Markwardt, F., & Klocking, H. P. (1977) *Haemostasis 6*, 370-374.
McLean, J. W., Tomlin, J. E., Kuang, W., et al. (1987) *Nature 330*, 132-137.
Miles, L. A., & Plow, E. F. (1987) *Thromb. Haemostasis 58*, 936-942.
Nakashima, Y., Ferrante, N. D., Jackson, R. L., & Pownall, H. J. (1975) *J. Biol. Chem. 250*, 5386-5392.
Nieuwenhuizen, W., Verheijen, J. H., Vermond, A., & Chang, G. T. (1983) *Biochim. Biophys. Acta 755*, 531-533.
Paques, E. P., Stohr, & Heimburger, N. (1986) *Thromb. Res. 42*, 797-807.
Rajagopalan, S., Gonias, S. L., & Pizzo, S. V. (1985) *J. Clin. Invest. 75*, 413-419.
Ranby, M. (1982) *Biochim. Biophys. Acta 704*, 461-469.
Reich, R., Thompson, E. W., Iwamoto, Y., et al. (1988) *Cancer Res. 48*, 3307-3312.
Rosenberg, R. D., & Damus, P. S. (1973) *J. Biol. Chem. 248*, 6490-6505.
Rosenberg, R. D., Bauer, K. A., & Marcum, J. A. (1986) in *Reviews in Hematology* (Murano, E., Ed.) pp 351-416, PJD Publications Ltd., Westbury, NY.
Shimada, K., Gill, P. J., Silbert, J. E., Douglas, W. H., & Fanburg, B. L. (1981) *J. Clin. Invest. 68*, 381-387.
Silverstein, R. L., Nachman, R. L., Leung, L. L., & Harpel, P. C. (1985) *J. Biol. Chem. 260*, 10346-10352.
Sprengers, E. D., & Kluft, C. (1987) *Blood 69*, 381-387.
Stein, P. L., van Zonneveld, A., Pannekoek, H., & Strickland, S. (1989) *J. Biol. Chem. 264*, 15441-15444.
Strickland, S., Reich, E., & Sherman, M. I. (1976) *Cell 9*, 231-240.
Urano, T., deSerrano, V. S., Chibber, B. K., & Castellino, F. J. (1987) *J. Biol. Chem. 262*, 15959-15964.
Wojta, J., Beckmann, R., Turcu, L., Wagner, O. F., van Zonneveld, A., & Binder, B. R. (1989) *J. Biol. Chem. 264*, 7957-7961.
Woods, A., Couchman, J. R., & Hook, M. (1985) *J. Biol. Chem. 260*, 10872-10879.

# Internal Transcribed Spacer 1 of the Yeast Precursor Ribosomal RNA. Higher Order Structure and Common Structural Motifs†

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ABSTRACT: The higher order structure of the first internal transcribed spacer between the 18S and the 5.8S rRNA sequences in the *Saccharomyces cerevisiae* precursor ribosomal RNA has been investigated. Sites of potential base pairing in the RNA region have been determined by using a combination of enzymatic and chemical structure sensitive probes. Data generated have been used to evaluate secondary structure models predicted by minimum free energy calculations. Several alternative suboptimal structures were also evaluated. The derived model contains several stable hairpins. Theoretical secondary structural models for the corresponding RNA region from *S. carlsbergensis*, *S. pombe*, *N. crassa*, *X. laevis*, and mung bean have also been derived from identical calculations and assumptions. Certain structural motifs appear to be conserved despite extensive divergence in the base sequence. The yeast model should be a useful prototype for investigation of structure and function of precursor ribosomal RNA molecules.

In *Saccharomyces cerevisiae*, the primary rRNA transcription product is a 35S RNA molecule that is processed into three mature rRNA molecules (5.8S, 18S, and 25S). These mature rRNA sequences in the 35S pre-rRNA[1] are separated by spacer sequences. These internal spacer sequences constitute about 10% of the total RNA in length. The necessary structures for the recognition of the proper cleavage sites and the role of the spacer sequences in the regulation of ribosome biogenesis are not clear. Solely on the basis of the terminal nucleotide sequence information of the processing intermediates, Veldman et al. (1981) postulated that small hairpin structures located near the processing sites may be involved in the maturation of the pre-rRNA and that all the sites are brought into one region through long-range RNA-RNA interactions. In an attempt to examine the structural features of the pre-rRNA, we have recently reported the successful cloning of the yeast rRNA gene in an expression vector and production of the 35S pre-rRNA molecule in vitro. Preliminary chemical modification studies with kethoxal and dimethyl sulfate on the internal transcribed spacer 1 (ITS-1) between the 18S and 5.8S rRNA sequences within the pre-rRNA molecule have also been reported (Thweatt & Lee, 1990).

The goal of the present study is to investigate in detail the solution structure of the ITS-1 sequence within the pre-rRNA molecule with a combination of chemical and enzymatic structural probes. By combining the structure probing experimental data and computer theoretical secondary structure modeling, we have constructed a refined secondary structure model. Several suboptimal structures have also been evaluated. They do not agree as well with the experimental data. In

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[1] Abbreviations: DMS, dimethyl sulfate; CMCT, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate; EDTA, ethylenediaminetetraacetic acid; pre-rRNA, precursor ribosomal RNA; ITS, internal transcribed spacer; LETS, left external transcribed spacer; ddATP, ddCTP, and ddTTP, dideoxyadenosine, dideoxycytosine, and dideoxythymidine triphosphates, respectively.

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addition, the existence of a previously proposed tertiary interaction (Veldman et al., 1981) within the pre-rRNA molecule has been tested by a new experimental protocol. The protocol involves binding of a specific complementary oligodeoxynucleotide to the suspected interacting RNA region followed by chemical modifications. In the presence of the competitive complementary oligomer, the putative interacting RNA partner sequence should now become available for chemical modifications and detectable by primer extension. Data obtained by this approach do not support the putative interaction.

RNA models for the ITS-1 region within the pre-rRNA from *S. carlsbergensis*, *S. pombe*, mung bean, *N. crassa*, and *X. laevis* have been constructed by using identical theoretical assumptions. Our analysis reveals several conserved structural motifs among the pre-rRNAs of these eukaryotes despite extensive divergence in their primary structures. Hence, the ITS-1 secondary structure model for *Saccharomyces cerevisiae* should be a useful prototype for future investigations of the structure, function, and processing of pre-rRNA.

## Materials and Methods

*Buffers.* CMK: 80 mM potassium cacodylate (pH 7.2), 20 mM magnesium acetate, 300 mM KCl. CE: 80 mM potassium cacodylate (pH 7.2), 1 mM EDTA. BMK: 50 mM potassium borate (pH 8.0), 20 mM magnesium acetate, 300 mM KCl. BE: 50 mM potassium borate (pH 8.0), 1 mM EDTA.

*In Vitro Transcription.* The plasmid (pD) containing the *S. cerevisiae* rDNA sequence coding for the 35S precursor rRNA in pGEM-2 (Thweatt & Lee, 1990) was used as the template. The plasmid was first treated with *Pst*I followed by Klenow DNA polymerase (Promega) to repair the resultant 3′-overhang structure and finally transcribed in vitro as described previously (Yeh & Lee, 1990). The pre-rRNA products were recovered following phenol/chloroform extraction and purified through a 31–34% sucrose gradient at 18 °C for 16 h at 24 000 rpm in a Beckman SW 50.1 rotor.

*Modifications of Pre-rRNA.* Purified pre-rRNA (1 pmol) was modified in CMK buffer (for enzymatic digestions and modification with DMS) or BMK buffer (for modification with CMCT). The RNA sample was pretreated by incubation at 42 °C for 20 min, slow cooled to room temperature for renaturation, and placed on ice for 5 min. Conditions for DMS and CMCT modifications were similar to those described previously (Yeh & Lee, 1990). Modification with kethoxal was as described previously (Thweatt & Lee, 1990). For modification under native conditions, the pre-rRNA molecule was incubated with DMS (0.35 μL of DMS/pmol of RNA) or CMT (84 μg of CMCT/pmol of RNA) for 15 min at 25 °C. Conditions for enzymatic modifications were similar to those described previously (Ehresmann et al., 1987; Yeh & Lee, 1990). Pre-rRNA was digested with RNase T1 (Calbiochem, 0.002 unit/pmol of RNA) or RNase V1 (Pharmacia, 0.01 unit/pmol of RNA) at 25 °C for 10 min. Both reactions were stopped by extraction with phenol/chloroform; the RNA was precipitated with sodium acetate and ethanol. For modifications under denaturing conditions, the RNA molecule was dissolved in CE buffer (for DMS modification) or BE buffer (for CMCT modification). Modifications were at 90 °C for 1 min with 2–4-fold lower reagent concentrations. For each type of modification, a control RNA sample was run simultaneously under identical conditions except for omission of the enzyme or the modifying chemical agent. Modified residues were located by primer extension with AMV reverse transcriptase (Promega). Oligodeoxynucleotide primers were synthesized by using an Applied Biosystem Model 380B DNA synthesizer (Table I), labeled at the 5′-end with [$\gamma$-$^{32}$P]ATP and T4 polynucleotide kinase and purified on 20% polyacrylamide gels in 1× TBE buffer as described (Yeh & Lee, 1990). Each primer was used three to four times with different preparations of pre-rRNA with reproducible results.

Table I: Oligodeoxynucleotides Used in This Study

| oligomer | residues to which the oligomer is complementary |
|---|---|
| A | 65–81 in ITS-1 |
| B | 206–222 in ITS-1 |
| C | 285–301 in ITS-1 |
| D | 56–72 in 5.8S |
| E | 16–32 in 18S |
| F | −5–12 at LETS/18S |

*Oligonucleotide Competition.* To determine whether the previously proposed interaction between the 3′-end of the LETS and the middle of the ITS-1 exists, oligodeoxynucleotides B and F (Table I), which are complementary to the corresponding sequences, were used. Each oligomer was allowed to hybridize with the pre-rRNA under "native" conditions, making the putative complementary pre-rRNA seequence available for chemical modifications. Three concentrations (0.3, 0.6, and 1.0 pmol per 0.1 pmol of pre-rRNA) of a specific competitor oligodeoxynucleotide were hybridized to the pre-rRNA molecule. The oligodeoxynucleotide–pre-rRNA complex was treated with CMCT, DMS, and kethoxal; modified bases in the pre-rRNA were located as described above. Primers E and B were used for probing when oligonucleotides B and F were used as competitors, respectively.

*Computer Analysis of RNA Structure.* The program FOLD in the Sequence Analysis Software Package (version 6) produced by the Genetics Computer Group (GCG) of the University of Wisconsin was used. This program predicts a secondary structure with minimum free energy (Freier et al., 1986) for a RNA molecule by the method of Zuker and Stiegler (1981). Alternative structures were generated by the suboptimal RNA folding program (MFOLD, v.2) written for the VAX (Jaeger et al., 1989a,b; Zuker, 1989).

## Results

*DMS and CMCT Modifications of Pre-rRNA.* The reactivity of bases in the ITS-1 sequence within the 35S pre-rRNA molecule was probed with DMS and CMCT under "native" conditions. DMS reacts with unpaired adenine at N1 and more slowly with cytosine at N3; CMCT reacts with unpaired uracil at N3 and more slowly with guanine at N1 [Ehresmann et al. (1987) and references therein; Noller et al., 1987]. The modified bases were detected by primer extension with reverse transcriptase (Stern et al., 1988). Four DNA primers (A–D in Table I), spaced along the RNA molecule, were used in order to provide overlapping information on the reactivity of bases within the entire ITS-1 sequence. The base sequence of each primer is complementary to a specific sequence in the pre-rRNA. The identity of the modified bases was facilitated by running the appropriate dideoxy sequencing lane on the same gel. Artifact bands were distinguished from modified sites by their presence in the DNA molecules by using unmodified control pre-rRNA. Modifications were also carried out under denaturing conditions to ensure that the lack of reactivity of those unreactive residues under native conditions is not due to an intrinsic property of the residue.

Figures 1 and 2 show representative autoradiograms of DMS and CMCT modifications of pre-rRNA under native

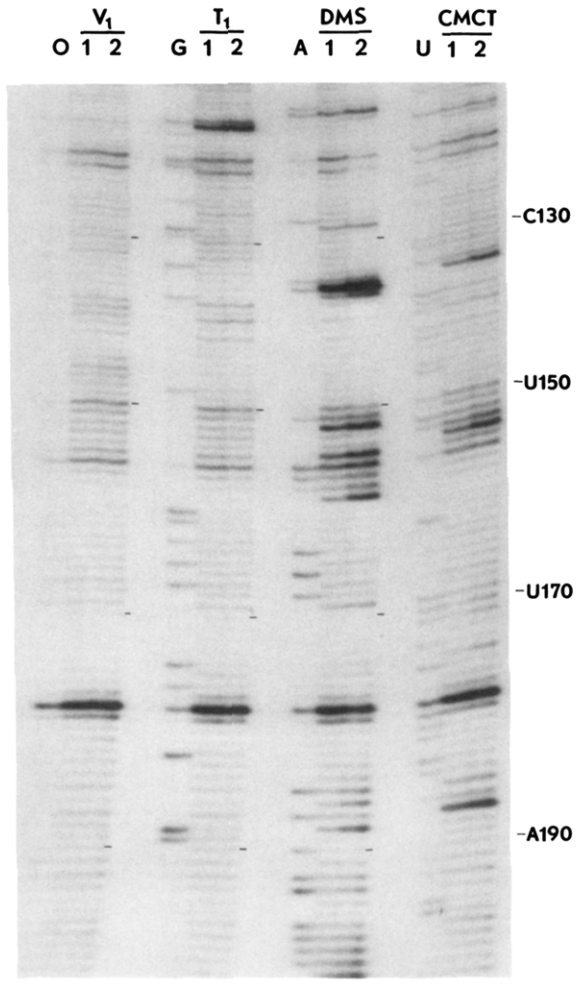


FIGURE 1: Autoradiograms showing sites within the ITS-1 sequence of *S. cerevisiae* that were chemically or enzymatically modified under native condition by using primer B. Four modifiers were used: V1, T1, DMS, and CMCT. Lane O is unmodified control, which was treated as the modified samples except for omission of the reagent. For each modifier, a control sample with unmodified pre-rRNA was run by dideoxy sequencing, generated by reverse transcription in the presence of ddCTP, ddTTP, and ddATP (lanes G, A, and U for T1, DMS, and CMCT, respectively). Two concentrations of each modifier were used (lanes 1 and 2). For V1, the concentrations were 0.01 and 0.02 unit/pmol of pre-rRNA. For T1, the concentrations were 0.002 and 0.004 unit/pmol. For DMS, the concentrations were 0.35 and 0.70 $\mu$L/pmol. For CMCT, the concentrations were 84 and 168 $\mu$g/pmol. Positions of the corresponding nucleotides are denoted on the side.

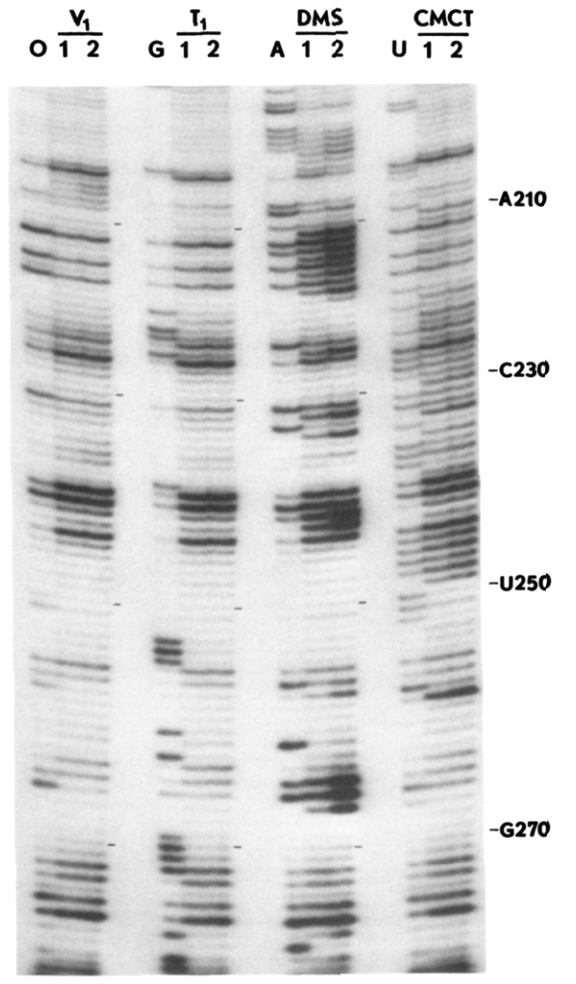


FIGURE 2: Autoradiograms showing sites within the ITS-1 sequence of *S. cerevisiae* that were chemically or enzymatically modified under native conditions by using primer C. Conditions and symbols are similar to those used in Figure 1.

conditions. Results of RNA modifications under denaturing conditions are shown in Figures 3 and 4. The data are summarized in Figure 5. Except residues 72, 75, 111, 193, 208, 262, 297, and 315, all unreactive adenine residues in the native RNA molecule became reactive upon denaturation of the RNA molecule. The absence of reactivity of these adenine residues cannot be readily explained at the present. Not all cytosine residues in the denatured RNA molecule were reactive under our experimental conditions, perhaps because DMS reacts with cytosine much slower than with adenine. The reactivity of several adenine residues (A135, A151, A158, A210, A241, A242, and A265) and cytosine residues (C154, C211, C214, C216, and C264) was enhanced in the native compared to the denatured state. Except residues 7–9, 66, 85, and 92, all unreactive uracil residues in the native RNA molecule became reactive upon denaturation of the RNA molecule. The reactivity of three uracil residues (U134, U153, and U186) was enhanced in the native compared to the denatured state.

*Reactivity of Bases As Probed by Enzymes.* The ITS-1 region of the pre-rRNA in the native state was probed with RNase T1 and V1. The former enzyme cleaves unpaired guanine residues and the latter paired or stacked nucleotides [Ehresmann et al. (1987) and references therein]. Thus, results generated from these studies should complement those from chemical modifications. Representative autoradiograms are shown in Figures 1 and 2 and the results are summarized in Figure 5. Very few guanine residues in the ITS-1 sequence in the "native" pre-rRNA were cleaved by RNase T1, suggesting that most of the guanine residues were hydrogen bonded. In agreement with the finding, many of these residues were cleaved by RNase V1. In fact, numerous residues in the native RNA molecule that are cleaved by V1 are not reactive with DMS or CMCT unless the RNA is denatured. However, several residues (C103, G137, G239, C240, and C243) are modified by DMS and cleaved by V1. Their apparent unexpected reactivities may be explained by their location at the end of a stem, which would render them to assume a flexible configuration.

*Probing a Previously Proposed Intramolecular Interaction.* To assess the existence of a previously proposed tertiary interaction between the sequence at the LETS-18S rRNA boundary and a sequence in the ITS-1 region, a complementary oligodeoxynucleotide (F in Table I) was allowed to hybridize to the LETS-18S rRNA boundary in the pre-rRNA molecule under native conditions. The concentrations of the competitor oligonucleotide selected were intended to displace

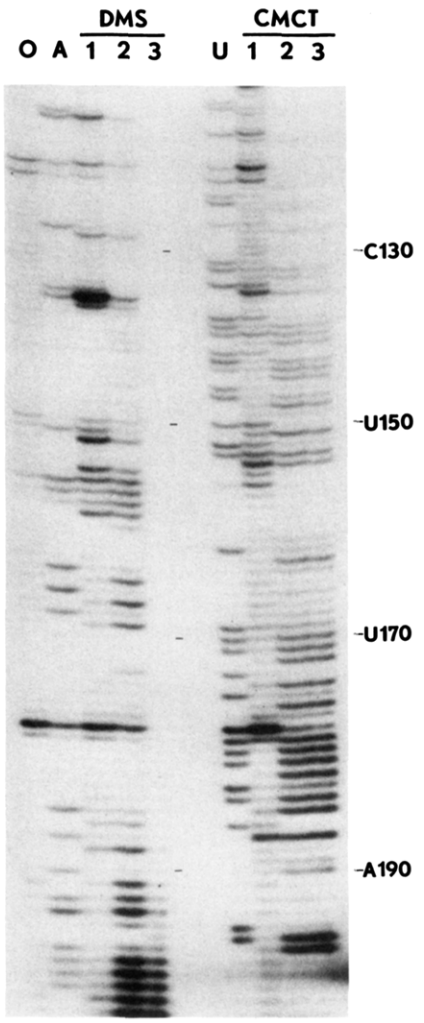


FIGURE 3: Autoradiograms comparing sites within the ITS-1 sequence of pre-rRNA that were chemically modified under native and denatured conditions by using primer B. Lane O is the unmodified RNA control under native conditions. Lanes A and U are dideoxy sequencing lanes, generated by reverse transcription in the presence of ddTTP and ddATP, respectively. Lane 1 is modified RNA under native conditions. Lanes 2 and 3 are RNA modified under denatured conditions. Two concentrations of each modifying reagent were used: for DMS, 0.09 and 0.18 $\mu$L/pmol; for CMCT, 21 and 42 $\mu$g/pmol. Nucleotide positions are indicated on the side. The number of bands in lane 3 (DMS) is small compared to that in the other lanes, presumably because at the higher DMS concentration, larger numbers of residues were modified; thus the cDNA products were shorter.

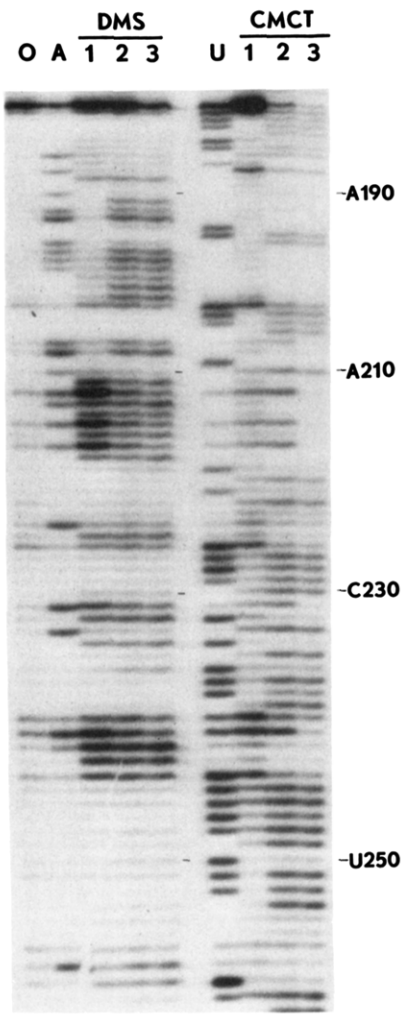


FIGURE 4: Autoradiograms comparing sites within the ITS-1 sequence of pre-rRNA that were chemically modified under native and denatured conditions by using primer C. Conditions and symbols are similar to those used in Figure 3.

the proposed intramolecular interaction with no or minimal perturbation of the remaining RNA structure. Subsequent probing of the complex with DMS, CMCT, and kethoxal using primer B showed that the reactivities of the residues at the putative interacting sequence (including flanking residues 88–202) in the ITS-1 region were not changed. Studies with a second oligodeoxynucleotide (B in Table I) that is complementary to the putative interacting sequence in the ITS-1 region and probing with primer E yielded the same results. In the latter case, the sequence containing residues −65 (in the ETS region) to 13 (in the 18S rRNA sequence) was examined for changes in chemical reactivities. Control experiments further showed that the "competitor" oligodeoxynucleotide hybridized only to the correct sequence in the pre-rRNA molecule. The control experiments involved hybridization of the $^{32}$P-labeled competitor oligonucleotide with the pre-rRNA molecule, primer-extend synthesis of cDNA molecules in the presence of ddATP and ddTTP, and determination of the nucleotide sequence by the dideoxy sequencing technique (Sanger et al., 1977). Only a single correct sequence was obtained, suggesting that the competitor hybridized to the correct sequence in the pre-rRNA molecule.

*Modeling of the RNA Structure.* Results of the chemical and enzymatic probing of the ITS-1 sequence within the yeast 35S pre-rRNA molecule are superimposed on a structural model for the ITS-1 region (Figure 6). The model was generated by theoretical minimum free energy calculations ($\Delta G$ = −105.7 kcal/mol). Potential influence from 50 bases flanking each end of the ITS-1 sequence was also considered in generating the theoretical model. The number of flanking bases selected was arbitrary but was deliberately small partly due to limitations in the computer program in handling the maximum number of bases. A different folding model was generated when flanking sequences were not taken into consideration in the folding and did not fit the experimental data as well (65%).

Analysis of the same RNA region by the suboptimal RNA folding program of Zuker (Jaeger et al., 1989a,b; Zuker, 1989) suggested several alternative structures with folding energies in the range −89.2 to −90.6 kcal. These alternative structures consist of a common structure with distinct alternatives at designated locations (I and II in Figure 6) of the RNA region. The salient characteristics of the alternative foldings for these locations are diagramed in Figure 7. For RNA region I, 90.5% of the residues experimentally shown to be in the correct configuration were situated in the predicted configuration

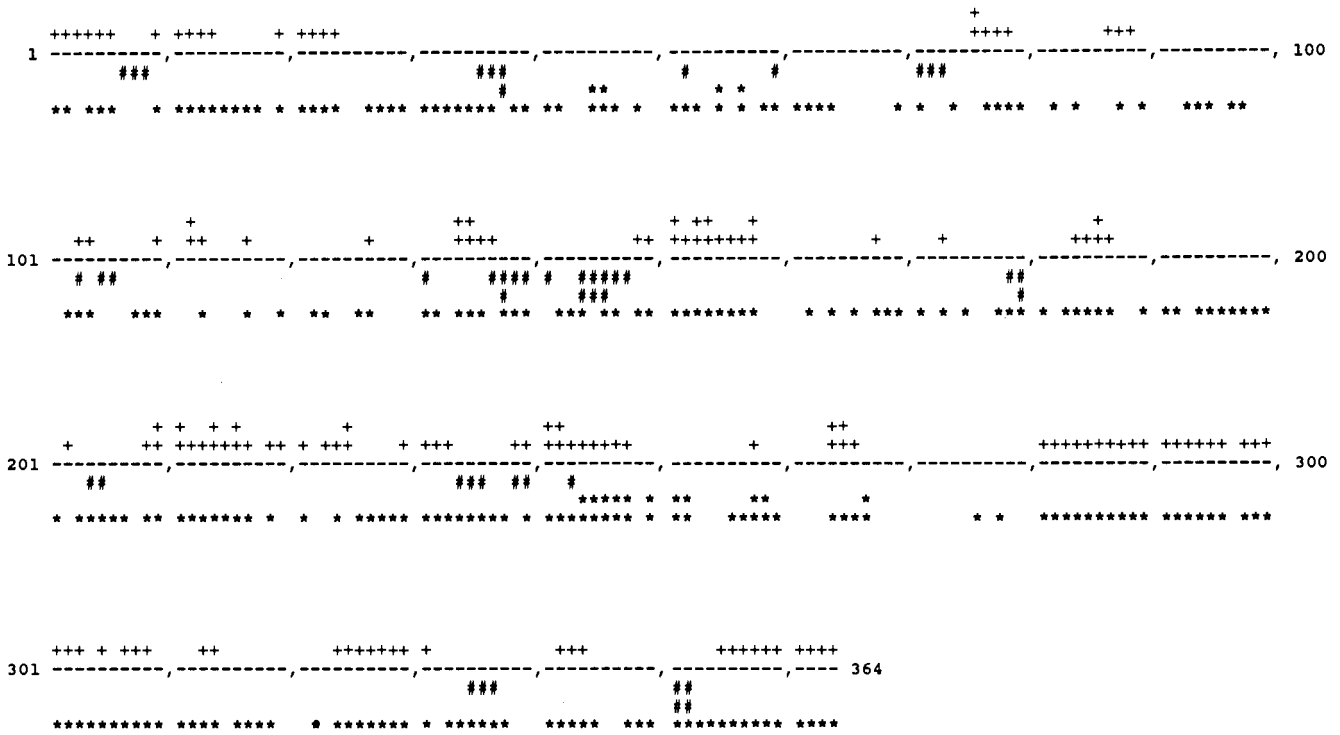

FIGURE 5: Summary showing the reactivity of individual nucleotides in the ITS-1 region of *S. cereviaiae*. Each nucleotide is represented by a straight line. Its reactivity toward chemical reagents and enzymes is indicated by a symbol above or below the line. Symbols above the line indicate that the nucleotide is reactive with DMS, CMCT, or RNase T1 when the RNA is in the native conditions (+, weakly reactive; ++, strongly reactive). Symbols below the line indicate that the nucleotide is cleaved by RNase V1 under native conditions (#, weakly reactive; ##, strongly reactive). An asterisk means that the nucleotide is reactive with DMS or CMCT when the RNA is denatured (*, weakly reactive; **, strongly reactive).

shown in Figure 6, only 81 and 71.4% for the alternative structures IA and IB in Figure 7. For RNA region II, both the structure shown in Figure 6 and its alternate in Figure 7, there was only 67.6% agreement between the experimental data and the predicted model. It is difficult to determine a priori which structure should predominate.

## Discussion

Previously, several structural models for the ITS-1 region have been proposed (Nazar et al., 1987; Thweatt & Lee, 1990; Veldman et al., 1981). The models proposed by Nazar et al. and Veldman et al. were based on theoretical calculations, whereas the model by Thweatt and Lee was based on theoretical calculations and limited experimental data generated by chemical modifications with kethoxal and DMS only. Data from the present study using enzymatic probes and additional chemical probes as well as an improved theoretical predicative computer program have allowed us to construct a more refined secondary structure model for the ITS-1 region. Additionally, several suboptimal RNA structures have been generated, but they are not as consistent with the data.

The processing scheme proposed by Veldman et al. (1981) involves clustering of all processing sites for the yeast pre-rRNA. The processing sites are located in a similar configuration consisting of a combination of type I and II consensus sequences flanked by short base-paired regions. According to the current model, both the type I processing site A2, involving the sequence UCAAUA (residues 205–210), and the type II site, involving UUUAAAAUA (residues 352–360), are located in a short stem and a loop region. Whether they are clustered as previously suggested cannot be deduced from the present data. The same authors further suggested that portions of the LETS and the ITS-1 regions interact to form a loop with the 18S rRNA region in the center. The interacting sequences are located in the LETS at the boundary of the 5′-end of the 18S rRNA sequence and the ITS-1 sequence GUUUCAAUA (residues 202–210). However, according to our competition experiments, this particular interaction does not appear to exist.

Since these experiments were done on the purified RNA molecule produced in vitro, the structure model is applicable to the RNA molecule in solution without the methylated residues that are present in the in vivo products or any other cellular components that might normally interact with the precursor. Nevertheless, studies from other laboratories on the in vitro *E. coli* pre-rRNA molecule lend credence to the idea that the synthetic RNA species are folded similarly to the in vivo molecules. For example, the synthetic RNA molecule can bind ribosomal proteins (Mougel et al., 1987; El-Baradi et al., 1987), form part of a functional ribosome (Krzyzosiak et al., 1987; Melancon et al., 1987), undergo correct processing (Stern et al., 1988), and be methylated correctly in vitro (Negre et al., 1989).

In the present study, secondary structure models for the ITS-1 region of *S. carlsbergensis* and of *S. pombe* were also generated by using published sequences (Veldman et al., 1981; Schaak et al., 1982) and identical computer programs and assumptions. Not surprisingly, models for *S. carlsbergensis* (Figure 8B) and *S. cerevisiae* (Figure 8A) are very similar, since the ITS-1 sequences for the two yeasts are identical except six residues. According to the present model, the variant nucleotides are located in single-stranded regions. The model for *S. pombe* (Figure 8C) is also similar to that proposed for *S. cerevisiae*, even though the *S. pombe* ITS-1 sequence is longer and has a lower GC content than the *S. cerevisiae* sequence (Table II). A search in the *S. pombe* ITS-1 region for sequences that are similar to those proposed for the *S. cerevisiae* processing sites fails to reveal their existence. Therefore, it is not clear that a consensus recognition sequence can be suggested. Perhaps the recognition signals for the

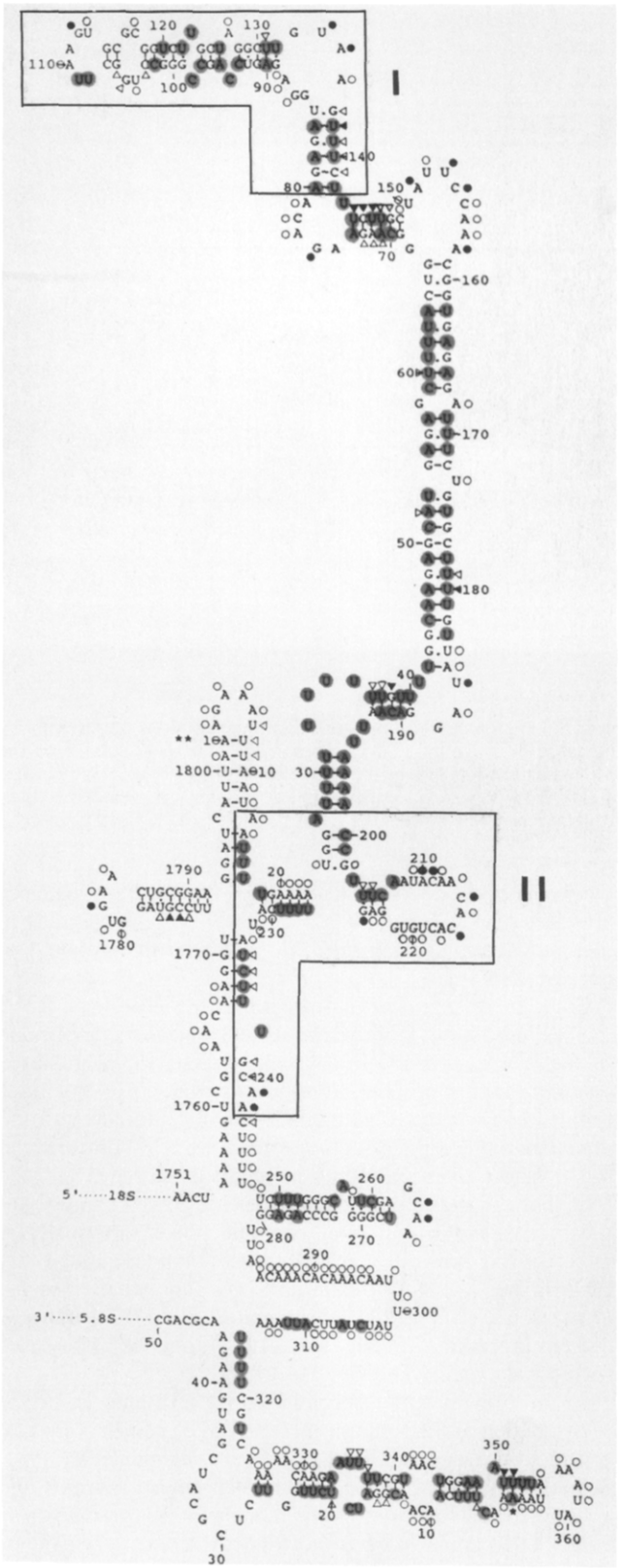

FIGURE 6: Proposed secondary structure model of the ITS-1 region of the pre-rRNA from *S. cerevisiae*. The reactivities of individual nucleotides to chemical and enzymatic probes under native and denatured conditions are indicated. (**) Beginning of the ITS-1 sequence; (*) beginning of the 5.8S rRNA sequence; (—) Watson–Crick base pairing; [•] noncanonical G·U interaction; [●] and [○] residues that were strongly and weakly, respectively, modified by DMS, CMCT, and RNase T1; [▲] and [△] residues that were readily and less readily, respectively, cleaved by nuclease V1. Shaded sequences are those that became reactive upon denaturation of the pre-rRNA molecule. The RNA regions with alternative folding structures are marked by large Roman numerals.

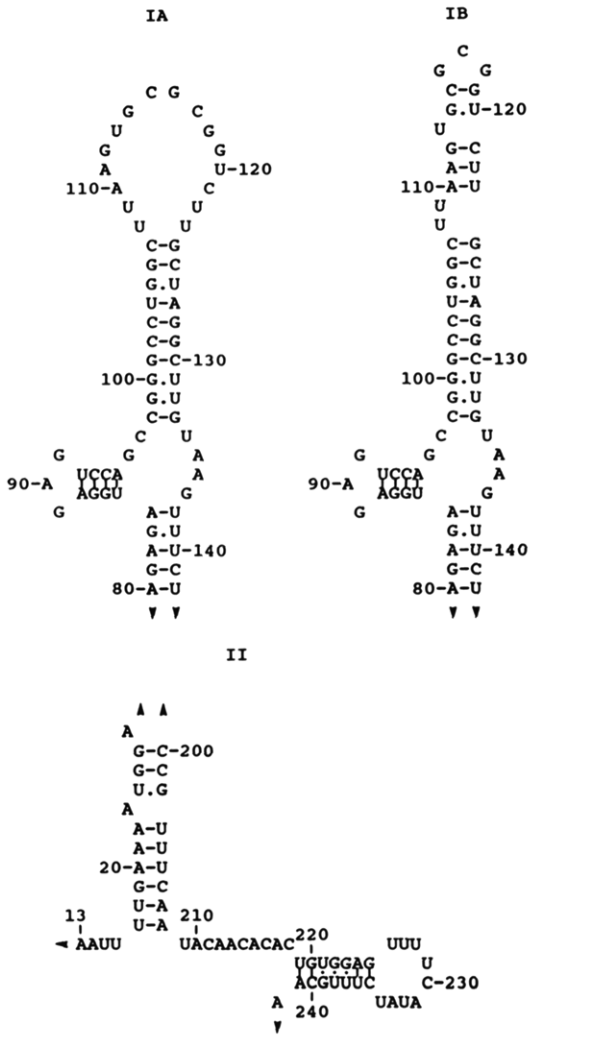


FIGURE 7: Alternative suboptimal RNA folding structures. The locations of the alternative structures within the main model as presented in Figure 6 are marked by the large Roman numerals. The alternative folding structures were generated by using the suboptimal RNA folding program (MFOLD) of Zuker. An energy level within 5% with 10 tracebacks and a window of 1 was used.

Table II: Size and GC Content of Internal Transcribed Spacer 1 in Pre-rRNAs

| organism | no. of bases | G + C (%) | ref |
|---|---|---|---|
| *S. cerevisiae* | 364 | 35.7 | this study |
| *S. carlsbergensis* | 362 | 35.6 | Veldman et al., 1981 |
| *S. pombe* | 420 | 19.3 | Schaak et al., 1982 |
| *N. crassa* | 185 | 55.1 | Chambers et al., 1986 |
| mung bean | 205 | 59.5 | Schiebel & Hemleben, 1989 |
| *X. laevis* | 557 | 84.2 | Hall & Maden, 1980 |

processing enzymes lie more in the higher order structure than in the primary structure. Validity of the model will have to be tested with additional experimental data.

From the rDNA sequence data of *N. crassa* (Chambers et al., 1986), mung bean (Schiebel & Hemleben, 1989), and *X. laevis* (Hall & Maden, 1980), secondary structure models for the ITS-1 region of these organisms were also generated (Figure 8D–F). Although *N. crassa* is classified as a fungus, the model for this organism is distinctly different from those for *S. pombe*, *S. cerevisiae*, and *S. carlsbergensis*. The *N. crassa* ITS-1 sequence is shorter than that of *S. cerevisiae* and has a higher G + C content (Table II). Models for mung bean and *X. laevis* are surprisingly similar in several features to that for *S. cerevisiae*, even though the ITS-1 sequences and the GC

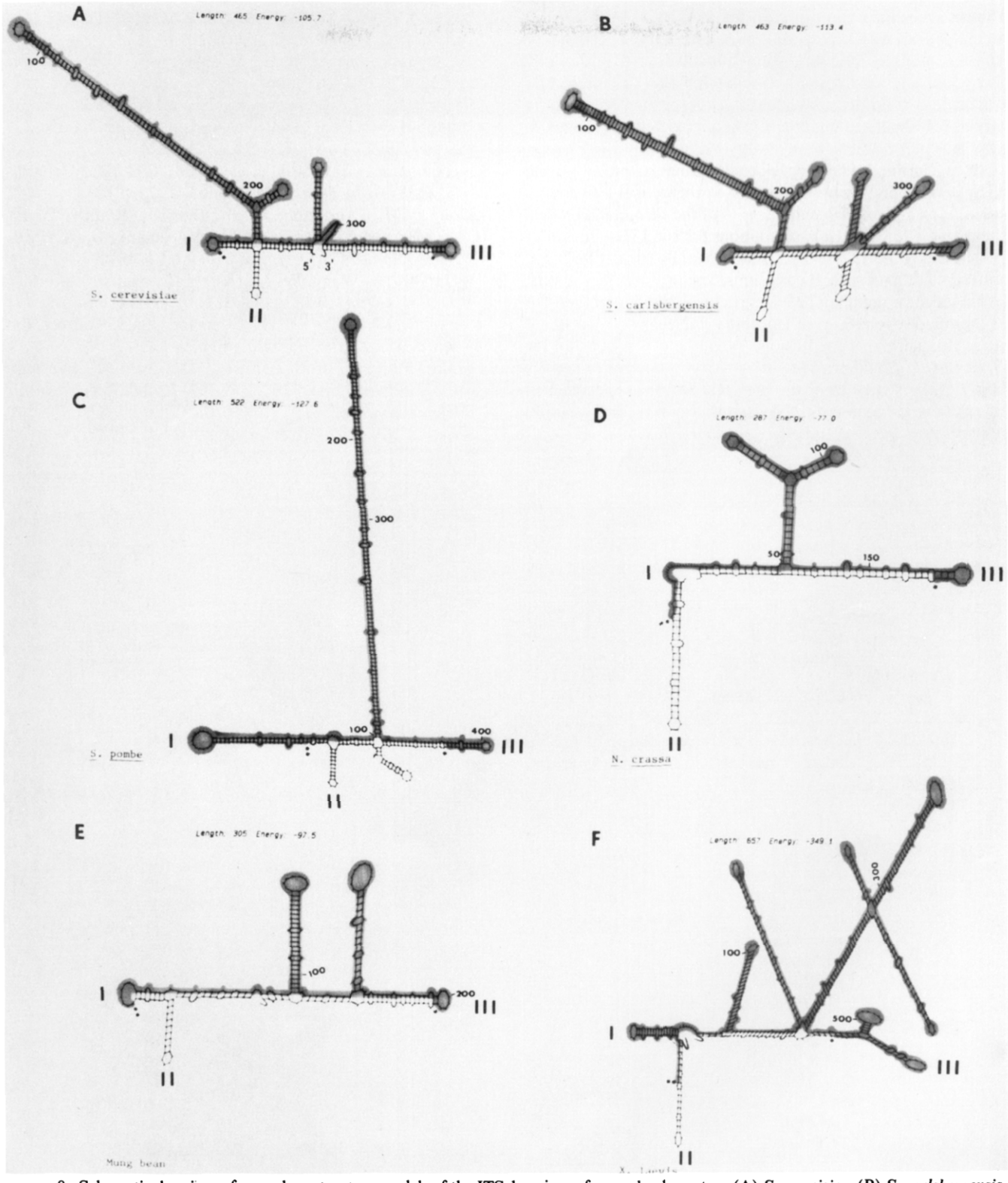


FIGURE 8: Schematic drawings of secondary structure models of the ITS-1 regions of several eukaryotes: (A) *S. cerevisiae*, (B) *S. carlsbergensis*, (C) *S. pombe*, (D) *N. crassa*, (E) mung bean, and (F) *X. laevis*. [**] Beginning of ITS-1 sequence; [*] beginning of the 5.8S rRNA sequence. The ITS-1 sequence is shaded. Length indicates the length of the entire sequence under consideration by the computer program in generating the secondary structure. Energy indicates the $\Delta G$ value (kcal/mol) for the proposed structure. The conserved loop structures I–III are indicated.

content of these organisms are very different (Table II). Even though the models for the different organisms are different in many aspects, three general structural motifs (loops I–III) appear to be conserved. For example, in all the models, the beginning of the ITS-1 sequence is located near a loop–stem structure (loop I), although the size of the stem varies. Similarly, the end of the ITS-1 sequence appears to be located near a loop–stem structure (loop III). Loop–stem structure II appears to be located near structure I, although its size also varies from organism to organism. Whether these common structure motifs have any physiological significance such as processing of the precursor RNA molecule must await further investigations.

Recently, the nucleotide sequence of the transcribed spacers of the human ribosomal RNA has been determined (Gonzalez et al., 1990). A secondary structure model based entirely on

the sequence information has been generated and is similar to the present model proposed for *S. cerevisiae* in that it also contains many stable long stem–loop structures. The long stem–loop structures in the model for the human rRNA molecule appear to match both in shape and in size the structures visualized under electron microscopy (Wellauer & Dawid, 1973).

In conclusion, we have developed a structural model for the ITS-1 region of *S. cerevisiae* based on theoretical free energy calculation and experimental data. All the theoretical models generated with identical assumptions for the ITS-1 region of *S. pombe*, *S. carlsbergensis*, mung bean, and *X. laevis* are different but have several common secondary structure motifs. Whether these common helical structures play any role in the recognition mechanism of ribosomal RNA processing must await further experimentation. Nonetheless, the model for *S. cerevisiae* should provide a reasonable prototype for further study of the structure of the pre-rRNA molecule as well as interactions between pre-rRNA and ribosomal proteins during assembly.

## References


Chambers, C., Dutta, S. K., & Crouch, R. J. (1986) *Gene 44*, 159–164.

Ehresmann, C., Baudin, F., Mougel, M., Romby, P., Ebel, J.-P., & Ehresmann, B. (1987) *Nucleic Acids Res. 15*, 9109–9128.

El-Baradi, T., de Regt, V., Planta, R. J., Nierhaus, K. H., & Raue, H. A. (1987) *Biochimie 69*, 939–948.

Freier, S. M., Ryszard, K., Jaeger, J. A., Sugimoto, N., Caruthers, M. H., Neilson, T., & Turner, D. H. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 9373–9377.

Gonzalez, I. L., Chambers, C., Gorski, J. L., Stambolian, D., Schmickel, R. D., & Sylvester, J. E. (1990) (in press).

Hall, L. M. C., & Maden, B. E. H. (1980) *Nucleic Acids Res. 8*, 5993–6005.

Jaeger, J. A., Turner, D. H., & Zuker, M. (1989a) *Proc. Natl. Acad. Sci. U.S.A. 86*, 7706–7710.

Jaeger, J. A., Turner, D. H., & Zuker, M. (1989b) *Methods Enzymol. 183*, 281–306.

Krzyzosiak, W., Denman, R., Nurse, K., Hellman, W., Boublik, M., Gehrke, C. W., Agris, P. F., & Ofengand, J. (1987) *Biochemistry 26*, 2353–2364.

Melancon, P., Gravel, M., Boileau, G., & Brakier-Gringras, L. (1987) *Biochem. Cell Biol. 65*, 1022–1030.

Mougel, M., Eyermann, F., Westhof, E., Romby, P., Expert-Bezancon, A., Ebel, J.-P., Ehresmann, B., & Ehresmann, C. (1987) *J. Mol. Biol. 198*, 91–107.

Nazar, R. N., Wong, W. M., & Abrahamson, L. A. (1987) *J. Biol. Chem. 262*, 7523–7527.

Negre, D., Weitzmann, C., & Ofengand, J. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 4902–4906.

Noller, H. F., Stern, S., Moazed, D., Powers, T., Svensson, P., & Changchien, L.-M. (1987) *Cold Spring Harbor Symp. Quant. Biol. 52*, 695–708.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.

Schaak, J., Mao, J., & Soll, D. (1982) *Nucleic Acids Res. 10*, 2851–2864.

Schiebel, K., & Hemleben, V. (1989) *Nucleic Acids Res. 17*, 2852.

Stern, S., Moazed, D., & Noller H. F. (1988) *Methods Enzymol. 164*, 481–489.

Thweatt, R., & Lee, J. C. (1990) *J. Mol. Biol. 211*, 305–320.

Veldman, G. M., Klootwijk, J., Van Heerikhuizen, H., & Planta, R. J. (1981) *Nucleic Acids Res. 9*, 4847–4862.

Wellauer, P. K., & Dawid, I. B. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2827–2831.

Yeh, L.-C. C., & Lee, J. C. (1990) *J. Mol. Biol. 211*, 699–712.

Zuker, M. (1989) *Science 244*, 48–52.

Zuker, M., & Stiegler, J. (1981) *Nucleic Acids Res. 9*, 133–148.