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# Fingerprinting the folding of a group I precursor RNA

(RNA structure/splicing/native gel electrophoresis)

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ABSTRACT **Evidence that folding of the *Tetrahymena* pre-rRNA follows a defined path and is rate-determining for splicing at physiological temperatures is presented. Structural isomers were separated by native polyacrylamide gel electrophoresis and their splicing activities were compared. GTP binding selectively shifts the active form of the pre-RNA to an electrophoretic band containing both spliced and unspliced RNA. *In situ* chemical modification provides evidence for base-pair rearrangements in the 5′ exon and structural alterations in the intron core of partially and fully active forms. Transition to the fully active precursor requires high temperature, but the activation energy is lower than expected for opening of RNA helices. Implications for control of RNA conformation during splicing are discussed.**

Pre-RNA structure is essential for both recognition of processing sites and chemical catalysis. As in the case of proteins (1), the formation of RNA structures is likely to involve a series of metastable intermediates, between which RNA interactions are broken and reformed. For example, there is evidence that significant changes in RNA contacts occur during spliceosome formation and pre-mRNA splicing (2, 3). These structural changes are associated with splice site alignment and proofreading functions (4, 5) and are linked to ATP hydrolysis. Control of alternative RNA folding pathways provides a mechanism for genetic regulation (6).

We have used the self-splicing intron from *Tetrahymena* as a model for examining folding pathways in large RNAs. Like pre-mRNAs, group I precursors undergo multiple conformational changes during splicing. For example, binding of small RNA substrates in the active site of the intron occurs in at least two steps (7, 8). In the pre-RNA, 5′ splice site recognition depends on thermal renaturation, suggesting that a significant rearrangement of the RNA is required (9). After the first step of splicing, another conformational change replaces the guanosine added to the 5′ splice site (G1) with the 3′ terminal guanosine (G414) in the G-binding site (10, 11).

Nondenaturing polyacrylamide gel electrophoresis has been used to study the conformation of small RNAs (12) and intermolecular RNA interactions (13). We apply this technique to separation of multiple conformers of a *Tetrahymena* pre-RNA. Isolation of RNA structures permits characterization or fingerprinting of folding intermediates that equilibrate in solution. Each form of the pre-RNA described here is distinguished on the basis of 5′ and 3′ splice site activity. We use this method to identify a folding pathway and to measure the activation energy for conversion of two of the pre-RNA conformers.

## MATERIALS AND METHODS

**Preparation of RNA and Nondenaturing Gel Electrophoresis.** The 657-nt pre-RNA used in these experiments is encoded by pSW012 (14) and includes 242 nt of rRNA flanking sequence in addition to the *Tetrahymena* intron. RNA was transcribed with T7 RNA polymerase in the presence of [$\alpha$-$^{32}$P]ATP and passed over a G-100 column (Clontech) as described (9). RNA samples (2–6 pmol; 0.4–1.2 $\mu$M) were electrophoresed on 6% polyacrylamide gels (29:1, acrylamide/bisacrylamide) containing 34 mM Tris·HCl, 66 mM Hepes (pH 7.5), 0.1 mM EDTA, 10 mM $MgCl_2$ (13). Gels were run for 5–6 hr (15 W) at 10°C and visualized by autoradiography or storage phosphorescence (Molecular Dynamics).

For two-dimensional analyses, lanes from a native gel containing precursor RNA were visualized by autoradiography, excised, and placed at the bottom of an empty gel frame. Solutions containing GTP and 5′ exon RNA were applied to the slice and allowed to stand 2 min. A solution of 10 M urea was added before the second-dimension gel (8% polyacrylamide/100 mM Tris borate, pH 8.3/1 mM EDTA/8 M urea) was cast (15). A small piece of acrylamide containing xylene cyanol was placed next to the gel slice as a marker for the second dimension.

The composition of native gel bands was determined by two-dimensional electrophoresis as described above. Alternatively, the RNA was soaked from the native gel, precipitated with ethanol, and analyzed by electrophoresis on a 4% denaturing polyacrylamide gel. Mole fractions of precursor and product RNAs were calculated from the radioactivity in each band of the denaturing gel, normalized to the total radioactivity in the lane, and adjusted for the relative specific activity of each RNA.

**Chemical Modification.** The RNA in the native gel bands was modified by soaking chemical reagents directly into the acrylamide matrix (16, 17). Gel slices containing pre-RNA corresponding to band 1 or band 4 were located by comparison to adjacent lanes containing radiolabeled RNA and excised. All manipulations were carried out on ice. Dimethyl sulfate (DMS): Gel pieces were incubated with 20 $\mu$l of DMS (1:40 in 50% ethanol) for 0.5–2 min. The reaction was stopped with addition of 300 $\mu$l of 0.2 M 2-mercaptoethanol/0.3 M sodium acetate/20 mM EDTA. Kethoxal: 20 $\mu$l of kethoxal (70 mg/ml) was applied to the gel for 3–8 min, followed by 300 $\mu$l of 0.3 M sodium acetate/20 mM EDTA. RNA was soaked from the gel and precipitated with ethanol. Radiolabeled 5′ end-labeled primer (0.4 pmol) was extended with avian myeloblastosis virus reverse transcriptase (18, 19). Sequencing gels were visualized by autoradiography or by storage phosphorescence. Sites of modification were identified relative to dideoxynucleotide sequencing reactions of the same RNA. Relative intensities of modifications in bands 1 and 4 were determined and compared to control lanes derived from unmodified samples.

**Rate Determinations for Isomerization.** Radiolabeled pre-RNA was incubated in splicing buffer and 10% (vol/vol) glycerol/0.1% xylene cyanol. Aliquots were removed at various times and incubated an additional 3 min at 30°C

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Abbreviation: DMS, dimethyl sulfate.

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before nondenaturing electrophoresis. The radioactivity in each lane was quantitated and relative fractions of bands 1 and 4 were determined. Rate constants ($k_{obs}$) were determined from linear fits to $\ln(1 - f_{band4})$ vs. time over the first 1–3 min of the reaction. The extent of conversion ranged from 25–30% at 30°C (3 min) to 70–90% at 95°C (1 min). Above 45°C, the semilogarithmic plot deviated from linearity at longer times.

## RESULTS

**Separation of pre-rRNA Conformers.** *Tetrahymena* pre-RNA was transcribed and isolated without denaturation in order to preserve any RNA structure that may have formed during synthesis (9). As shown in Fig. 1*A*, electrophoresis in a 6% native polyacrylamide gel resulted in several distinct bands with a mobility that could correspond to the pre-RNA. The identity of the RNA in each band on the gel was determined by two-dimensional electrophoresis or by isolation of the RNA from the native gel and electrophoresis on separate denaturing polyacrylamide gels (data not shown). The results confirmed that bands 1 and 4 in Fig. 1 contain predominantly pre-RNA, while other bands correspond to spliced products that accumulate during transcription.

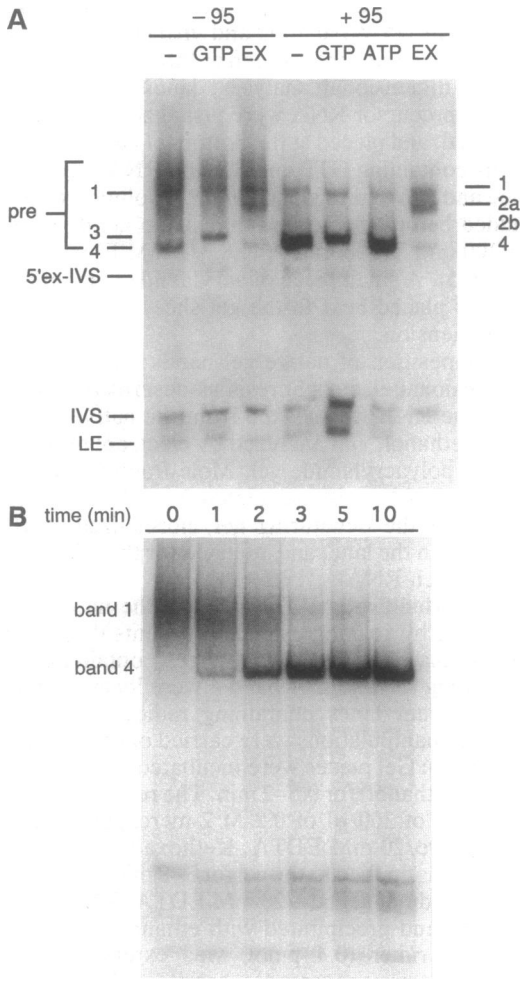



FIG. 1. Native gel electrophoresis of *Tetrahymena* pre-RNA. (*A*) A 657-nt precursor was applied to a 6% native polyacrylamide gel. Prior to loading, RNA was either heated to 95°C for 1 min and cooled rapidly in the presence of splicing buffer [100 mM $(NH_4)_2SO_3$/50 mM Hepes/5 mM $MgCl_2$, pH 7.5] (+95) or diluted in splicing buffer at room temperature (−95). Samples were also electrophoresed after the addition of 0.1 mM GTP, 1 mM ATP, or 70 μM 5′ exon RNA, 5′-rGGCUCUCU-3′ (lanes EX). Bands 1–4 denote species with the molecular weight of the pre-RNA. The positions of the 5′ exon-linear intron (IVS), linear IVS, and ligated exon (LE) products that accumulate during transcription are indicated. (*B*) Newly transcribed precursor RNA was incubated at 45°C in splicing buffer for 0–10 min and then cooled to 30°C before nondenaturing electrophoresis as described above. Positions of bands 1 and 4 are indicated.

We previously observed that pre-rRNA transcripts do not self-splice at the optimal rate when isolated from transcription reaction mixtures and that the reactivity of the precursors is increased by a heat/cool renaturation treatment (9, 20). Accordingly, the pre-RNA was heated to 95°C and cooled in the presence of buffer containing 5 mM $MgCl_2$ before electrophoresis. After renaturation, almost all of the radioactivity is concentrated in band 4 (Fig. 1*A*). Diffuse radioactivity in the lane is nearly eliminated, suggesting that the conformational heterogeneity of the RNA has been reduced. Incubation for a longer time (10 min) at 45°C resulted in nearly complete conversion of band 1 to band 4, as shown in Fig. 1*B*.

One explanation of these results might be that the slower band (band 1) is a pre-RNA dimer. Dimerization has been observed for other transcripts containing the *Tetrahymena* intron (G.-W. Fang and T. R. Cech, personal communication). To test this, the 657-nt transcript was mixed with a 1.3-kb *Tetrahymena* precursor before native gel electrophoresis. No bands corresponding to the expected heterodimer or homodimer were observed. Moreover, the pattern shown in Fig. 1 is highly reproducible and does not depend on RNA concentration over the range tested (0.4–1.2 μM).

The simplest interpretation of the data is that bands 1 and 4 represent two different conformations of single pre-RNA molecules. The large mobility shift between bands 1 and 4 indicates that the overall dimensions of the molecule change significantly. Moreover, the distribution of RNA between these two forms is altered by thermal renaturation, in agreement with results from self-splicing reactions (9). To verify that band 1 was related to band 4, the RNA was recovered from each region of the gel and applied to another nondenaturing gel. Prior to the second round of electrophoresis, half of each sample was heated to 95°C and cooled in splicing buffer. As shown in Fig. 2, most of the RNA recovered from band 1 is converted to band 4 after renaturation.

**Self-Splicing Activity.** To determine whether the RNA within these bands could self-splice, each lane was excised from the native gel, and a solution containing GTP was soaked in the gel matrix. This was followed by separation of

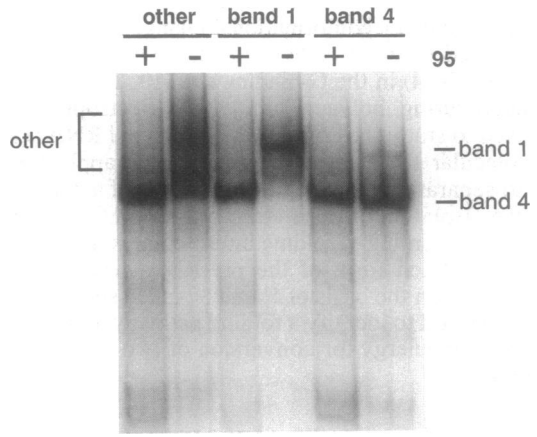



FIG. 2. Interconversion of RNA conformers. After electrophoresis as in Fig. 1, fragments of the polyacrylamide gel containing band 1, band 4, or other regions of the lane were excised. RNA was eluted from the gel fragments and applied to a second native gel as shown. Before loading, the sample was heated to 95°C (lanes +) or diluted in splicing buffer at room temperature (lanes −) as described in the legend to Fig. 1.

the RNA in a second dimension of denaturing electrophoresis. As shown in Fig. 3*B* and *E*, much of the RNA that originally migrated in band 4 appears as spliced products in the second dimension after the addition of GTP. Very little of band 1, however, is able to splice in the gel.

This experiment was repeated but with addition of an oligoribonucleotide containing the sequences of the 5′ exon instead of GTP. This oligomer, 5′-rGGCUCUCU-3′, can bind to the free internal guide sequence of the intron and participate in intermolecular exon ligation (trans-splicing) if the 3′ splice site is correctly activated (21). When the 5′ exon RNA is added after native gel electrophoresis, band 1 appears to trans-splice at least as well as band 4 RNA (Fig. 3*C* and *F*).

From these experiments, we conclude that band 4 contains an active form of the precursor RNA that undergoes both G addition to the 5′ splice site and exon ligation at the 3′ splice site. Band 1 pre-RNA is partially active, as it does not react rapidly with GTP but can readily participate in exon ligation. This result implies that band 1 represents a pre-RNA conformer where the 3′ splice site, but not the 5′ splice site, is activated. These observations correlate well with results from self-splicing reactions in solution (9), in which thermal renaturation of this pre-RNA is required for optimal intramolecular splicing but not for intermolecular splicing. Thus, native gel electrophoresis can separate precursor molecules with different activities.

**Effect of GTP and RNA Substrates.** This approach was next used to determine the effect of substrates on the structure of the pre-RNA. GTP or ATP was mixed with the precursor immediately prior to electrophoresis. As shown in Fig. 1*A*, addition of GTP causes a noticeable shift in the migration of the pre-RNA (band 3), whereas ATP, which is not a substrate for splicing, has no effect. In experiments not shown, 1 mM dGTP and ddGTP, which are weak competitive inhibitors of splicing (22), also do not cause a shift in gel mobility. To determine the composition, the RNA in band 3 was soaked from the gel and analyzed on denaturing gels. Band 3 contains a mixture of precursor and spliced products (free intron and ligated exons) in a 1:1 (mol/mol) ratio, with an average of 30% precursor and 70% products. The ratio of spliced and unspliced RNA depends on the time of incubation with GTP. Addition of 5′ exon oligonucleotide also causes new species to appear (bands 2a and 2b in Fig. 1*A*) that contain a mixture of unspliced RNA and products of intermolecular splicing. The precursor/products ratios in bands 2a and 2b are 40:60 and 70:30, respectively.

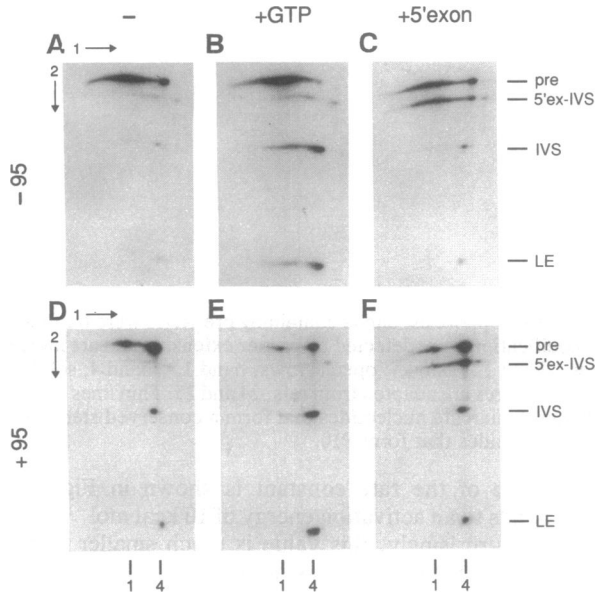



FIG. 3. Two-dimensional electrophoresis of pre-RNA conformers. After native gel electrophoresis as in Fig. 1, pre-RNA was run in a second-dimension polyacrylamide gel containing 8 M urea. In the first dimension, RNA was used before (*A–C*) or after (*D–F*) 95°C renaturation. Before the second dimension, gel slices were treated as follows: *A* and *D*, no treatment; *B* and *E*, addition of GTP; *C* and *F*, addition of 5′ exon RNA. Positions of bands 1 and 4 in the first dimension are indicated at the bottom. Pre, precursor; IVS, linear intron; LE, ligated exon RNA.

The fractions of RNA in each band of the native gel are listed in Table 1. Examination of the results with or without renaturation shows that almost all of the radioactivity in band 4 is shifted to band 3 in the presence of GTP, whereas the fraction of band 1 remains nearly constant. This is consistent with the idea that only band 4 pre-RNA contributes to formation of band 3. The nearly complete conversion to band 3 at 0.1 mM GTP is perhaps surprising, as the $K_m$ for splicing of this precursor is 0.04 mM (V.L.E., unpublished data) and $K_d$ (pG) is 0.09 mM for the L-21 Sca form of the intron (23). Since it is unlikely that the molecular weight of GTP can account for the observed mobility shift, we infer that GTP binding causes a reorganization of the pre-rRNA. The fact that band 3 contains an appreciable amount of unspliced RNA suggests that it is not merely a product complex but rather represents a pre-RNA structure in which no further rate-determining conformational change need occur before chemistry.

***In Situ* Chemical Modification.** The observed alteration in electrophoretic mobility and 5′ splice site activity must arise from a significant change in conformation of the pre-RNA. As a first step toward determining the nature of this structure difference, chemical modification experiments were carried out on the RNA in band 1 or band 4. Differences in DMS and kethoxal modification of form 1 and form 4 RNAs are summarized in Fig. 4. Many changes in the protection pattern are located in a conserved rRNA stem upstream of the splice junction. The ability of 5′ exon sequences to form an alternative secondary structure has been correlated with self-splicing efficiency (26). This change in secondary structure could account for the difference in 5′ splice site recognition between band 1 and band 4 RNA. Modification protection provides evidence for base-pairing changes in this region of the pre-rRNA.

Other differences in pre-RNA modification include increased protection of the first nucleotides of the intron in band 4 relative to band 1. This pattern is consistent with stronger base pairing between the 5′ exon and the internal guide sequence (P1) in band 4 pre-RNA. There are also changes in the modification of residues within the catalytic core of the intron. In particular, the nucleotides adjoining P3 (J2.1/3, J3/8, and J3/7) are less accessible in band 4 than in band 1. Differential modification of nucleotides joining double helices was also seen during partial unfolding of the sun Y intron (27). Our results could be explained either by increased rigidity of the intron structure in the more active complex or by movement of P1 relative to the core (28).

Table 1. Fraction of RNA in native gel bands

| | – 95 | | + 95 | |
|---|---|---|---|---|
| | – GTP | + GTP | – GTP | + GTP |
| Band 1 (3′ splice site active) | 0.89 | 0.86 | 0.11 | 0.19 |
| Band 3 (+ GTP) | — | 0.10 | — | 0.81 |
| Band 4 (both splice sites active) | 0.11 | 0.01 | 0.89 | — |

The amount of radioactivity in each lane of a 6% nondenaturing polyacrylamide gel was quantitated and the fraction of RNA in bands 1, 3, and 4 was computed relative to the sum of radioactivity in these bands. Values shown are averages of six experiments. + 95, Pre-RNA was renatured prior to electrophoresis; – 95, no treatment; + GTP, addition of 100 μM GTP; – GTP, none added; —, no radioactivity detected above background.

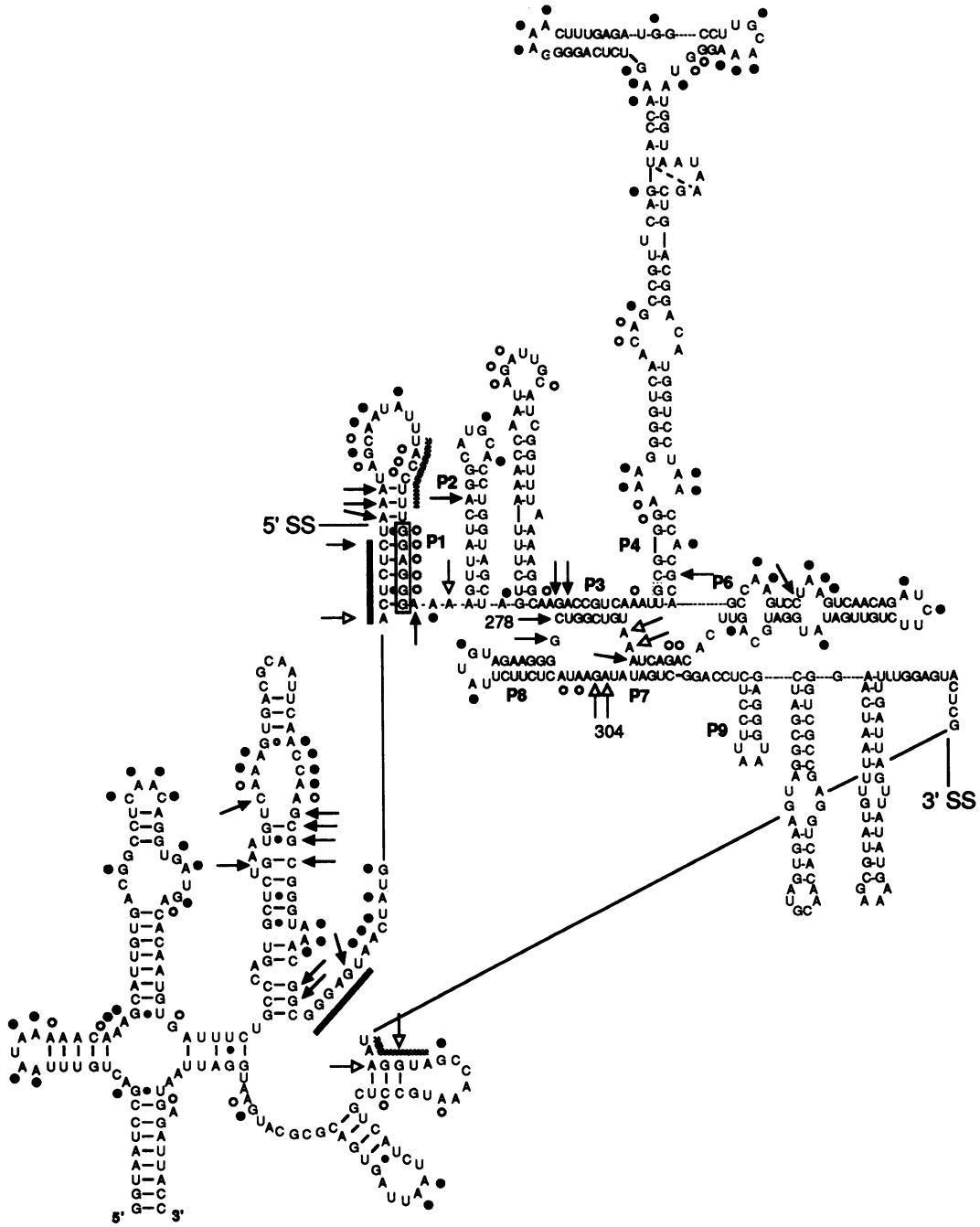



FIG. 4. *In situ* chemical modification of pre-RNA conformers. After native gel electrophoresis, bands containing pre-RNA were treated with either DMS or kethoxal by direct application of reagent to the gel slice. Sites of modification were detected by primer extension and are indicated relative to the secondary structure of the intron and the 26S rRNA. Solid arrows, band 1 > band 4; open arrows, band 1 < band 4; solid dots, band 1 = band 4 (medium strong); open dots, band 1 = band 4 (weak). Secondary structures are adapted from refs. 24 and 25. Thin lines represent covalent bonds between the intron and exon portions of the precursor. Heavy lines demarcate nucleotides that form a conserved stem–loop in the mature 26S RNA that competes with formation of P1; dashed lines denote nucleotides that form P10.

Interestingly, DMS modification of A304, near the region of contact between the P1 helix and the intron core (29), is enhanced in band 4. Accessibility of this nucleotide may be a result of the complete precursor structure, since it was not modified in earlier studies on the L-21 ribozyme form of the intron (29).

**Activation Barrier Between RNA Structures.** Facile conversion of band 1 to band 4 requires incubation at a high temperature, which ordinarily implies that the activation energy for the process is significant. To measure the energy barrier between forms 1 and 4, rates of formation of band 4 were measured at temperatures from 30°C to 95°C using the native gel assay (for example, see Fig. 1*B*). The temperature dependence of the rate constant is shown in Fig. 5 and corresponds to an activation energy of 10 kcal·mol$^{-1}$ (1 cal = 4.184 J). Surprisingly, this value is much smaller than expected for opening of an RNA hairpin (30, 31), although the midpoint of the transition is high.

## DISCUSSION

The results presented here suggest a sequence of conformational rearrangements, involving both exon and intron sequences, that leads to the first chemical step in splicing. Based on partial splice site recognition and stability in solution, the band 1 isomer can be regarded as a late folding

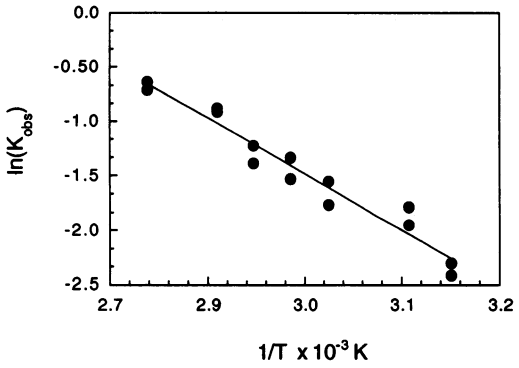
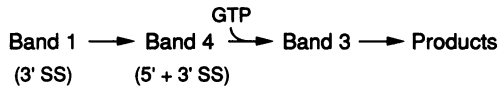



FIG. 5. Temperature dependence of band 1 to band 4 transformation. Logarithm of the rate of formation of band 4 RNA from band 1 ($min^{-1}$) is plotted vs. $1/T$ (K). Fractions of bands 1 and 4 were determined after native gel electrophoresis. Rate constants were taken from early times (1–3 min) over which the isomerization followed a single exponential. Line represents the best fit (least squares) to the data points in the linear range of the Arrhenius plot, which was 45°C to 85°C. Apparent activation energy is 9.9 $kcal \cdot mol^{-1}$.

intermediate of the pre-RNA. From these data, Scheme I can




Scheme I

be proposed. Band 1 RNA, in which only the 3′ splice site (3′ SS) is activated, isomerizes to band 4, where both splice sites (5′ + 3′ SS) are recognized. In the presence of GTP, band 4 converts to band 3. The precursor then appears to splice rapidly, followed by slow product dissociation.

Chemical modification and differences in 5′ splice site activity suggest that isomerization of forms 1 and 4 involves rearrangement of base pairs, accompanied by adjustment of non-Watson–Crick interactions. The low Arrhenius activation energy for this process indicates that few of these interactions are simultaneously broken in the transition state. A mechanism of base-pair exchange similar to branch migration would account for the shallow temperature dependence (12) and is consistent with a proposed model for the alternative structure of the pre-rRNA (32). On the other hand, the rates of interconversion are slow (0.09–0.8 $min^{-1}$), indicating a substantial free energy of activation. This in turn suggests that the entropy of activation for this process may be high.

This study demonstrates that self-splicing of the *Tetrahymena* pre-rRNA involves critical structural changes in the RNA. In particular, refolding of the 5′ exon is required for recognition of the 5′ splice site. This is in turn necessary for formation of an apparent "activated" splicing complex in the presence of GTP. The rate of isomerization (0.09 $min^{-1}$) is much slower than the rate of self-splicing (0.7 $min^{-1}$; ref. 9) at physiological temperatures (30°C). The presence of well-populated intermediates and a late transition state are reminiscent of protein folding and may indicate general features of biopolymers (1, 33, 34). These results highlight the general importance of folding mechanisms in determining the rate of RNA processing and emphasize the likelihood that acceleration of these conformational changes will play an important role in genetic regulation of RNA expression.

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1. Kim, P. S. & Baldwin, R. L. (1990) *Annu. Rev. Biochem.* **59**, 631–660.
2. Guthrie, C. (1991) *Science* **253**, 157–163.
3. Green, M. R. (1991) *Annu. Rev. Cell Biol.* **7**, 559–599.
4. Burgess, S. M. & Guthrie, C. (1993) *Cell* **73**, 1377–1391.
5. Sontheimer, E. J. & Steitz, J. A. (1993) *Science* **262**, 1989–1996.
6. Olender, D. L., Zurawski, G. & Yanofsky, C. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5524–5528.
7. Bevilacqua, P. C., Kierzek, R., Johnson, K. A. & Turner, D. H. (1992) *Science* **258**, 1355–1358.
8. Herschlag, D. (1992) *Biochemistry* **31**, 1386–1399.
9. Emerick, V. L. & Woodson, S. A. (1993) *Biochemistry* **32**, 14062–14067.
10. Been, M. D. & Perrotta, A. (1991) *Science* **252**, 434–437.
11. Michel, F., Hanna, M., Green, R., Bartel, D. P. & Szostak, J. W. (1989) *Nature (London)* **342**, 391–395.
12. LeCuyer, K. A. & Crothers, D. M. (1993) *Biochemistry* **32**, 5301–5311.
13. Pyle, A. M., McSwiggen, J. A. & Cech, T. R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8187–8191.
14. Woodson, S. A. (1992) *Nucleic Acids Res.* **20**, 4027–4032.
15. Branch, A. P., Benedfeld, B. J., Paul, C. P. & Robertson, H. P. (1989) *Methods Enzymol.* **180**, 418–442.
16. Law, R., Kuwabara, M. D., Briskin, M., Fasel, N., Hermanson, G., Sigman, D. S. & Wall, R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9160–9164.
17. Straney, D. C. & Crothers, D. M. (1987) *J. Mol. Biol.* **193**, 279–292.
18. Inoue, T. & Cech, T. R. (1985) *Proc. Natl. Acad. Sci. USA* **76**, 1760–1764.
19. Moazed, D., Stern, S. & Noller, H. F. (1986) *J. Mol. Biol.* **187**, 399–416.
20. Walstrum, S. & Uhlenbeck, O. C. (1990) *Biochemistry* **29**, 10573–10576.
21. Inoue, T., Sullivan, F. X. & Cech, T. R. (1985) *Cell* **43**, 431–437.
22. Bass, B. L. & Cech, T. R. (1985) *Biochemistry* **25**, 4473–4477.
23. McConnell, T. S., Cech, T. R. & Herschlag, D. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 8362–8366.
24. Murphy, F. L. & Cech, T. R. (1993) *Biochemistry* **32**, 5291–5300.
25. Gutell, R. R., Schnare, M. N. & Gray, M. W. (1990) *Nucleic Acids Res.* **18** (Suppl.), 2319–2330.
26. Woodson, S. A. & Cech, T. R. (1991) *Biochemistry* **30**, 2042–2050.
27. Jaeger, L., Westhof, E. & Michel, F. (1993) *J. Mol. Biol.* **234**, 331–346.
28. Wang, J. F. & Cech, T. R. (1992) *Science* **256**, 526–529.
29. Pyle, A. M., Murphy, F. L. & Cech, T. R. (1992) *Nature (London)* **358**, 123–128.
30. Freier, S. M., Kierzek, R., Caruthers, M. H., Neilson, T. & Turner, D. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9373–9377.
31. Crothers, D. M., Cole, P. E., Hilbers, C. W. & Shulman, R. G. (1974) *J. Mol. Biol.* **87**, 63–88.
32. Woodson, S. A. & Emerick, V. L. (1993) *Mol. Cell. Biol.* **13**, 1137–1145.
33. Camacho, C. J. & Thirumalai, D. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6369–6372.
34. Sosnick, T. R., Mayne, L., Hiller, R. & Englander, S. W. (1994) *Nat. Struct. Biol.* **1**, 149–156.