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Secondary structure of the *Tetrahymena* ribosomal RNA intervening sequence: Structural homology with fungal mitochondrial intervening sequences

(RNA splicing/free energy of RNA folding/computer prediction of RNA structure/maturase)

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ABSTRACT **Splicing of the ribosomal RNA precursor of *Tetrahymena* is an autocatalytic reaction, requiring no enzyme or other protein *in vitro*. The structure of the intervening sequence (IVS) appears to direct the cleavage/ligation reactions involved in pre-rRNA splicing and IVS cyclization. We have probed this structure by treating the linear excised IVS RNA under nondenaturing conditions with various single- and double-strand-specific nucleases and then mapping the cleavage sites by using sequencing gel electrophoresis. A computer program was then used to predict the lowest-free-energy secondary structure consistent with the nuclease cleavage data. The resulting structure is appealing in that the ends of the IVS are in proximity; thus, the IVS can help align the adjacent coding regions (exons) for ligation, and IVS cyclization can occur. The *Tetrahymena* IVS has several sequences in common with those of fungal mitochondrial mRNA and rRNA IVSs, sequences that by genetic analysis are known to be important *cis*-acting elements for splicing of the mitochondrial RNAs. In the predicted structure of the *Tetrahymena* IVS, these sequences interact in a pairwise manner similar to that postulated for the mitochondrial IVSs. These findings suggest a common origin of some nuclear and mitochondrial introns and common elements in the mechanism of their splicing.**

Genes coding for rRNA are interrupted by intervening sequences (IVSs) in several species of *Tetrahymena* and in *Physarum polycephalum* (1). These split genes are transcribed to give an IVS-containing pre-rRNA which is then spliced in the nucleus (1). In the case of *Tetrahymena*, the IVS is excised as a unique linear molecule which is subsequently cyclized (2, 3). Both linear and circular forms of the IVS RNA appear to be rapidly degraded in the nucleus and not exported to the cytoplasm (4). The presence of stop codons in all possible reading frames (5, 6) also makes it unlikely that the excised IVS could serve as a mRNA. Splicing of the *Tetrahymena* pre-rRNA is a novel case of an autocatalytic reaction that, at least *in vitro*, requires no enzyme or other protein (7). It remains possible that proteins accelerate the rate of splicing or perhaps even regulate the process *in vivo*.

RNA splicing is also required for the expression of genes coding for the large rRNA and various mRNAs in the mitochondria of fungi. Many of the mitochondrial IVSs contain long open reading frames in phase with the upstream exon. The occurrence of *trans*-recessive splicing-defective mutants that map within three of the cytochrome *b* gene (*cob*) introns (8) has led to the proposal of intron-encoded maturases (9). The introns in yeast and *Neurospora* large mitochondrial rRNA genes also contain long open reading frames (10, 11); these apparently do not encode maturases because splicing of yeast mitochondrial rRNA continues in the absence of protein synthesis (12). The occurrence of nuclear mutants in mitochondrial mRNA and rRNA splicing (13, 14) makes it seem likely that proteins other than the maturases are required for the process. Finally, there is a class of splicing-defective mutants mapping within yeast mitochondrial mRNA gene introns that are *cis*-dominant (15–18). Several groups have proposed that these sequences define secondary/tertiary structure elements that fold the pre-mRNA into a configuration that allows it to serve as a substrate for the splicing process (16, 19, 20).

Based on the considerations given above, the splicing of nuclear pre-rRNA and mitochondrial pre-mRNA appear to be dissimilar processes. It therefore was surprising when Burke and RajBhandary (11) found that one of the *cis*-dominant sequences of the yeast mitochondrial mRNA introns that is highly conserved among fungal mitochondrial mRNA and rRNA introns is also present in the *Tetrahymena* and *Physarum* nuclear rRNA introns. We now find that several other sequences are highly conserved between nuclear rRNA and mitochondrial introns. Furthermore, we present experimental evidence for a secondary structure of the *Tetrahymena* IVS in which the conserved sequences interact in a pairwise manner similar to that postulated for the mitochondrial introns (19, 20).

MATERIALS AND METHODS

Preparation of End-Labeled IVS RNA. Unspliced pre-rRNA was transcribed from pIVS11 DNA *in vitro* by using *Escherichia coli* RNA polymerase (7). After deproteinization, the RNA was incubated with [α-^{32}P]GTP under splicing conditions at 30°C. This results in autoexcision of the IVS and covalent addition of the GTP to its 5′ end (7). Each preparation of purified IVS RNA consisted mainly of molecules with a native structure as judged by their ability to undergo autocyclization.

Nuclease Treatment of Native RNA. Purified IVS RNA was treated with RNase T1 or RNase T2 (both from Sankyo) in 10 μl of 200 mM NaCl/5 mM $MgCl_2$/10 mM Tris·HCl, pH 7.5, containing 0.25 μg of deproteinized tRNA per μl. The effect of varying the $MgCl_2$ concentration in the range 0–15 mM was also tested for RNase T1. Treatment with S1 nuclease (New England Nuclear) was normally done in 200 mM NaCl/5 mM $MgCl_2$/0.1 mM $ZnSO_4$/25 mM $NaC_2H_3O_2$, pH 5.5, containing

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Abbreviations: IVS, intervening sequence or intron; exon, coding sequence interrupted by an IVS; *cob*, cytochrome *b* gene.

tRNA (0.25 μg/μl). Treatment with cobra venom nuclease (a gift from J. Vournakis) was done in 200 mM NaCl/2 mM $MgCl_2$/10 mM Tris·HCl, pH 7.5, containing tRNA (0.25 μg/μl). For all four enzymes, temperatures of 0°C, 10°C, 20°C, and 30°C were tested. Reactions were stopped by chilling the mixture on ice and adding 10 μl of 100 mM Tris/80 mM boric acid/1 mM EDTA/10 M urea/0.5% sodium dodecyl sulfate/0.1% bromophenol blue/0.1% xylene cyanole containing 2 μg of proteinase K.

Free Energy Values. The secondary structures were calculated by using values for the free energies that are different from those used earlier (21). The free energy values we now use are those tabulated by Salser (22) except: (*a*) all values are rounded to two significant figures; (*b*) the values for bulges of two or more bases are increased by 2.2 kcal (1 cal = 4.184 J) (23); (*c*) hairpin loops are given the same free energy whether they are closed by a G·C or an A·U base pair [the values are +7.0 kcal for three unbonded bases in the loop, +4.5 kcal for four bases, +4.1 for five bases, +4.3 for six bases, and +4.5 for seven bases, and the values for hairpins closed by a G·C base pair (tabulated in ref. 22) for all other hairpin loops (24, 25)].

Calculation of Secondary Structure. A computer program was used to find the lowest-free-energy secondary structure (26). The program allows one to incorporate nuclease-digestion data by specifying that certain bases be base-paired or not base-paired.

SECONDARY STRUCTURE OF THE *TETRAHYMENA* IVS RNA

The 5′-end-labeled IVS was digested under nondenaturing conditions with RNase T1, which can be used as a probe for guanosine residues in single-stranded regions (27). Cleavage sites were located by denaturing the RNA and subjecting it to electrophoresis on a sequencing gel (Fig. 1*a*). The strong RNase T1 cleavages occurred independently of temperature in the range 0–30°C. At 30°C, a slight degree of cleavage at most of the guanosines in the molecule was apparent. Temperatures above 30°C were not routinely used because most of the RNA cyclized, making the 15-mer that is released upon cyclization the predominant end-labeled species. The same RNase T1 cleavage sites were found over a wide range of nuclease digestion, from an average of less than one cut per molecule to the higher levels shown in Fig. 1*a*.

The lowest-free-energy secondary structure of the linear IVS was computed. When no experimental data were included in the analysis, the predicted structure (not shown) had a calculated ΔG of −134.0 kcal/mol. The structure was then recomputed subject to the constraint that the guanosines at positions 7, 22, 23, 44, 73, and 77, which were major sites of RNase T1 cleavage, be unpaired or followed by unpaired bases. The resulting secondary structure, shown in Fig. 2, had a calculated ΔG of −131.7 kcal/mol; it is only 2.3 kcal less stable than the unconstrained structure. For several reasons, this free energy is well within the range of reasonable structures expected from this calculation. The free energy parameters were obtained from a limited collection of model oligonucleotides. No attempt was made to correct for the effect of solution composition or to include tertiary interactions, both of which are poorly understood.

After the structure shown in Fig. 2 was derived, we mapped additional RNase T1 cleavage sites near the 3′ end of the molecule. Sites of cleavage of the double-strand-specific cobra venom RNase (29) and of single-strand-specific S1 nuclease (27) and RNase T2 were similarly determined (Fig. 1) and are summarized in Fig. 2 *Upper*. These cleavages were consistent with the predicted secondary structure, which provided some confidence that the prediction was largely correct.

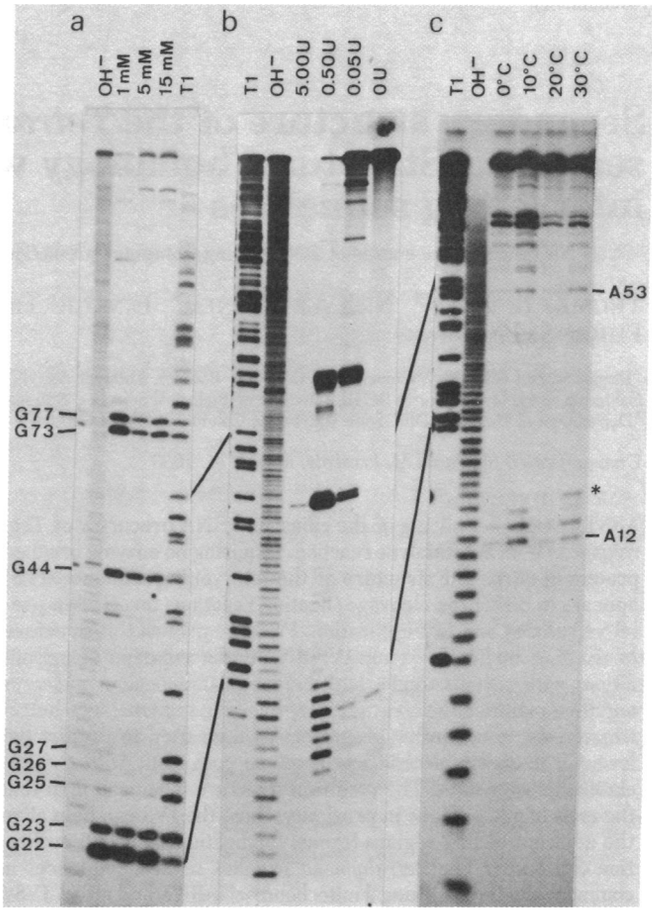

FIG. 1. Nuclease digestion of 5′-end-labeled IVS RNA under native conditions. Autoradiograms of three representative sequencing gels are shown. Size markers (lanes OH^- and T1) on gels are ladder of all the nucleotides produced by partial alkaline hydrolysis and ladder of all the guanosine residues produced by partial RNase T1 digestion under totally denaturing conditions (28). (*a*) RNase T1 digestion for 15 min at 30°C in 1, 5, and 15 mM $MgCl_2$. Minor cleavage sites at G32 and G39 apparent here were not seen when digestion was performed at lower temperatures, whereas minor sites at G25–G27 were apparent at all temperatures. (*b*) S1 nuclease digestion for 10 min at 30°C at pH 4.5, with decreasing concentrations of nuclease. Digestion at pH 5.5 gave the same results. (*c*) Cobra venom RNase digestion for 30 min at 0°C and 10°C and for 15 min at 20°C and 30°C. On the original autoradiogram and in other experiments, slight digestion was apparent at nucleotides 18–21. *, 15-mer produced by autocyclization of the IVS RNA, which begins to occur at 30°C. This 15-mer, like the S1 nuclease and cobra venom RNase products, has a 3′-hydroxyl terminus and therefore runs as if it were one nucleotide larger compared to the T1 and OH^- markers. Electrophoresis was on 12% polyacrylamide/7 M urea gels in *a* and *b* and 20% polyacrylamide/7 M urea gel in *c*.

The existence of some of the helical stems is supported by compensatory base changes (30) in the IVS of a different species, *T. pigmentosa* (5). Compensatory base changes occur in the a and j helices (Fig. 2 *Lower*). In addition, single base changes at positions 51 and 116 result in the conversion of a G·U to an A·U base pair, preserving the structure. Major differences between the two sequences occur at the ends of three of the hairpins (f, h, and k), as already noted by Kan and Gall (6). Because of their location, these deletion-substitutions do not disrupt the core structure of the molecule. Consistent with this view, a cloned variant IVS (6) that contains a deletion-substitution in a peripheral region (e in Fig. 2 *Lower*) still excises and cyclizes itself *in vitro* (7).

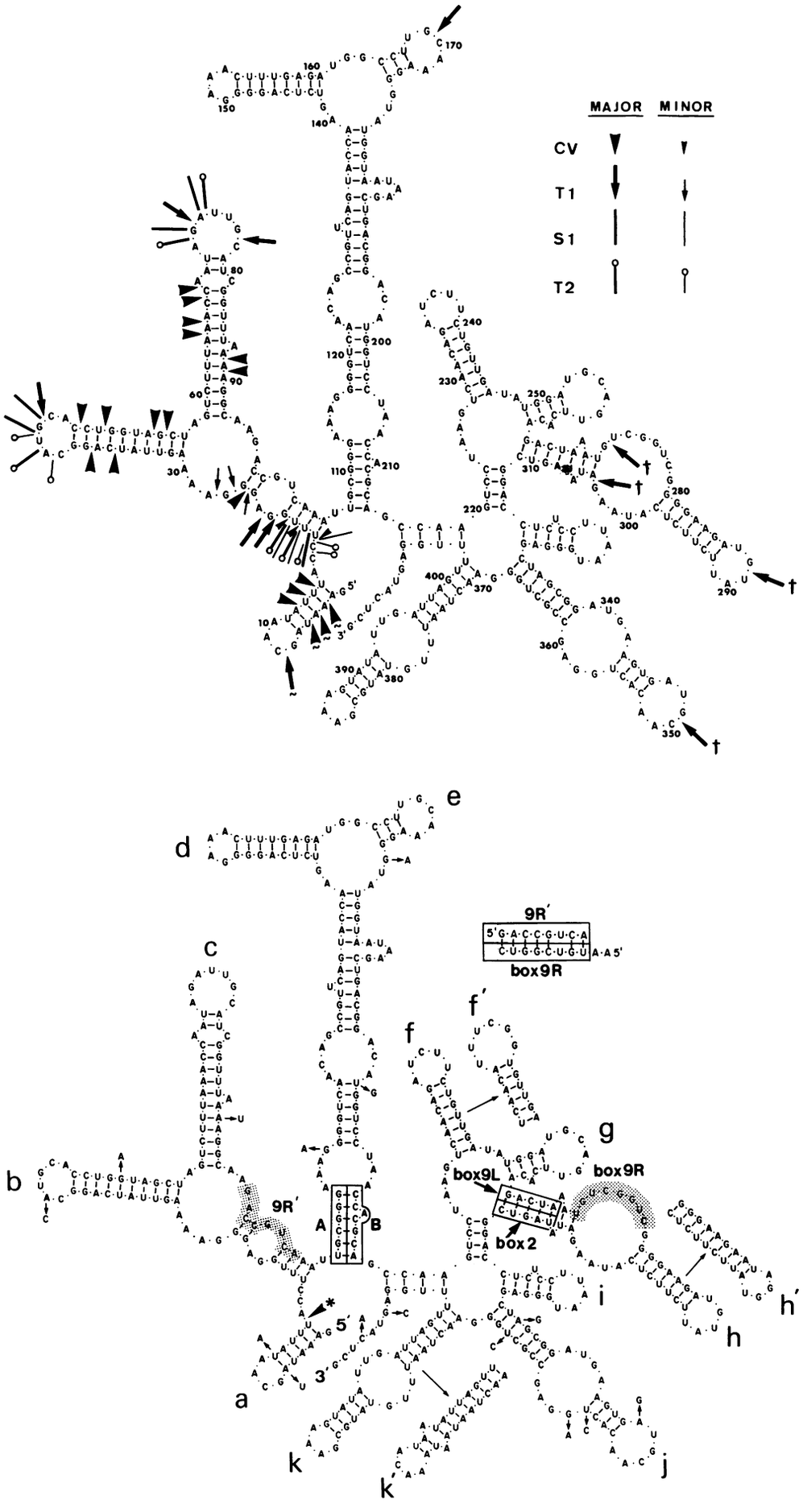

FIG. 2. Model of the secondary structure of the excised IVS RNA. (*Upper*) Sites of cleavage of the RNA under native conditions by double-strand-specific cobra venom RNase (CV) and by single-strand-specific nucleases T1, S1, and T2. Gel composition and electrophoresis time were varied to allow resolution of different regions of the molecular weight spectrum. Except for RNase T1, cleavage sites beyond position 100 have not yet been accurately mapped. The data support the conclusion that the middle of the molecule is relatively inaccessible to the single-strand-specific nucleases. There were no apparent S1 nuclease cleavages between nucleotides 75 and ≈212. Cobra venom RNase, on the other hand, did cleave in the middle region of the RNA. †, At these positions on the sequencing gel, individual G residues could no longer be seen on the T1 ladder; cleavage sites were therefore determined by reading clusters of Gs. ~, Cleavage seen only at higher levels of digestion; therefore, we do not know that these cleavages are occurring on intact molecules. (*Lower*) Phylogenetic comparisons. Boxes denote base-paired portions of those sequences that are conserved between nuclear rRNA introns and mitochondrial introns. Shading denotes the *box*9R and 9R' regions, which are conserved in position but not in nucleotide sequence. These may pair in a tertiary interaction, as detailed in the *Inset*. Arrows, single bases and hairpins that differ in the IVS of *T. pigmentosa* (5). *, Site of addition of the 3' end to form circular IVS with release of hairpin a.

SEQUENCE HOMOLOGY BETWEEN NUCLEAR AND MITOCHONDRIAL INTRONS

Several groups have identified *cis*-dominant, splicing-defective mutations within mitochondrial introns (15–18). To date, such mutants define four functionally important regions of intron 4 of the yeast *cob* gene: *box*9L, *box*9R, *box*2, and 9R′ (Fig. 3*a*). Based on their sequences, these four regions appear to be capable of forming two base-paired stems. The *box*9L·*box*2 pairing is shown in Fig. 4. Several groups have noted that the *box*9L and *box*2 sequences are present in five other yeast mitochondrial introns and in mitochondrial introns in other fungi (11, 16, 19, 20). The *box*9L homologs in intron 4 of the yeast *oxi3* gene (coding for subunit 1 of cytochrome oxidase) and in intron 5 of the yeast *cob* gene are also sites of *cis*-dominant splicing-defective mutations (ref. 16; unpublished data), confirming the importance of this conserved sequence in splicing.

There is strong genetic evidence for base-pairing of the other pair of mutable sequences in *cob* intron 4, denoted here as *box*9R and 9R′. A second-site revertant of a *box*9R mutation is located in 9R′, and DNA sequence analysis shows that the double mutant could form an A·U base pair instead of the wild-type G·C base pair (36). This pair of sequences is not conserved in the other mitochondrial introns. However, the possibility of forming a base-paired stem between *box*9R (sequences a few nucleotides downstream from *box*9L) and 9R′ (sequences nearer to the 5′ exon–intron boundary) is conserved.

Waring *et al.* (20) noted the presence of another pair of sequences, termed A and B, between the 9R′ and *box*9L sequences. They pointed out that A and B are present in many yeast and *Aspergillus* introns and that five bases of A are complementary to five bases of B (Fig. 4). No splicing-defective mutants have yet been located in these sequences.

Burke and RajBhandary (11) previously noted that the *box*9 sequence is present in the nuclear rRNA introns of *Tetrahymena* and *Physarum*. We now note that the other conserved mitochondrial sequences—A, B, and *box*2—also occur in the *Tetrahymena* intron (Figs. 3 and 4). In addition, *Physarum* intron 1 contains all four sequences; intron 2 contains clear homologs of the *box*9L and *box*2 sequences but lacks A and B. The *box*9L sequence in *Physarum* intron 1 contains a single base change in the pentanucleotide sequence thought to be involved in base pairing, but a compensatory change in the *box*2 sequence preserves the potential for pairing. The conserved sequences occur in the same order in the nuclear introns as they occur in the mitochondrial introns. The distance between adjacent conserved sequences is highly variable in both systems.

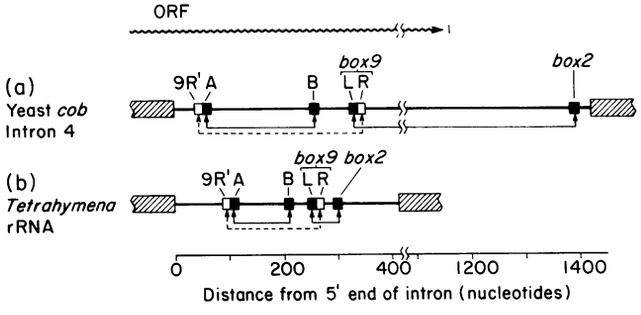

FIG. 3. Organization of conserved sequences in mitochondrial and nuclear introns. Cross-hatched areas, exons; solid boxes, conserved intron sequences; open boxes, intron sequences conserved only in their potential for base-pairing. Boxed sequences are enlarged slightly for clarity. Solid and dashed double-headed arrows show postulated secondary and tertiary base-pairing interactions. (*a*) Interactions are based on model building and genetic data, as described in the text. (*b*) Interactions are taken from Fig. 2.

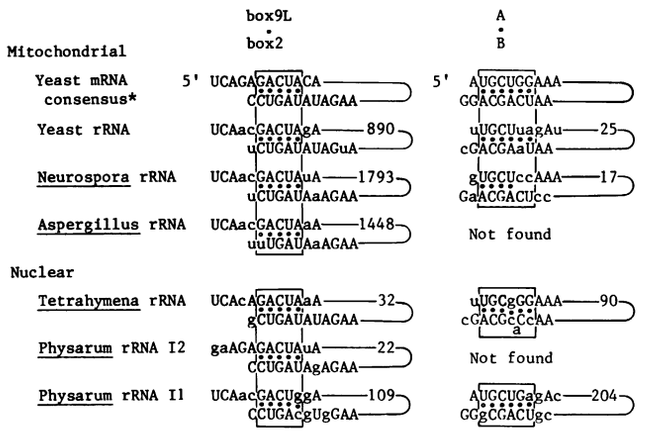

FIG. 4. Conservation of the *box*9L, *box*2, A, and B sequences in mitochondrial and nuclear introns. Lowercase letter indicates that the nucleotide differs from the mitochondrial mRNA consensus sequence. *Sequences shown are found in *cob* intron 4 (18, 31) and in introns 3 and 4 of the *oxi3* (cytochrome oxidase) gene (32). Several other *cob* introns have similar sequences. Mitochondrial rRNA intron sequences are from refs. 10, 11, and 33; nuclear rRNA intron sequences are from refs. 5, 6, 34, and 35.

The conserved sequences are designated in the secondary structure model of the *Tetrahymena* intron in Fig. 2 *Lower*. The *box*9L·*box*2 and A·B interactions are both predicted. It is important to note that these sequences were not given preferential treatment in the folding program, so their occurrence as two helices is not biased by any preconceived notion that they should be paired. Sequences to the right of *box*9L are unpaired and are complementary to sequences to the left of A, such that a *box*9R·9R′ tertiary interaction could be formed. By disrupting two G·C base pairs (nucleotides 25 and 26 paired with 98 and 99), the sequence G-A-C-C-G becomes available to pair with C-G-G-U-C in *box*9R. As shown in the *Inset* in Fig. 2 *Lower*, this helix could also be extended to seven base pairs with a single mismatch.

DISCUSSION

We have presented a model for the secondary structure of the IVS excised from the pre-rRNA of *Tetrahymena*. The model is based on the susceptibility of various regions of the molecule to cleavage by nucleases and on calculations of the free energies of alternative structures. Although the proposed structure is consistent with all of the nuclease digestion data, it cannot be considered proven. Phylogenetic comparison of IVS sequences from other species of *Tetrahymena* and examination of the effects of altering the sequence by *in vitro* mutagenesis will provide additional evidence for substantiating or modifying the proposed structure.

The *Tetrahymena* IVS contains the *box*9L, *box*2, A, and B elements that are highly conserved in fungal mitochondrial introns. In our structural model, these sequences form two short base-paired helices (Fig. 2 *Lower*). Michel *et al.* (19) and Davies *et al.* (37) proposed models for the structure of many of the mitochondrial mRNA and rRNA introns in which the conserved sequence elements form a common structural core. Their folded "group I" mitochondrial introns bear a strong resemblance to our folded *Tetrahymena* and *Physarum* introns. Michel *et al.* (19) proposed that the *box*9R·9R′ pairing is a secondary structure interaction and mentioned the possibility of a *box*9L·*box*2 tertiary interaction; we calculate more stable structures with *box*9L and *box*2 paired in a secondary structure and *box*9R and

9R′ left single-stranded for a tertiary interaction, as in the models of Davies *et al.* (37).

The various conserved sequences in the mitochondrial introns are the sites of *cis*-acting splicing mutants, so it is clear that they are involved in structures necessary for splicing. The same sequences occur in the self-splicing *Tetrahymena* IVS, where we know that the structure of the RNA directly lowers the activation energy for highly specific cleavage/ligation reactions (7) and probably also provides a binding site for the guanosine cofactor required for splicing (unpublished data). It therefore seems likely that mitochondrial RNA splicing follows a similar mechanism.

What then would be the role of the intron-encoded polypeptides ("maturases") and nuclear products necessary for mitochondrial RNA splicing? They could be specific RNA binding proteins that are necessary for a very large mitochondrial intron to fold into the correct conformation to splice itself. On the other hand, these proteins could be real enzymes, needed because the RNA by itself was capable of performing only part of the splicing reaction.

Finding RNA sequences and structures conserved between *Tetrahymena* nuclear rRNA introns and fungal mitochondrial introns is certainly unexpected and has implications for the evolution of these introns. One possibility is that the introns had their origin in transposable elements that were able to enter both nuclear and mitochondrial compartments of a cell. Another possibility is that the introns were always present in various mitochondrial genomes and are occasionally transferred to the nucleus. Transfer of DNA from mitochondria to nucleus has been documented (38, 39).

The structure we have characterized is that of native IVS RNA, as judged by its ability to undergo autocyclization when incubated at 42°C. The structure is largely invariant from 0°C to 30°C. It is possible that minor structural changes occur at higher temperatures where cyclization takes place. It is also possible that cyclization might occur by a reaction path in which a different structure, present only in very small quantities at equilibrium, is the active form.

One pleasing feature of the structure is that the 3′ terminal G-OH is not locked in an interaction that would preclude it from reacting with the cyclization site at A^{16} (3). Rather, the way in which the helical domains emanate from a central core constrains A^{16} and G^{414} to be in the vicinity of one another (Fig. 2 *Lower*). We have no experimental data concerning the structure of the IVS within the pre-rRNA and therefore do not yet know the extent to which structural rearrangements take place during splicing. If the IVS does exist within the pre-rRNA in much the same form shown in Fig. 2, then the folding of the IVS can be seen to be a major factor that brings the adjacent exon sequences into proximity for ligation.

After this work was completed, we learned that Michel and Dujon (40) and Waring *et al.* (41) have also proposed secondary structures for the *Tetrahymena* IVS and adjacent exon sequences. Their structures, which are based on homology with yeast mitochondrial introns rather than the use of structural probes, are similar but not identical to ours. We find it encouraging that two quite different approaches have converged on a common structure.

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1. Cech, T. R., Zaug, A. J., Grabowski, P. J. & Brehm, S. L. (1982) in *The Cell Nucleus*, eds. Busch, H. & Rothblum, L. (Academic, New York), Vol. 10, pp. 171–204.
2. Grabowski, P. J., Zaug, A. J. & Cech, T. R. (1981) *Cell* **23**, 467–476.
3. Zaug, A. J., Grabowski, P. J. & Cech, T. R. (1983) *Nature (London)* **301**, 578–583.
4. Brehm, S. L. & Cech, T. R. (1983) *Biochemistry*, in press.
5. Wild, M. A. & Sommer, R. (1980) *Nature (London)* **283**, 693–694.
6. Kan, N. C. & Gall, J. G. (1982) *Nucleic Acids Res.* **10**, 2809–2822.
7. Kruger, K., Grabowski, P. J., Zaug, A. J., Sands, J., Gottschling, D. E. & Cech, T. R. (1982) *Cell* **31**, 147–157.
8. Lamouroux, A., Pajot, P., Kochko, A., Halbreich, A. & Slonimski, P. P. (1980) in *The Organization and Expression of the Mitochondrial Genome*, eds. Saccone, C. & Kroon, A. (North-Holland, New York), pp. 152–156.
9. Lazowska, J., Jacq, C. & Slonimski, P. P. (1980) *Cell* **22**, 333–348.
10. Dujon, B. (1980) *Cell* **20**, 185–197.
11. Burke, J. & RajBhandary, U. L. (1982) *Cell* **31**, 509–520.
12. Morimoto, R., Locker, J., Synenki, R. M. & Rabinowitz, M. (1979) *J. Biol. Chem.* **254**, 12461–12470.
13. Mannella, C. A., Collins, R. A., Green, M. R. & Lambowitz, A. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2635–2639.
14. Dieckmann, C. L., Pape, L. K. & Tzagoloff, A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1805–1809.
15. DeLaSalle, H., Jacq, C. & Slonimski, P. P. (1982) *Cell* **28**, 721–732.
16. Netter, P., Jacq, C., Carignani, G. & Slonimski, P. P. (1982) *Cell* **28**, 733–738.
17. Weiss-Brummer, B., Rodel, G., Schweyen, R. J. & Kaudewitz, F. (1982) *Cell* **29**, 527–536.
18. Anziano, P. Q., Hanson, D. K., Mahler, H. R. & Perlman, P. S. (1982) *Cell* **30**, 925–932.
19. Michel, F., Jacquier, A. & Dujon, B. (1982) *Biochimie* **64**, 867–881.
20. Waring, R. B., Davies, R. W., Scazzocchio, C. & Brown, T. A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6332–6336.
21. Nussinov, R., Tinoco, I. & Jacobson, A. (1982) *Nucleic Acids Res.* **10**, 341–350.
22. Salser, W. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42**, 993.
23. Yuan, R. C., Steitz, J. A., Moore, P. B. & Crothers, D. M. (1979) *Nucleic Acids Res.* **7**, 2399–2418.
24. Wickstrom, E. & Tinoco, I. (1974) *Biopolymers* **13**, 2367–2383.
25. Ninio, J. (1979) *Biochimie* **61**, 1133–1150.
26. Zuker, M. & Stiegler, P. (1981) *Nucleic Acids Res.* **9**, 133–148.
27. Wurst, R. M., Vournakis, J. N. & Maxam, A. M. (1978) *Biochemistry* **17**, 4493–4499.
28. Donis-Keller, H., Maxam, A. M. & Gilbert, W. (1977) *Nucleic Acids Res.* **4**, 2527–2538.
29. Vassilenko, S. K. & Rait, V. K. (1975) *Biokhimiya* **40**, 578–583.
30. Fox, G. & Woese, C. R. (1975) *Nature (London)* **256**, 505–507.
31. Nobrega, F. G. & Tzagoloff, A. (1980) *J. Biol. Chem.* **255**, 9829–9837.
32. Bonitz, S. G., Coruzzi, G., Thalenfeld, B. E. & Tzagoloff, A. (1980) *J. Biol. Chem.* **255**, 11927–11941.
33. Netzker, R., Köchel, H. G., Basak, N. & Küntzel, H. (1982) *Nucleic Acids Res.* **10**, 4783–4794.
34. Nomiyama, H., Kuhara, S., Kukita, T., Otsuka, T. & Sakaki, Y. (1981) *Nucleic Acids Res.* **9**, 5507–5520.
35. Nomiyama, H., Sakaki, Y. & Takagi, Y. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1376–1380.
36. Weiss-Brummer, B., Holl, J., Schweyen, R. J., Rodel, G. & Kaudewitz, F. (1983) *Cell*, in press.
37. Davies, R. W., Waring, R. B., Ray, J. A., Brown, T. A. & Scazzocchio, C. (1982) *Nature (London)* **300**, 719–724.
38. Farrelly, F. & Butow, R. A. (1983) *Nature (London)* **301**, 296–301.
39. Wright, R. M. & Cummings, D. J. (1983) *Nature (London)* **302**, 86–88.
40. Michel, F. & Dujon, B. (1983) *EMBO J.* **2**, 33–38.
41. Waring, R. B., Scazzocchio, C., Brown, T. A. & Davies, R. W. (1983) *J. Mol. Biol.*, in press.