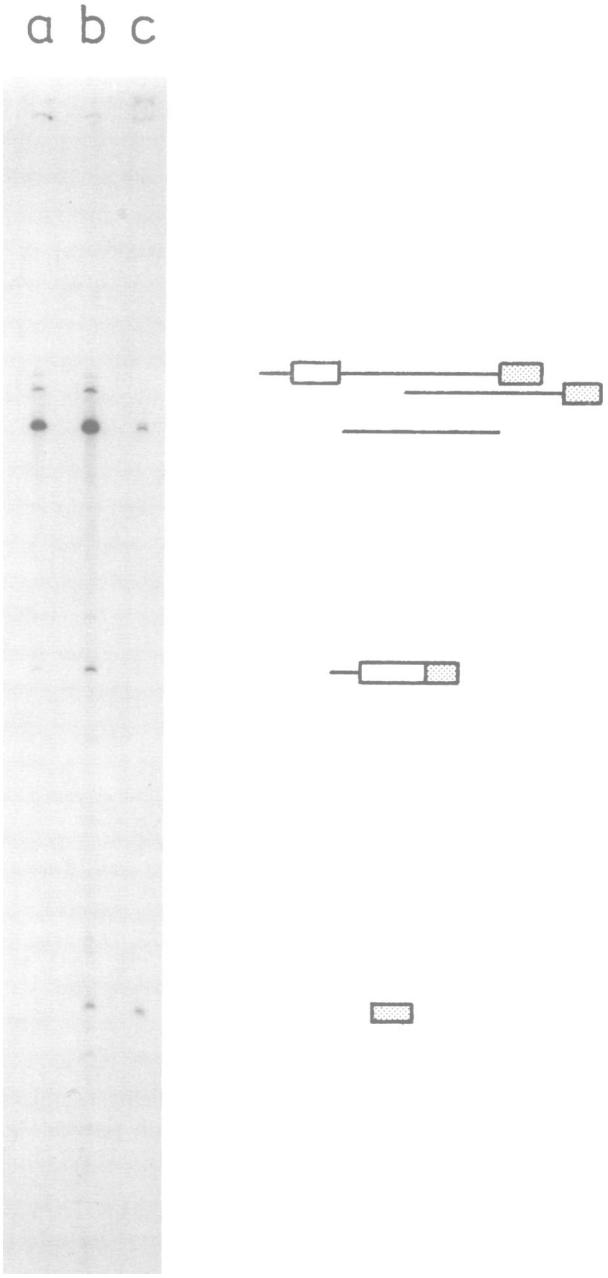

Fig. 1. Self-splicing of unlabelled ribosomal precursor RNA in the presence of [α-^{32}P]GTP. Precursor RNA was incubated for 1 h at 40°C under self-splicing conditions in the presence of a fixed amount of [α-^{32}P]GTP (0.5 μM) and increasing amounts of unlabelled GTP. RNA products were analysed by polyacrylamide gel electrophoresis. **Lane a**, without added GTP; **lane b**, with 5 μM GTP; **lane c**, with 50 μM GTP.

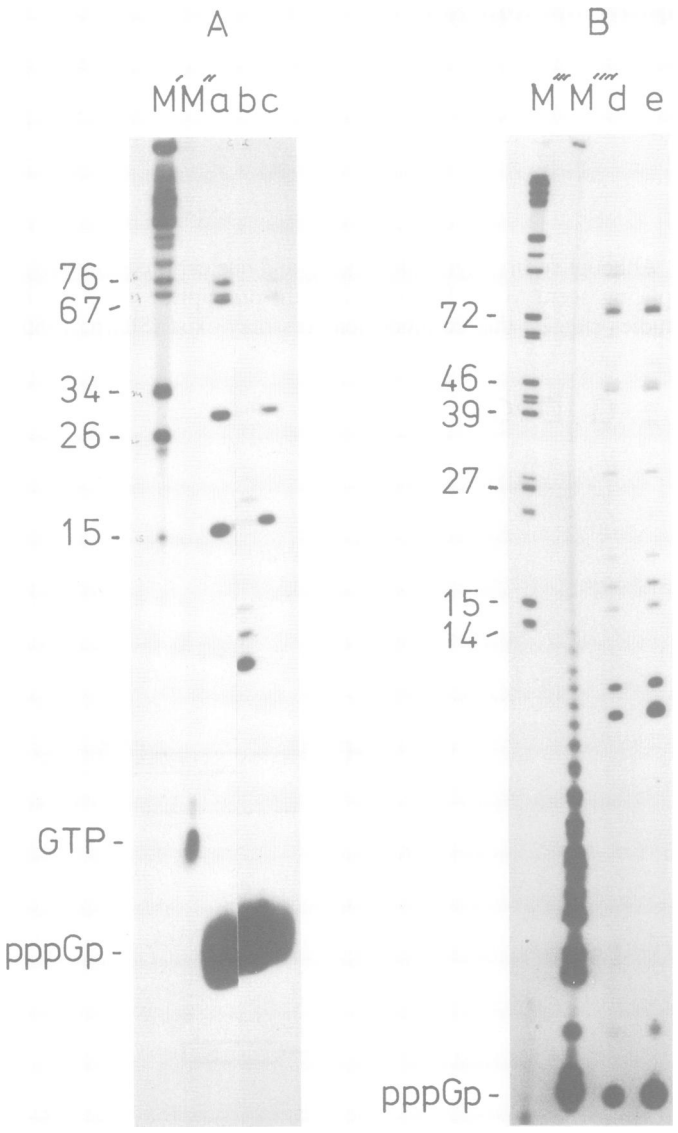

Fig. 2. Analysis of [α-^{32}P]GTP labelled splicing products by partial T_1 ribonuclease digestion. [α-^{32}P]GTP end-labelled RNA products were isolated from a polyacrylamide gel and incubated with T_1 ribonuclease. Digestion products were analysed by polyacrylamide gel electrophoresis. **Lane a**, intron RNA; **lane b** , 3′ exon RNA; **lane c**, intron and downstream exon RNA; **lane d**, precursor RNA; **lane e**, exon ligation product. M are various marker lanes: M′, pBR322 × Msp, 15, 26, 34, 67, 76, 90, 110 bp, etc.; M″, [α-^{32}P]GTP; M‴, mp7 × *Dde*I, 14, 15, 24, 27, 28, 39, 42, 46, 63, 72, 128 bp, etc.; M⁗, partial alkaline breakdown products from [α-^{32}P]GTP end-labelled intron RNA.

Cech, 1986). Most interesting among these is the observation that during nuclear RNA splicing and self-splicing a similar RNA product is formed: a branched RNA molecule (lariat). A lariat was first identified in nuclear RNA splicing (Ruskin *et al.*, 1984; Padgett *et al.*, 1984; Domdey *et al.*, 1984) and the branch-point demonstrated to consist of the 5′ phosphate of the first nucleotide of the intron connected via a 5′−2′ phosphodiester bond to an intron−internal nucleotide (Konarska *et al.*, 1985). Later it was found that a lariat is also formed during self-splicing of group II intron-containing precursor RNAs (Peebles *et al.*, 1986; Van der Veen *et al.*, 1986; Schmelzer and Schweyen, 1986). Formation of a common splicing product could suggest that both splicing pathways, despite their apparent differences, have mechanistic principles in common. This may extend to self-splicing of group I-containing precursor RNA as well. Although the normal fate of the excised group I intron is circularization we nevertheless observed that lariats can be produced in low yield under *in vitro* self-splicing conditions as side products of the reaction (Arnberg *et al.*, 1986).

During our work on group I self-splicing we noticed two other reactions that could not easily be fitted into the group I self-splicing model (Tabak *et al.*, 1985; Arnberg *et al.*, 1986). Since they occur in the absence of protein their characterization may be useful to illustrate the potential range of RNA-mediated reactions and to look for the possible existence of additional features that may be involved in self-splicing and nuclear RNA splicing reactions.

A diagnostic property of group I self-splicing is the initiation of splicing by guanosine nucleotide attack at the 5′ splice site, resulting in covalent addition of the guanosine nucleotide to the 5′ end of the intron (Zaug and Cech, 1982). This reaction can easily be studied by incubating unlabelled group I intron-containing precursor RNA under self-splicing conditions in the presence of [α-^{32}P]GTP. Apart from RNA products expected to become labelled, the intron still attached to the downstream exon and to the excised intron itself, we noticed in earlier studies (Tabak *et al.*, 1985; Arnberg *et al.*, 1986), that a number of other labelled products can arise. Here we characterize these RNAs in more detail and show that the guanosine nucleotide attack can also be directed at the 3′ splice site followed by addition of [α-^{32}P]GTP to the downstream exon and that the first 5′ nucleotide of the artificial RNA precursor initiated at the phage SP6 promoter and the first 5′ nucleotide of the excised intron RNA can be exchanged for [α-^{32}P]GTP present in the incubation mixture.

Results

Guanosine nucleotide-mediated opening of the 3′ splice site

Truncated precursor RNA was synthesized *in vitro* from a recombinant DNA clone consisting of a SP6 promoter followed by an intron-containing yeast mitochondrial DNA fragment from the large rRNA gene (Van der Horst and Tabak, 1985). The unlabelled gel-purified precursor RNA was incubated with a fixed amount of [α-^{32}P]GTP and increasing amounts of unlabelled GTP (Figure 1). Prominently labelled RNAs migrated at the position of the excised intron and intron still attached to the 3′ exon (Figure 1, lanes a and b). Labelling of these RNAs is diminished in lane c due to the decrease in the specific activity of [α-^{32}P]-GTP. Faintly labelled RNA products also appear at positions at which we expect the unspliced precursor RNA, the ligated exons and the free 3′ exon.

The [α-^{32}P]GTP end-labelled RNA products were isolated from a polyacrylamide gel and subjected to partial breakdown by ribonuclease T_1. Since yeast mtDNA is AT-rich, the number of guanosine nucleotides is rather limited and a very characteristic pattern of digestion products is obtained for each RNA (Figure 2 and Table I). The size and order of the partial T_1 fragments derived from RNAs comigrating with the intron and intron + downstream exon (Figure 2A, lanes a and c) is exactly what one would expect from RNA labelled at the 5′ end of the intron by [α-^{32}P]GTP addition [as demonstrated before (Van der Horst and Tabak, 1985)]. The T_1 RNA products from the RNA comigrating with the 3′ exon are indeed one nucleotide longer than those predicted to arise on the basis of the DNA sequence downstream of the 3′ splice site (Figure 2A, lane b). This demonstrates that the RNA consists of the 3′ exon end-labelled by addition of [α-^{32}P]GTP to its 5′ end. While labelling of the precursor

Table I. RNA sequences in ribosomal precursor RNA downstream of positions at which guanosine nucleophilic attack occurs

SP6 Precursor RNA

```
GAAUACACGG AAUUCGAGCU CGCCCGGGAU CCGUCGACCU GAGGUCGGGA ACUUAAAUAA AAAGGAAAGA
1          9 10       16 18     22    26       33 36     41 43    47                  64      69
```

T7 Precursor RNA

```
GGGAGACCAC AACGGUUUCC CUCUAGCGGA UCGAUCCGAA UUCGAGCUCG CCCGGGGAUC
1 3 5            14              26 29     33      38       44       49     53
```

Intron RNA

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GAAUUUACCC CCUUGUCCCA UUAUAUUGAA AAAUAUAAUU AUUCAAUUAA UUAUUUAAUU GAAGUAAAUU
1                  15                  28                                  61 64
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3′ Exon

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GAACAGGGUA AUAUAGCGAA AGAGUAGAUA UUGUAAGCUA UGUUUGCCAC CUCGCUGUCG ACUCAUCAUU
1       6 7 8      16 18     22 24 27       33     37       42     46       54 57 60
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Numbers below the RNA sequences refer to the length predicted for partial T_1 ribonuclease digestion products.

RNA, intron + 3′ exon, excised intron and ligated exons (see below) diminish when the unlabelled GTP concentration increases, the radioactivity in the labelled 3′ exon RNA remains about the same (Figure 1, lane c). This suggests that the rate of 3′ splice site opening or the number of molecules converted increases even further at higher GTP concentration thus compensating for the decrease in [α-^{32}P]GTP specific activity. Opening of the 3′ splice site is specific for GTP. No labelling of the 3′ exon takes place when unlabelled precursor RNA is incubated with [γ-^{32}P]ATP or [α-^{32}P]UTP (experiment not shown).

The 5′ terminal GTP exchange reaction

When the RNA comigrating with precursor RNA (Figure 1) is subjected to partial T_1 digestion, the pattern of the RNA products resolved by polyacrylamide gel electrophoresis conforms to expectations for 5′-end-labelled precursor RNA initiated at the SP6 promoter (Figure 2B, lane d). In this case the size of the T_1 RNA products obtained indicates that labelling is not due to [α-^{32}P]GTP addition, as in the examples discussed before, but due to exchange with the first guanosine nucleotide of the precursor RNA. The first partial T_1 product found is nine nucleotides long and not the dinucleotide GG expected in the case of guanosine nucleotide addition. When 5′-end-labelled precursor RNA undergoes self-splicing it is clear also that a labelled exon to exon ligation product will be expected. This is indeed confirmed by the partial T_1 analysis shown in Figure 2 (lane e). Since the exon ligation product is an end product of the RNA splicing reaction, it accumulates to a higher extent than the end-labelled RNA precursor itself, which only reaches a certain steady state level as intermediate in the reaction (Figure 1). End labelling is not caused by exchange of the 5′ triphosphate group present at the beginning of the precursor RNA since labelling also occurs with GTP labelled with ^{3}H in the purine ring (Figure 3). It demonstrates that the complete nucleotide moiety is exchanged.

The exchange reaction shows the following properties: (i) it is specific for GTP, no 5′ terminal exchange of nucleotides is observed, for instance, when GTP is replaced by ATP (Figure 4, lanes a–d) and (ii) the exchange reaction shows sequence specificity. The yeast mtDNA insert was recloned behind a T7 phage promoter and run-off precursor RNA synthesized with T7 RNA polymerase. The 5′ terminal nucleotides of this precursor RNA differ from the precursor RNA synthesized from the SP6 phage promoter (see Table I). When incubated in the presence of [α-^{32}P]GTP various RNA splicing products (intron, intron + downstream exon and 3′ exon) are labelled but precursor RNA or the product of exon ligation, remain unlabelled, however (Figure 4, lanes g–i). The 5′ end of the SP6 precursor RNA reads 5′-GAA-3′ which can base-pair with the Internal Guide Sequence (IGS) (see Discussion). During the normal course of RNA splicing a guanosine nucleotide is added onto the 5′ end of the intron RNA providing the excised intron RNA with the sequence motif 5′-GAA-3′. We were therefore interested to see whether the intron displays the exchange reaction, too. Unlabelled intron RNA was gel purified and re-incubated under self-splicing conditions in the presence of [α-^{32}P]GTP. Figure 4 (lanes e and f) shows that the exchange reaction indeed occurs and rather efficiently for that matter. It has been observed that the *Tetrahymena* intron RNA can be labelled too at its 5′ end when incubated with [α-^{32}P]GTP, albeit under different conditions, which was postulated to be due to exchange of its 5′ terminal G (Zaug and Cech, 1985).

The 5′ terminal exchange reaction and GTP-mediated opening of the 3′ splice site are not restricted to the group I intron of the rRNA gene. Similar reactions were observed during self-splicing of another group I intron present in truncated precursor RNA transcribed from a cytochrome oxidase subunit I gene segment containing intron aI3 cloned behind the SP6 promoter (Figure 5). The number of splicing products from overall [α-^{32}P]UTP precursor RNA is more than usual due to a second position within the intron itself at which guanosine attack can occur (lane a; Tabak *et al.*, 1987). This is also the reason for the extra number of labelled RNA products that arise from splicing of unlabelled precursor RNA in the presence of [α-^{32}P]GTP (lane b). Among them are, however, the precursor RNA labelled due to the exchange reaction and splicing product of such labelled precursor RNA, the ligated exons, and the labelled 3′ exon due to attack at the 3′ splice site.

Discussion

During self-splicing of *in vitro* synthesized precursor RNA derived from the yeast mitochondrial split gene coding for large ribosomal RNA, we observed a number of RNAs that do not fit in with the normal group I splicing products. These include lariats (Arnberg *et al.*, 1986) and unexpected RNA products that arise from guanosine nucleotide attack at positions different from the normal site of nucleophilic attack: the 5′ splice site. By carrying out the self-splicing reaction with unlabelled precursor RNAs in the presence of [α-^{32}P]GTP and characterization of the labelled RNA products by partial T_1 ribonuclease digestion, these

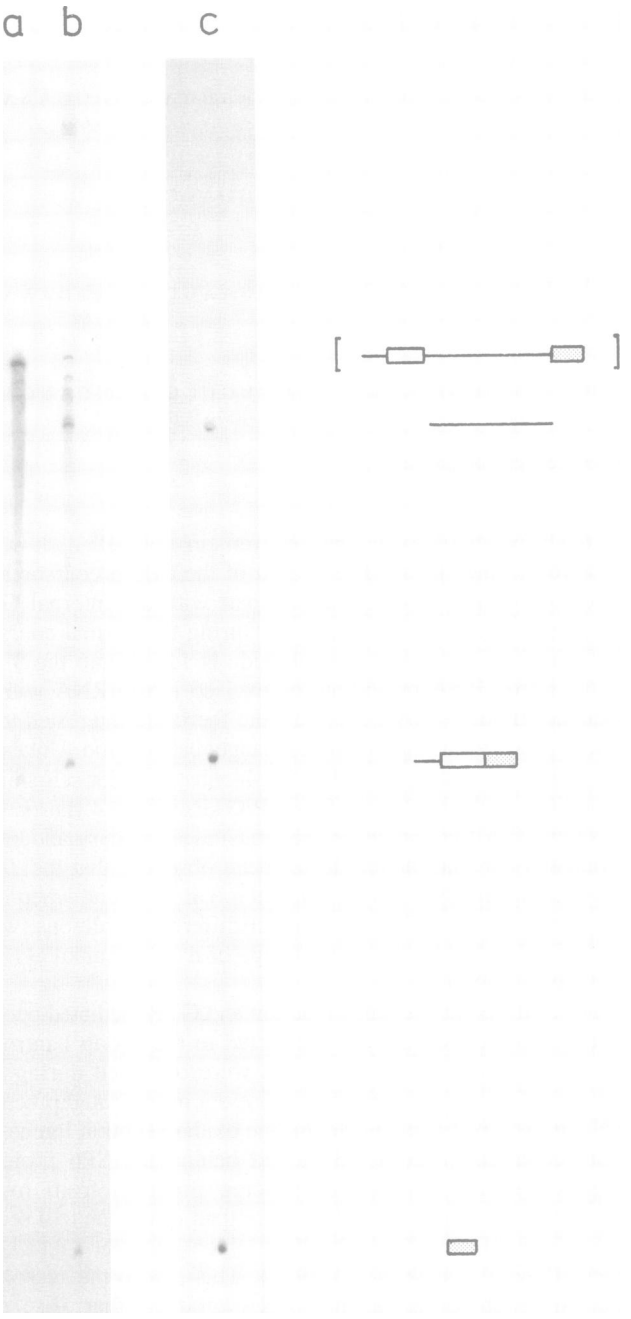

Fig. 3. self-splicing of unlabelled ribosomal precursor RNA in the presence of ^{3}H-labelled GTP. Gel purified unlabelled precursor RNA was incubated for 1 h at 40°C in the presence of [^{3}H]GTP. Splicing products were analysed by polyacrylamide gel electrophoresis. **Lane a**, overall [α-^{32}P]UTP-labelled precursor RNA (input); **lane b**, self-splicing products of RNA described in lane a; **lane c**, [^{3}H]GTP end-labelled splicing products from unlabelled precursor RNA.

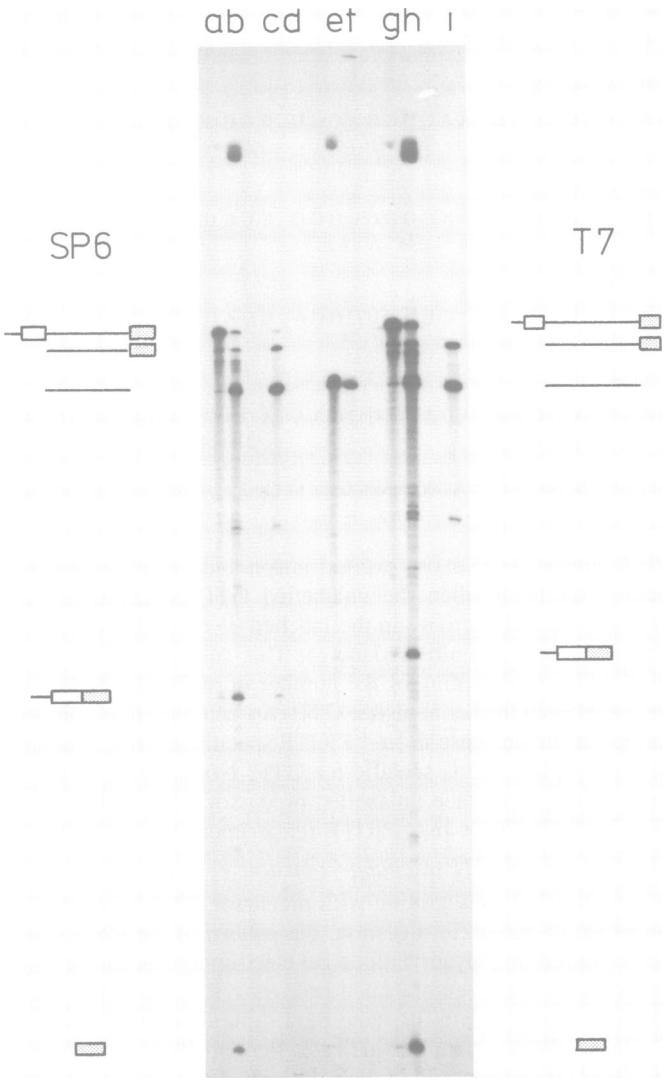

Fig. 4. Characteristics of the 5′ terminal guanosine nucleotide exchange reaction. Ribosomal precursor RNAs were synthesized from a SP6 promoter containing recombinant DNA **(lanes a–f)** or a T7 promoter containing recombinant DNA **(lanes g–i)**, see Materials and methods, and incubated under self-splicing conditions for 1 h at 40°C. RNA products were separated by polyacrylamide gel electrophoresis. **Lane a**, isolated [α-^{32}P]UTP-labelled precursor RNA (input); **lane b**, self-splicing products of [α-^{32}P]UTP-labelled precursor RNA; **lane c**, splicing products from unlabelled precursor RNA in the presence of [α-^{32}P]GTP (5 μM); **lane d**, similar to lane c but in the presence of [γ-^{32}P]ATP (0.5 μM) instead of [α-^{32}P]GTP, a concentration similar to that used in Figure 1, lane a, to detect the exchange reaction; **lane e**, isolated overall [α-^{32}P]UTP-labelled intron RNA (input); **lane f**, unlabelled intron RNA incubated under self-splicing conditions in the presence of [α-^{32}P]GTP; **lane g**, isolated [α-^{32}P]UTP-labelled precursor RNA synthesized from the T7 promoter (input); **lane h**, splicing products from RNA described in lane g; **lane i**, self-splicing products from unlabelled precursor RNA incubated in the presence of [α-^{32}P]GTP (5 μM).

positions were identified as the 3′ splice site and the phosphodiester bond between the first and second ribonucleotide of the precursor RNA and of excised intron RNA. Evidence for GTP attack at or near the 3′ splice site has also been reported for self-splicing of precursor RNA containing the first intron of the *Neurospora crassa* mitochondrial apocytochrome *b* gene (Garriga and Lambowitz, 1984).

Although both GTP-mediated reactions are rather inefficient, they were nevertheless very specific and therefore of interest if one wishes to extend the knowledge of RNA-mediated reactions in general. Cech and coworkers have proposed that the normal splicing reactions as well as the many partial reactions mediated by the *Tetrahymena* intron are carried out in one reaction centre in which the IGS functions as the active site and the GTP-cofactor and the last guanosine nucleotide of the intron RNA act as equivalent nucleophiles in the various reactions (Inoue *et al.*, 1986). The RNA structure illustrating these principles is shown in Figure 6a. It is attractive to assume that the IGS also partici-

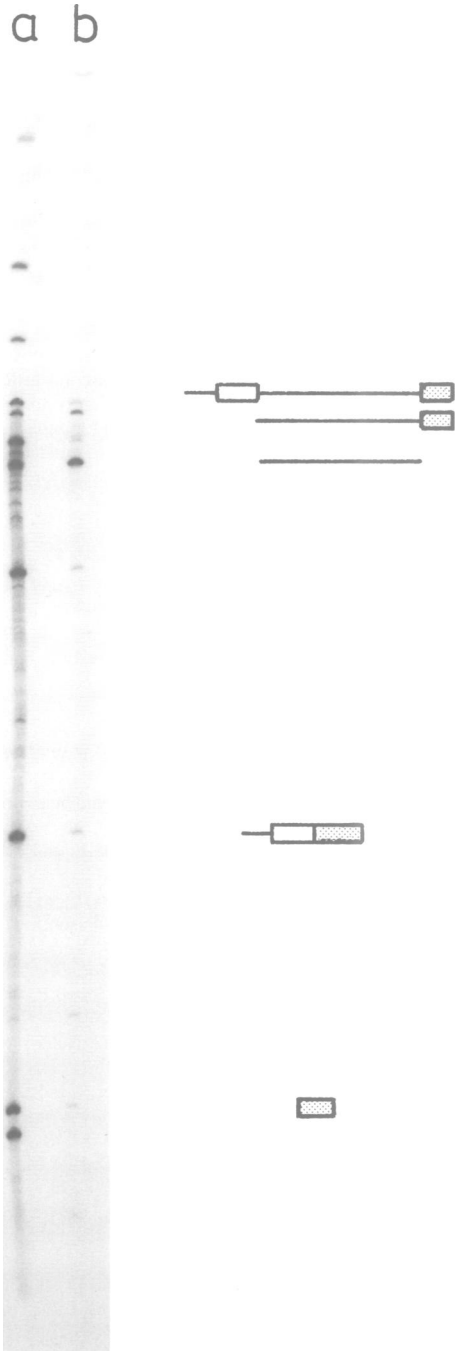

Fig. 5. Self-splicing RNA products from aI3-intron-containing precursor RNA. Sketches indicate the nature of some of the RNA products. For a full description of self-splicing of the aI3-intron-containing precursor RNA see Tabak *et al.* (1987). **Lane a**, self-splicing products of [α-^{32}P]UTP-labelled precursor RNA incubated for 1 h at 40°C. **Lane b**, self-splicing of unlabelled precursor RNA in the presence of [α-^{32}P]GTP.

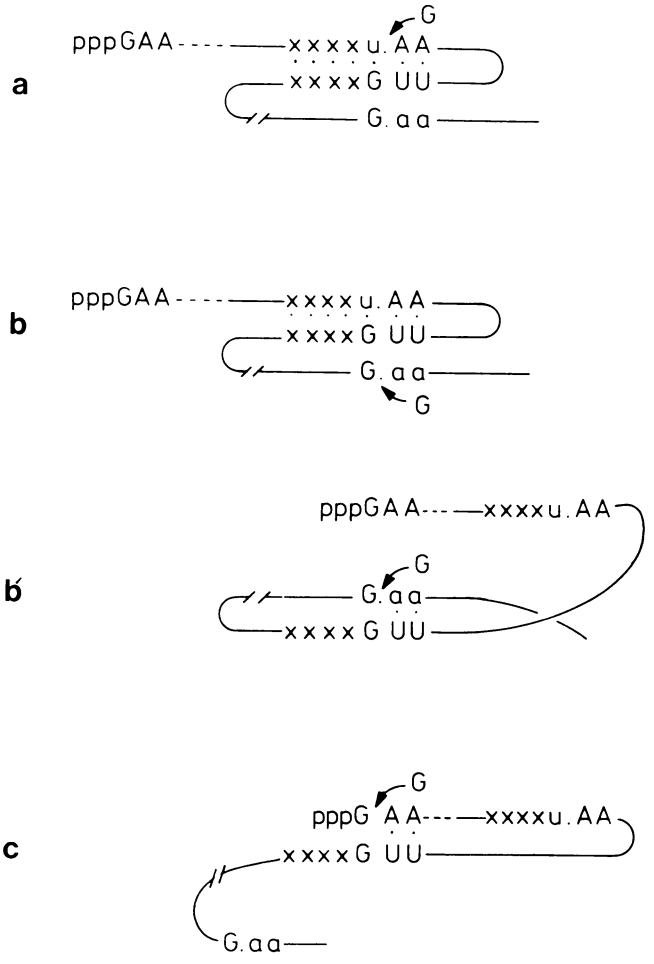

Fig. 6. Schematic presentation of possible base-pairing interactions with the IGS. The IGS is indicated by the sequence 3′-xxxxGUU-5′. Intron RNA nucleotides are indicated by capital letters, mitochondrial exon nucleotides by lower case letters, the SP6 RNA 5′ end is indicated by pppGAA . . . **(a)** Guanosine nucleotide attack at the 5′ splice site. **(b)** Guanosine nucleotide attack at the 3′ splice site. **(b′)** Other representation of (b). **(c)** Guanosine nucleotide attack at the first internucleotide bond of precursor RNA.

pates in carrying out the new reactions that we have described in this paper: the 5′ terminal exchange reaction and GTP-mediated opening of the 3′ splice site. In all cases a 5′-GAA-3′ sequence motif is involved which can base-pair with the IGS (Figure 6, lanes b, b′ and c). This GAA sequence is present at the 5′ end of precursor RNA initiated at the SP6 promoter, at the 5′ end of the excised ribosomal intron RNA and at the 3′ splice site. The aI3 intron of the coxI gene mediates the same reactions. Part of its IGS (3′-GUU-5′) is identical to the IGS of the ribosomal RNA intron and the GAA sequence is also present at the 3′ splice site of the aI3 intron. The rRNA intron and aI3-intron-mediated reactions can therefore all be explained on the same basis: guanosine nucleophilic attack opens the phosphodiester bond 5′ to the AA dinucleotide sequence, which can be aligned with the IGS. Alteration of the pppGAA sequence at the 5′ end of the precursor RNA abolishes the exchange reaction which is in support of the interpretation given in Figure 6.

GTP-mediated opening of the 3′ splice site is not completely unexpected since the phosphodiester bond at this position is strained and susceptible to site-specific hydrolysis in the case of the *Tetrahymena* intron (Inoue *et al.*, 1986). More surprising is the GTP exchange reaction at the 5′ end of precursor RNA initiated from the SP6 promoter. The first 27 nucleotides are derived from the SP6 bacteriophage genome and we consider it unlikely that this part of the RNA fortuitously participates in folding together with the intron core structure to put the first phosphodiester bond under strain in a way comparable with the 3′ splice site. It suggests that only very short RNA sequences need to interact with the reaction centre to result in sequence specific cleavage. In this respect the behaviour of this intron is remarkably

similar to the action of DNA restriction endonucleases and compares well with the *Tetrahymena* intron, a shortened form of which can act like a restriction endonuclease (Zaug *et al.*, 1986). An interesting difference is that in the *Tetrahymena* intron-catalysed reaction the RNA to be cut interacts with the 3′ part of the IGS resembling the first step of the normal self-splicing reaction: opening of the 5′ splice site. In the yeast mitochondrial intron mediated reactions the GAA sequence is proposed to interact with the 5′ part of the IGS which is in that respect analogous to the second step of the splicing reaction: opening of the 3′ splice site.

The rather low efficiency of the exchange reaction at the 5′ end of the precursor may be due to competition with the 3′ splice site for the same position of the IGS. Indeed, although we have not yet systematically compared the rates of both reactions, the qualitative results presented here suggest that the exchange reaction at the 5′ end of the excised intron occurs much more readily.

We cannot exclude the possibility that to explain the mechanism of the two reactions there is no need at all for base-pairing interactions with the IGS. Also, for some of the more conventional self-splicing reactions there is insufficient support for the contribution of base-pairing. Although from site-directed mutagenesis experiments there is firm support for the interaction of the 5′ exon border with the IGS (Waring *et al.*, 1986; Been and Cech, 1986) as proposed in the model of Davies *et al.* (1982). such evidence is thus far lacking for the interaction of the 3′ exon with the IGS (Been and Cech, 1985). It remains striking, however, that also in new examples of group I self-splicing introns the positions in the RNA at which cleavage occurs can always be aligned via base-pairing with the IGS (Tabak *et al.*, 1987).

Whatever the nature of interaction with the IGS one may wonder why every GAA sequence in the RNA does not run the risk of being cut. The 3′ splice site is of course designed to come into close contact with the reaction centre; the 5′ end of the precursor RNA may behave like a rather flexible unbase-paired arm which can easily diffuse into the reaction centre. This may be much more difficult for other parts of the intron RNA which are restrained in their movement by participating in the formation of the core structure.

We have tried to explain the two new reactions characterized in this paper following established principles of group I self-splicing as worked out by Cech and coworkers. Further work is needed to see whether some of our ideas concerning these reactions can withstand a critical test or will reveal that entirely new principles are involved.

Materials and methods

Restriction enzymes were from New England Biolabs or Boehringer Mannheim. SP6 RNA polymerase was purchased from Amersham. Plasmid pT7-13 was from Gibco-BRL. T7 RNA polymerase was purified according to Davanloo *et al.* (1984). [α-^{32}P]GTP (400 Ci/mmol), [α-^{32}P]UTP (3000 Ci/mmol), [γ-^{32}P]ATP (3000 Ci/mmol) and [^{3}H]GTP (10–20 Ci/mmol) were from Amersham.

The recombinant DNA clones containing an intron-containing DNA fragment from the yeast mitochondrial large rRNA gene (pSP65-C21) and the third intron (aI3) from the cytochrome oxidase subunit I gene (pSP64-aI3) have been described before (Van der Horst and Tabak, 1985; Tabak *et al.*, 1987). The ribosomal intron DNA fragment (*Bam*HI fragment from pSP65-C21), which consists of 160 bp of 5′ exon sequences, the 1143-bp intron, 60 bp of the 3′ exon and 40–50 bp of vector DNA, was recloned in the *Bam*HI site behind the T7 promoter (T7-C21) in plasmid pT7-13.

In vitro transcription

pSP65-C21 was linearized by digestion with *Pvu*II, extracted with phenol, precipitated with ethanol and redissolved in 10 mM Tris–HCl (pH 7.5), 0.5 mM EDTA. The *in vitro* transcription mixture (20 μl) contained 0.5–1 μg linear plasmid DNA, 40 mM Tris–HCl (pH 7.5), 6 mM $MgCl_2$, 10 mM DTT, 4 mM spermidine, 500 μm NTPs, 5–10 μCi [^{32}P]UTP and 5 U SP5 RNA polymerase. The reaction was carried out for 30 min at 40°C brought to 1% SDS, extracted with phenol, passed through a Sephadex G50 column and nucleic acids were precipitated with ethanol.

In vitro splicing

Precursor RNA was isolated from gel and 200–400 ng of the RNA incubated with 100 mM $(NH_4)_2SO_4$, 50 mM Tris–HCl (pH 7.5), 60 mM $MgCl_2$, 0.2 mM GTP or 40 μCi [^{32}P]GTP for 1 h at 40°C in a final volume of 20 μl. The reaction was stopped by addition of EDTA to a final concentration of 20 mM. The volume was increased to 100 μl, the RNA was passed through a Sephadex G50 column and precipitated with ethanol. T_1 digestion was carried out as described in Van der Horst and Tabak (1985).

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References

Abelson,J.A. (1979) *Annu. Rev. Biochem.*, **48**, 1035–1069.
Arnberg,A.C., Van der Horst,G. and Tabak,H.F. (1986) *Cell*, **44**, 235–242.
Bass,B.L. and Cech,T.R. (1986) *Annu. Rev. Biochem.*, **55**, 599–629.
Been,M.D. and Cech,T.R. (1985) *Nucleic Acids Res.*, **13**, 8389–8408.
Been,M.D. and Cech,T.R. (1986) *Cell*, **47**, 207–216.
Cech,T.R. (1983) *Cell*, **34**, 713–716.
Cech,T.R. (1986) *Cell*, **44**, 207–210.
Davanloo,P., Rosenberg,A.H., Dunn,J.J. and Studier,F.W. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 2035–2039.
Davies,R.W., Waring,R.B., Ray,J.A., Brown,T.A. and Scazzocchio,C. (1982) *Nature*, **300**, 719–724.
Domdey,H., Apostel,B., Liu,R.J., Newman,A., Brody,E. and Abelson,J. (1984) *Cell*, **39**, 611–621.
Garriga,G. and Lambowitz,A.M. (1984) *Cell*, **38**, 631–641.
Greer,Ch.L., Peebles,C.L., Gegenheimer,P. and Abelson,J. (1983) *Cell*, **32**, 537–546.
Inoue,T., Sullivan,F.X. and Cech,T.R. (1986) *J. Mol. Biol.*, **189**, 143–165.
Konarska,M.M., Grabowski,P.J., Padgett,R.A. and Sharp,P.A. (1985) *Nature*, **313**, 552–557.
Padgett,R.A., Konarska,M.M., Grabowski,P.J., Hardy,J.F. and Sharp,P.A. (1984) *Science*, **225**, 898–903.
Padgett,R.A., Grabowski,P.J., Konarska,M.M., Seiler,S. and Sharp,P.A. (1986) *Annu. Rev. Biochem.*, **55**, 119–150.
Peebles,C.L., Gegenheimer,P. and Abelson,J. (1983) *Cell*, **32**, 525–536.
Peebles,C.L., Perlman,P.S., Mecklenburg,K.L., Petrillo,M.L., Tabor,J.H., Jarrell,K.A. and Chen,H.L. (1986) *Cell*, **44**, 213–223.
Ruskin,B., Krainer,A.R., Maniatis,T. and Green,M.R. (1984) *Cell*, **38**, 317–331.
Schmelzer,C. and Schweyen,R.J. (1986) *Cell*, **46**, 557–565.
Sharp,P.A. (1985) *Cell*, **42**, 397.
Tabak,H.F., Arnberg,A.C. and Van der Horst,G. (1985) In van Knippenberg,P.H. and Hilbers,C.W. (eds), *Structure and Dynamics of RNA*. Plenum Press, NATO ASI Series, Vol. 110, pp. 309–315.
Tabak,H.F., Van der Horst,G., Kamps.A.M.J.E. and Arnberg,A.C. (1987) *Cell*, **48**, in press.
Van der Horst,G. and Tabak,H.F. (1985) *Cell*, **40**, 759–766.
Van der Veen,R., Arnberg,A.C., Van der Horst,G., Bonen,L., Tabak,H.F. and Grivell,L.A. (1986) *Cell*, **44**, 225–234.
Waring,R.B., Towner,P., Winter,S.J. and Davies,R.W. (1986) *Nature*, **321**, 133–139.
Zaug,A.J. and Cech,T.R. (1982) *Nucleic Acids Res.*, **10**, 2823–2838.
Zaug,A.J. and Cech,T.R. (1985) *Science*, **229**, 1060–1064.
Zaug,A.J., Been,M.D. and Cech,T.R. (1986) *Nature*, **324**, 429–433.

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Note added in proof

A similar GTP exchange reaction at the 5′ end of precursor RNA is mediated by the *Tetrahymena* intron and explained on the same basis [J.V.Price, J.Engberg and T.R.Cech (1987) *J. Mol. Biol.*, in press].