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Partial Enzyme Digestion Studies on *Escherichia coli, Pseudomonas, Chlorella, Drosophila,* HeLa and Yeast 5S RNAs Support a General Class of 5S RNA Models

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Summary. Fox and Woese (1975a) have shown that a model of 5S RNA secondary structure similar to the one originally derived for *Chlorella* 5S RNA can be generalized with relatively minor variations to all sequenced 5S RNA molecules, i.e. that corresponding base paired regions can be formed at approximately the same positions. We present experimental data in favour of this hypothesis and show that the points at which ribonucleases T1, T2 and pancreatic ribonuclease cleave six different 5S RNA molecules under 'mild' conditions (high ionic strength, low temperature, low RNAase concentration) nearly always fall in the proposed single-stranded regions. We conclude that this model is a good approximation to the conformation of 5S RNA in solution.

Key words: Ribosome – 5S RNA – Conformation – Evolution

Introduction

Much less is known about the conformation of 5S ribosomal RNA than about that of transfer RNA. The first tRNA sequence was determined in 1965 (Holley et al., 1965) and the "cloverleaf" model which was proposed at the same time became quickly accepted as the general model of tRNA conformation. On the other hand, although the first 5S RNA sequences were published in 1967 (Forget and Weissman, 1967; Brownlee et al., 1967) it is only now that a general model for the conformation of these molecules begins to emerge. This large time lag is due to a number of reasons: 5S RNA is appreciably larger than tRNAs and as computer studies show (Richards, 1969; Jordan, 1971a) the number of possible conformations increases more or less exponentially with the length of the RNA chain; the precise function of 5S RNA is still unknown so that there are no limitations on possible models similar to those

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imposed by the necessity of an accessible anticodon loop in the case of tRNA. Moreover, the question of whether or not the conformation of 5S RNA is identical in the ribosome and in solution is still open. Although a number of studies have shown that the differences should not be very extensive (Bellemare et al., 1972; Noller and Herr, 1974; Delihas et al., 1975), some results which suggest base pairing between 5S and 23S rRNAs in the *E. coli* ribosome (Herr and Noller, 1975) would imply substantial alterations in the secondary structure of 5S rRNA as it enters the ribosome.

Many physico-chemical studies, mostly dealing with *E. coli* 5S RNA, have been performed to obtain information on the conformation of these molecules (for a review and references see Fox and Woese, 1975a, b). Optical methods have given estimates of the amount of base pairing in the molecule; oligonucleotide binding, partial nuclease digestion and chemical modification experiments have delineated "accessible" and "hidden" parts of the molecule. Nuclear magnetic resonance spectra of 5S RNA have also been studied and interpreted as providing evidence for a specific model. However it is probably fair to say that none of these studies have definitely proved the correctness of a particular model. Apart from technical difficulties such as those due to uncertainties on the really "native" state of the 5S RNA preparations used (Monier, 1974) this results from the sad fact that delineation of accessible regions does not directly determine the pairing scheme of an RNA molecule; several different schemes are usually compatible with the data.

A different approach has been followed by Fox and Woese (1975a, b). Supposing (a) that all 5S RNA molecules fulfill at least some of the same function(s), (b) that molecules which perform the same functions have similar shapes, these authors examined whether a similar pairing scheme can be formed with all the known procaryotic 5S RNA sequences. It turns out that this is the case, i.e. all know procaryotic 5S RNA sequences can be folded into a pattern containing four helical regions. Three of these regions were found to be conserved in eucaryotic 5S RNAs as well while the position of the fourth is somewhat variable in eucaryotes. If assumptions (a) and (b) above are valid then this pairing scheme might be expected to represent the functional conformation of 5S RNA. As discussed by Fox and Woese (1975a) most of the available experimental data are compatible with the model which is similar to that earlier proposed for *Chlorella* 5S RNA on the basis of partial digestion studies (Jordan et al., 1974).

In this article we present data from partial ribonuclease digestion studies on six different 5S RNA species and examine whether they support the proposed general model. We have determined the location of the cleavages produced in these 5S RNA molecules by ribonucleases T1, T2 and pancreatic A ribonuclease under "mild" conditions – that is at low enzyme concentration, high ionic strength, high magnesium concentration and low temperature. Under these conditions the ribonucleases which we have used are known (Litt and Ingram, 1964; Wagner and Ingram, 1966) to cleave preferentially the single stranded regions of RNA molecules. The question which we then ask is whether or not these cleavages tend to cluster in the regions of the 5S RNA molecule which the proposed model shows as single-stranded. Some of the data included here have already been published: we then only show the location of the cleavage points on the sequence of the 5S RNA molecule; for new results the supporting evidence is briefly presented.

Methods

1. Preparation of Native ^{32}P-Labelled 5S RNA

This has been described previously for 5S RNA from *E. coli* (Jordan, 1971b), *Ps. fluorescens*, HeLa cells, yeast (Bellemare et al., 1973), *Chlorella* (Jordan et al., 1974) and *Drosophila* (Benhamou et al., 1977). Essentially, 5S RNA was purified by non-denaturing acrylamide gel electrophoresis of whole cell or partially purified RNA, and care was taken to avoid any conditions which could alter its conformation, such as lyophylization, dissolution in water or low ionic strength buffers, and repeated freezing and thawing.

2. Preparation and Analysis of Partial Digests

Partial digestion was in all cases performed at 0–4° C in 0.2 M NaCl 0.02 M Magnesium acetate 0.05 M Tris-HCl pH 7.5 buffer at RNA concentrations of 1–2 mg ml^{-1}. The digest was then fractionated by two-dimensional acrylamide gel electrophoresis

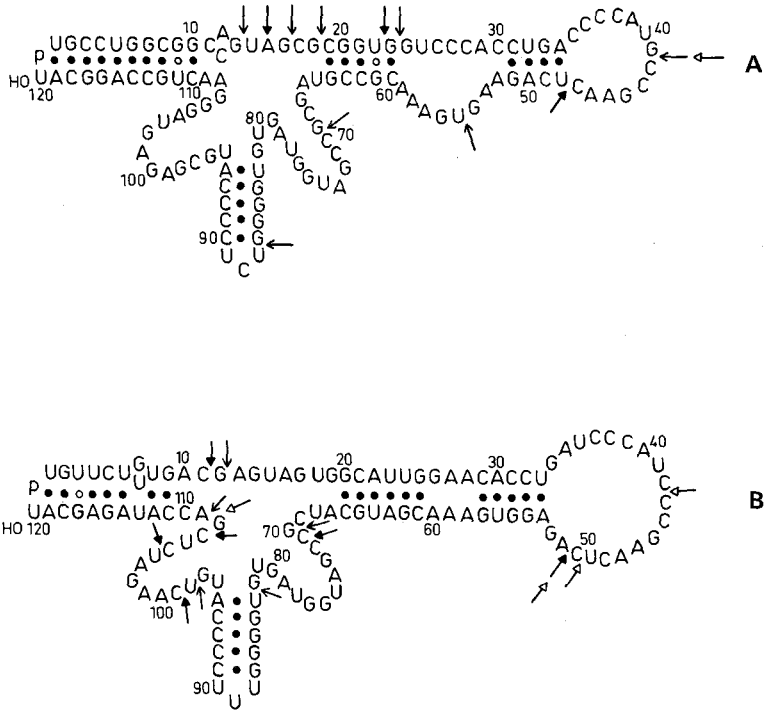

Fig. 1. Models and cleavage points for prokaryotic 5S RNA molecules **A)** *Escherichia coli* K 12 5S RNA (Brownlee et al., 1967) **B)** *Pseudomonas fluorescens* 5S RNA (Dubuy and Weissman, 1971). The detected cleavage points are shown by arrows according to the following code: → RNAase T1 cleavage ⇢ Pancreatic RNAase cleavage ⇢ RNAase T2 cleavage. The shapes of the single-stranded stretches in this and the following figures are not meant to imply any particular conformation (stacked or unstacked) for these regions

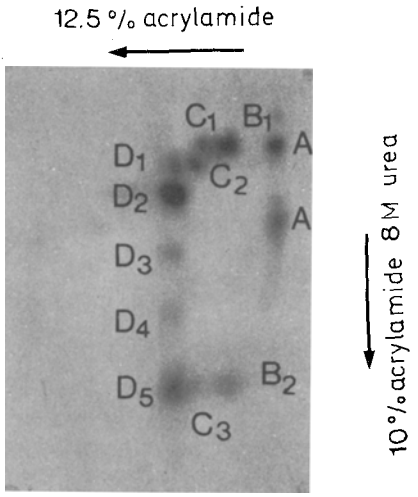

Fig. 2. Two dimensional fractionation of a partial RNAase T1 digest of *Escherichia coli* 5S RNA. First dimension (right to left) 12.5 % acrylamide; second dimension (top to bottom) 10% acrylamide, 8 M urea (Vigne and Jordan, 1971). Digestion was performed with 100 units per ml of RNAase T1. The two fragments referred to in the text are D3, 87–120 and D4, 70–86. The other fragments visible on the autoradiograph are A1 = B1 = C1, 42–120; As, 1–41; B2 = C3 = D5, 1–13; C2 = D1, 55–120; and D2, 70–120. The sequences were determined by two-dimensional fingerprinting after complete RNAase T1 digestion.

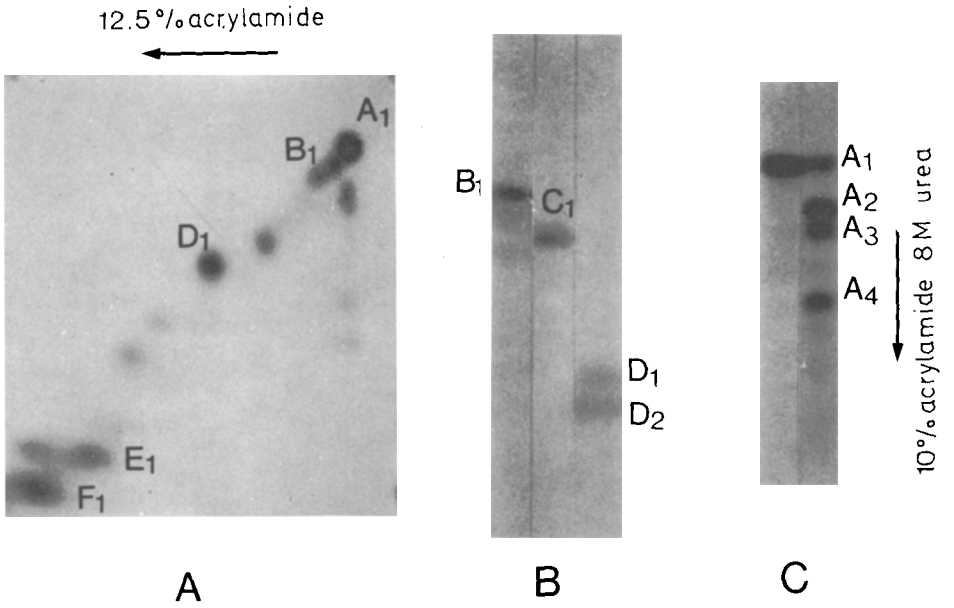

Fig. 3. Fragments obtained by partial digestion of *Pseudomonas fluorescens* 5S RNA. **A)** Digestion with RNAase T1 (100 units per ml) and two-dimensional fractionation. The fragments which were analysed by fingerprinting are A1, 1–120; B1, 13–108; D1, 13–70; E1, containing 1–12 and 109–120, and F1, 83–97. **B)** Digestion with pancreatic RNAase (0.1 μg per ml). In this case three fragments B, C and D were obtained in the first dimension (not shown) and rerun separately in the second dimension. B gives B1, 51–107; C gives C1, 72–98 and D gives D1, 105–120 and D2, 1–11. **C)** Digestion with RNAase T2 (150 units per ml). Fractionation as in (B): most of the material ran like "intact" 5S RNA in the first dimension gel. This "5S" band was run in the second dimension and gave A1, 1–120; A2, 51–120; A3, 51–108, and A4, 1–42

(Vigne and Jordan, 1971). The fragments obtained after the second electrophoretic run were eluted and analyzed by fingerprinting (Brownlee, 1972).

Results

1. Cleavage Points in Prokaryotic 5S RNAs

a) Escherichia coli 5S RNA. Fig. 1A summarizes the data, which have been published (Jordan, 1971b; Vigne and Jordan, 1971; Vigne et al., 1973) except for one cleavage point, G86, which corresponds to two fragments, 87–120 and 70–86, found in relatively low yields in RNAase T1 digests of the molecule (Fig. 2).

b) Pseudomonas Fluorescens 5S RNA. The location of two RNAase T1 cleavages (G12 and G108) and of two RNAase T2 cleavages (C42 and U49) have been reported (Vigne et al., 1973). Further experiments with this 5S RNA molecule yield the following results

(a) RNAase T1 digestion (Fig. 3A): cleavage at G12, G70, G82, G97 and G108
(b) pancreatic RNAase digestion (Fig. 3B): cleavage at C11, C50, C71, U98, U104 and C 107.
(c) RNAase T2 digestion (Fig. 3C): C50 and G108

These cleavage points are shown in Fig. 1B with the model for *Pseudomonas fluorescens* 5S RNA.

2. Cleavage Points in Eukaryotic 5S RNAs

The sequenced eukaryotic 5S RNAs can be folded into a model which is closely similar to that proposed for *Escherichia coli* and *Pseudomonas fluorescens* 5S RNAs except that the location of the fourth paired region (the "side loop" or "prokaryotic loop" of Fox and Woese (1975a, b) is somewhat different and fluctuates from one 5S species to another (Fig. 4).

a) In Chlorella 5S RNA. The available data (Jordan et al., 1974) are presented in Fig. 4A. The agreement with the model is good. The observation of some cleavages in the "stem" base-paired region indicates that this may be less stable in *Chlorella* 5S RNA than in other species.

b) In Drosophila 5S RNA. Fig. 4B shows the cleavage points found in *Drosophila* 5S RNA (Benhamou et al., 1977) which are also in very good agreement with the model, except for cleavage at G18 which was however only observed once in a fragment obtained in low yield.

c) In HeLa Cell 5S RNA. Fig. 5 shows a two-dimensional fractionation of a RNAase T1 partial digest which provides evidence for three cleavage points, G_{21}, G_{81} (or G_{82}) and G_{87} in addition to those previously reported (Vigne et al., 1973). The model with the observed cleavage points is shown in Fig. 4C.

d) In yeast 5S RNA. For the sake of completeness we show in Fig. 4D the model and cleavage points for yeast (*Saccharomyces cerevisiae*) 5S RNA (Vigne et al., 1973). The

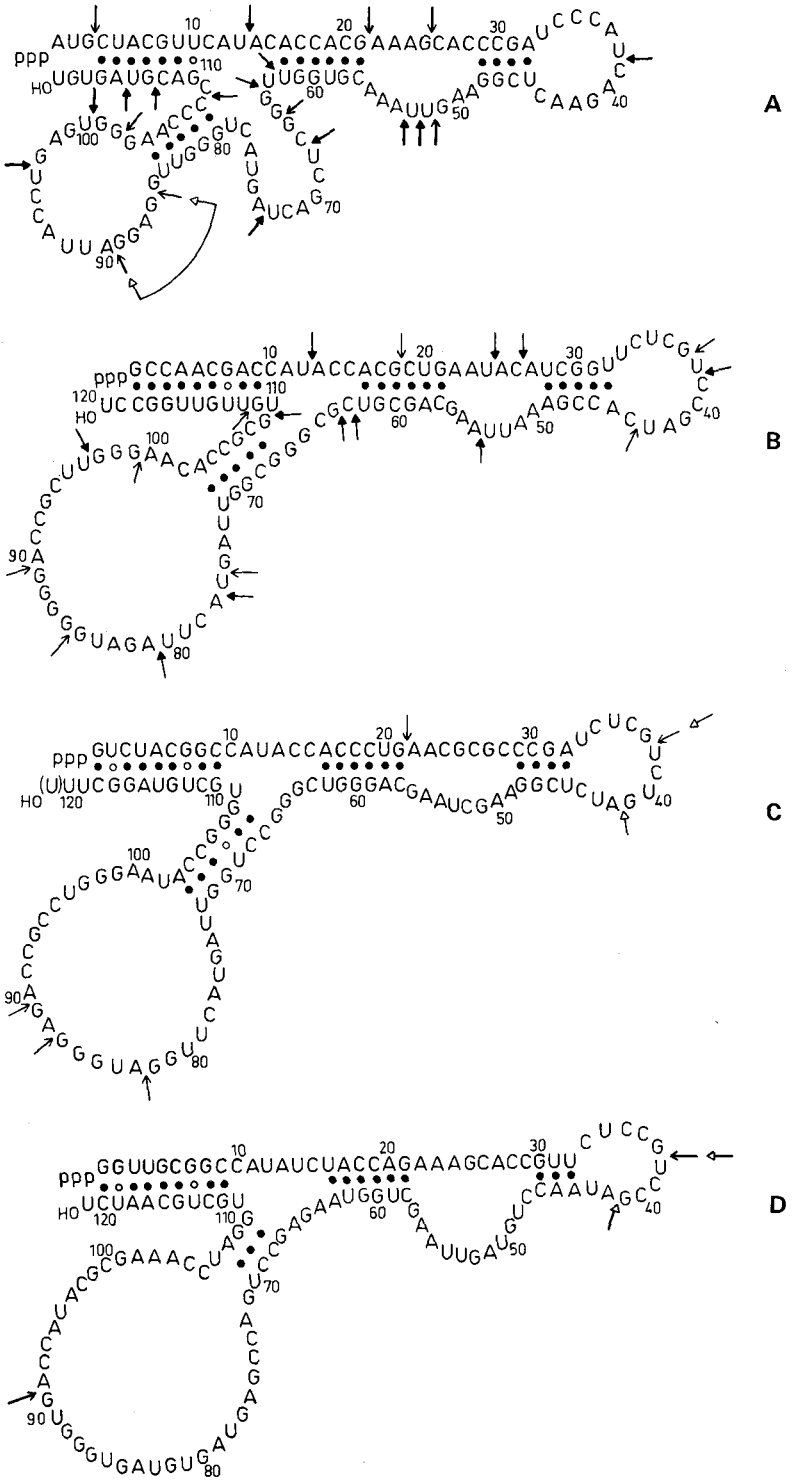

Fig. 4.

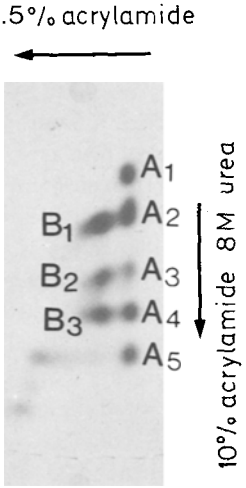

Fig. 5. Two dimensional fractionation of a partial digest of HeLa cell 5S RNA with RNAase T1 (20 units/ml). The sequences of the main fragments seen on this autoradiograph are A1, 1–120; A2, 38–120 plus 1–89; A3, 38–89; A4 = B3, 1–37; A5, 90–120; B1, 22–81 or 82 and B2, 38–87.

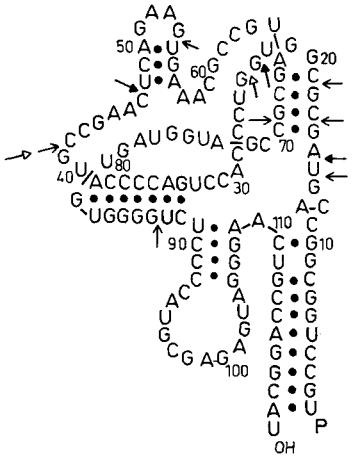

Fig. 6. Test of the Kearns and Wong model. The model proposed by Kearns and Wong (1974) for the secondary structure of *E. coli* 5S RNA at room temperature has been redrawn with the K12 strain sequence; also three errors present in the original figure (one A inserted between A_{29} and C_{30}, C_{42} and A_{73} deleted) have been corrected. The cleavage points observed in our partical digestion studies (→ cleavage by RNAase T1; ⇢, by pancreatic RNAase; ↛by RNAase T2) do not support this model

undetermined sequence in region 13–19 (Hindley and Page, 1972) has been arranged for maximum homology with the very closely related *Torulopsis utilis* sequence (Nishikawa and Takemura, 1974).

Fig. 4. Models and cleavage points for eukaryotic 5S RNA molecules. **A)** *Chlorella* 5S RNA (Jordan et al., 1974) **B)** *Drosophila melanogaster* 5S RNA (Benhamou and Jordan, 1976) **C)** HeLa cell 5S RNA (Forget and Weissman, 1967) **D)** *Saccharomyces cevérisiae* 5S RNA (Hindley and Page, 1972). The detected cleavage points are shown by arrows according to the following code: → RNAase T1 cleavage ⇢ Pancreatic RNAase cleavage ↛ RNAase T2 cleavage. The Chlorella model might also be represented with pairing between C_{106}CC and G_{64}GG. This would make it more similar to the other eukaryotic 5S RNAs but would give a shorter base paired region and would be in disagreement with the cleavage observed at G_{65}

Discussion

It is clear from the preceding section that the location of cleavage points produced by ribonucleases under "mild" conditions is very much in agreement with the proposed 5S RNA models. Since these can accomodate all known 5S RNA sequences into a reasonably similar pairing scheme it is extremely likely that they represent accurately at least the main features of the conformation of 5S RNA in solution. The available experimental evidence, although still rather sketchy, is in favour of the idea that this conformation is largely retained in the ribosome. It would be strange to find such a well-defined similar structure for 5S RNA molecules of different origins if this model was irrelevant.

In this report we have only used the *location* of the cleavage points to draw conclusions on the conformation of the molecule. We had previously interpreted the results of two-dimensional acrylamide gel electrophoresis of partially digested E. coli 5S RNA molecules as evidence for the absence of significant pairing between sequence 14–41 and the rest of the molecule (Vigne and Jordan, 1971). This interpretation is clearly in disagreement with the models shown in Figs. 1 and 4 in which this region is paired to the rest of molecule by a total of 8 to 11 base pairs. However as we have outlined (Vigne et al., 1973) even such stable structures as the "stem" of the yeast 5S RNA molecule (9 contiguous base pairs) can dissociate during electrophoresis in a moderate ionic strength environment (0.04 M Tris-Acetate). It is thus necessary to reexamine the significance of these conclusions.

Assuming that the 5S RNA preparation on which the partial digestion is performed is homogeneous with respect to its conformational state, three situations can arise:

(I) The pairing pattern is stable and remains stable during partial digestion (even after several nicks have been introduced in the molecule) and during electrophoresis in the first dimension (non-denaturing). Only during the second dimension (in 8 M urea) do paired fragments dissociate.

(II) The pairing pattern remains stable during the digestion; when however the digest is loaded on the gel, hydrogen bonding is weakened to such an extent that some paired fragments may dissociate during the first dimension. This will depend on the extent of base pairing and also on the constraints (size of adjoining loops, etc.) due to rest of the molecule.

(III) Once one or two cleavages have been introduced in the molecule its conformation is completely perturbed; the cleavages which then take place have little significance with respect to the conformation of the intact molecule.

The results which we obtained with yeast 5S RNA, that is, the opening up of the "stem" region of yeast 5S RNA on acrylamide gel electrophoresis in the absence of urea (Vigne et al., 1973) show clearly that assumption (I) is not necessarily true; thus one cannot assume that fragments held together by 5 to 10 base pairs will not dissociate in the first dimension since the yeast "stem" (9 base pairs) does. On the other hand if situation (III) was the real one then one should expect more or less random cleavages especially in heavily digested samples. The aggregate data collected in this report show that this in *not* the case. Thus the most likely case is situation (II): the location of the cleavage points is significant; the occurrence of fragments paired together in the first dimension (and dissociated in the second) shows that these *are* paired in the original

molecule; however the appearance of isolated fragments in the first dimension does *not* necessarily imply that these were not hydrogen bonded to the rest of the molecule. Thus the interpretation of results from our two-dimensional method is not as straightforward as we initially proposed and it is necessary to use mainly the *location* of the cleavage points under partial hydrolysis conditions in order to derive the pairing scheme of the molecule. As already outlined this is very much in favour of the proposed model which we therefore consider as a very probable representation of the 5S RNA molecule.

It must be emphasized that 5S RNA molecules may contain some short base-paired regions in addition to those shown in Figs. 1 and 4: different loops may be paired and this may help to obtain a compact folded structure. For example in all four eukaryotic molecules the loop located around position 40 and the large loop at 80–100 can be paired together in an almost identical fashion (G_{89}AU to A_{37}UC in *Chlorella*, G_{88}GAC to G_{37}UCC in *Drosophila*, A_{88}GAC to G_{37}UCU in HeLa and G_{90}AC to G_{37}UC in yeast). This type of pairing is probably not stable when 5S RNA is in solution (we *do* observe RNAase cleavages in these positions, see Fig. 4) but might be significant when 5S RNA is embedded in the ribosome.

It is also interesting to discuss some apparent differences between these molecules. In *Escherichia coli* 5S RNA a small base paired region can be formed close to the "stem", between U_{95}GC and G_{102}UA whereas similar pairing is not possible with the *Pseudomonas fluorescens* sequence. This may be correlated with the observation (Fig. 1) that several cleavage points are found in this region for *Pseudomonas fluorescens* 5S RNA and none for *Escherichia coli* 5S RNA.

Another experimental finding which seems to correlate well with the model is the instability of the yeast 5S molecule: we observed that after cleavage at positions 91 or 37 the two resulting fragments separated when analysed on 12.5 % acrylamide gel in 0.04 M Tris-acetate, that is, without denaturation (Vigne et al., 1973). When the model shown in Fig. 4D is considered it is apparent that, although the "stem" region is similar to that of the other eukaryotic 5S RNA, the two loops around position 40 and position 80–100 are definitely unstable according to the rules proposed by Tinoco et al. (1973) while they are stable (or marginally stable) in the other 5S RNAs.

The 5S RNA model does incorporate some features of earlier proposals, in particular the fairly obvious "stem" region which has been included in nearly all previous models (Brownlee et al., 1967; Forget and Weissman, 1967). In its general architecture it is rather similar to the pairing scheme proposed by Madison (1968) for *E. coli* 5S RNA. That the data reported here *does* allow discrimination between different models is shown by Fig. 6 in which the cleavage points which we have determined are indicated on the sequence of *E. coli* 5S RNA arranged as proposed by Kearns and Wong (1974) on the basis of nuclear magnetic resonance data. It is quite clear from Fig. 6 that our data argue strongly against this model. More precisely, if the "stem" region is left out, the base-paired regions proposed by this model cover 36 of the remaining 99 nucleotides i.e. 36 %; 4 out of 12 cleavage points, i.e. 33 % do fall into these paired region: there is no protection of the postulated paired regions. The disagreement between the Kearns and Wong model and our results may be due to the difficulties in interpretation of NMR spectra of molecules about which fairly little is known – in contrast to the case of transfer RNA molecules where the method can be used to refine the already established cloverleaf model.

We hope that the existence of a fairly well founded, general 5S RNA model (or rather family of models) will now help to define the still rather obscure role played by this molecule in the mechanism of protein synthesis.

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