Accessibility of phosphodiester bonds in the yeast ribosomal 5 S RNA protein complex

John McDougall and Ross N. Nazar

Department of Molecular Biology and Genetics, University of Guelph, Guelph N1G 2W1, Canada

Received 10 October 1986

The tertiary structure of the protein-associated yeast ribosomal 5 S RNA was examined using ethylnitrosourea reactivity as a probe for phosphodiester bonds. A reduced reactivity was consistently observed in at least nine residues within four distinct regions of the RNA sequence. Seven of these were also observed in three regions of the free RNA molecule while two, A_{27} and G_{30}, were only present in the ribonucleoprotein complex. The results strongly suggest that the tertiary structure of the free eukaryotic 5 S RNA is largely conserved in the 5 S RNA-protein complex although it appears to be further stabilized in interaction with the ribosomal protein.

5 S rRNA *Ribosome* *Ribonucleoprotein* *Tertiary structure*

1. INTRODUCTION

Although RNA-protein interactions are fundamental to ribosome structure and function, the mechanisms of interaction are still poorly understood. A number of protein binding sites in ribosomal RNA have been defined at least partially (e.g. [1–10]), but the studies have been limited to primary and secondary structures and there is little understanding for either the role of the tertiary structure or any effects of protein binding on the higher order structure. Because 5 S RNA-protein complexes are relatively stable and easily prepared in high yields, they have become attractive models for ribosomal RNA-protein interactions and perhaps RNA-protein interactions in general. Furthermore, since in the course of protein synthesis the 5 S RNA has been postulated to contribute to the peptidyl transferase activity [11], interact with tRNA [12], have a role in ribosomal translocation [13] or even subunit association [14], the 5 S RNA-protein complex may also be of considerable importance in mRNA translation.

While a great deal of progress has been made in our understanding of the primary and secondary structure for the 5 S RNA (reviews [7,15]) and the relationship of this structure to ribosomal protein binding, very little is known about the higher order structures or their role in the 5 S RNA-protein complex. Alternative experimental approaches have been used to describe the tertiary structures of the 5 S RNA from *Escherichia coli* and *Saccharomyces cerevisiae* (yeast) and somewhat differing models have been proposed [15–20]. These differences may simply reflect differences in the probing techniques, but more recent comparative studies now suggest that the free eukaryotic 5 S RNA does have a more organized tertiary structure [20]. Here, the use of ethylnitrosourea reactivity as a probe for RNA tertiary structure [15,21] was extended to the yeast 5 S RNA-protein complex. The results indicate that the higher order structure which is present in the free RNA molecule is retained in the complex and may be further stabilized in the presence of ribosomal protein.

2. MATERIALS AND METHODS

2.1. *Preparation and labeling of the 5 S RNA-protein complex*

Yeast (*S. cerevisiae*, strain S288C) was grown aerobically and both the 5 S RNA and 5 S RNA-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

protein complexes were isolated from 60 S ribosomal subunits as described [22]. The purified RNA was labeled with cytidine 3',5-[5'-^{32}P]bisphosphate at the 3'-end using RNA ligase (Pharmacia) and repurified on a 12% polyacrylamide sequencing gel [23]. The 5 S RNA-protein complex was then labeled by incubating purified yeast complex with labeled RNA of high specific activity to allow an exchange of RNA components. In a typical experiment ^{32}P-labeled 5 S RNA was dissolved in 25 μl of 25 mM EDTA, pH 7.0, containing 5–6 A_{260nm} units/ml of unlabeled yeast complex and incubated on ice for 30–45 min. The labeled complex was characterized by electrophoresis on an 8% polyacrylamide gel [10,22]; 50–90% of the labeled RNA was associated with the ribonucleoprotein complex.

2.2. *Determination of ethylnitrosourea-reactive sites*

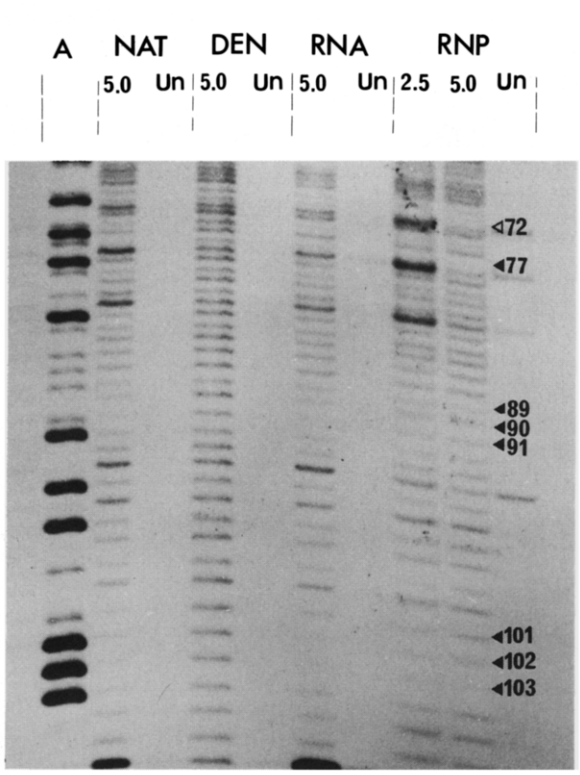
Purified RNA and the 5 S RNA-protein complex were treated with ethylnitrosourea and the modified phosphodiester bonds were identified essentially as described by Vlassov et al. [21]. Briefly, 20-μl aliquots of labeled RNA or ribonucleoprotein complex either in 2 mM EDTA, 0.3 M Na cacodylate, pH 8.0 (denatured condition), or in 0.1 M NaCl, 20 mM $MgCl_2$, 0.3 M Na cacodylate, pH 8.0 ('physiological-like' condition), or in 25 mM EDTA, pH 7.5 (RNP exchange buffer) were reacted with 2.5–7.5 μl ethylnitrosourea-saturated ethanol for 2 min at 80°C (denatured condition) or 1 h at 20°C. After alkylation, 20 μg carrier RNA was added to each sample followed by ethanol precipitation. The alkylated ribonucleoprotein samples were further deproteinized by an SDS/phenol treatment [22] and after alkali cleavage all samples were analyzed by gel electrophoresis on 12% polyacrylamide sequencing gels [15]. In each case samples, not treated with ethylnitrosourea, were included as controls for non-specific degradation.

3. RESULTS AND DISCUSSION

Previous studies on the accessibility of 3'-end-labeled eukaryotic 5 S rRNAs to ethylnitrosourea modification [15] indicated that three common regions of the sequence contained phosphodiester bands which showed a reduced reactivity to ethylnitrosourea under physiological like conditions. These results were subsequently used [15] to estimate a working model for the tertiary structure of the eukaryotic 5 S rRNA in which two arms of the secondary structure interact to form a loop or 'lollipop'-like structure. As shown in fig.1, in the present study these regions of reduced reactivity were observed not only in the free 5 S RNA but also in the yeast ribonucleoprotein complex. While some quantitative differences are evident, at least seven residues (G_{77}, G_{89}, U_{90}, G_{91}, G_{101}, A_{102} and A_{103}) in three different regions of the sequence showed a reduced reactivity in both RNA samples. Since the ribonucleoprotein complex was previously found to be most stable in very low salt buffers [24], the modification experiments with both the free (RNA) and protein-associated (RNP) 5 S RNA molecule were conducted under these optimized conditions. When compared to RNA treated under more physiological-like conditions (NAT) only one, somewhat variable difference was observed. As shown in fig.1, residue A_{72} (open arrow) was sometimes slightly less reactive in the absence of Mg^{2+}. Furthermore, when less ethylnitrosourea reagent was added (2.5 μl) as a control to reduce any artifacts due to the ethanol solvent, the results were essentially identical. Taken together, therefore, these results strongly suggest that the tertiary structure of the free 5 S RNA is largely retained within the RNA-protein complex. In addition, to avoid cleavages which are induced by conditions of electrophoresis [20] the RNA was extracted directly after modification.

Since previous physicochemical studies on 5 S RNA-protein complexes indicated RNA conformation changes during complex formation [24], other regions of the molecule were examined for reduced reactivity. As shown in fig.2, nucleotides in at least one region of the 5 S RNA were affected by the presence of ribosomal protein. Two residues (A_{27} and G_{30} showed a clearly reduced reactivity in the ribonucleoprotein complex.

The presence of these additional restrictions in the accessibility of phosphodiester bonds raises at least two possibilities; that these two residues interact directly with the ribosomal protein or alternately, that the ribosomal protein induces or stabilizes further higher order structure in the 5 S rRNA. As shown in fig.3, the two residues are estimated to play a role in the secondary structure



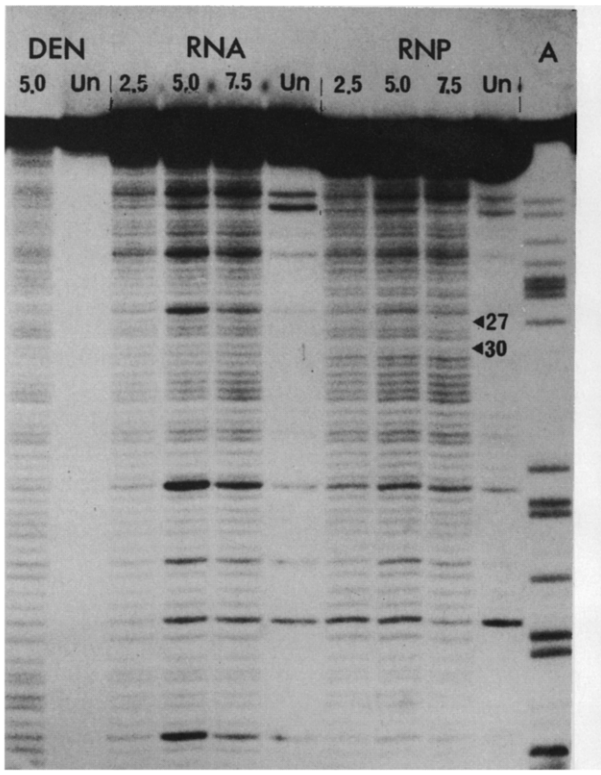


Fig.1. (Right) Common sites of reduced phosphate alkylation in the free and protein-associated *S. cerevisiae* 5 S rRNA. 20 μl aliquots of 3′-end-labeled free or protein-associated RNA were treated with 25 or 5.0 μl of ethanol-saturated ethylnitrosourea as described in section 2. The partially degraded fragments were analyzed on a 12% polyacrylamide gel at 1000 V for 6 h; fragments from a diethyl pyrocarbonate-induced aniline degradation [23] were included as markers (A) for residue number determinations. Residues which were partially resistant to ethylnitrosourea modification as compared to 5 S rRNA treated under denaturing conditions (DEN) are indicated with arrows. NAT, free 5 S RNA treated with ethylnitrosourea under physiological-like conditions; RNA, free 5 S rRNA treated with ethylnitrosourea under conditions which favour the formation of the RNA-protein complex; RNP, ribonucleoprotein complex treated with ethylnitrosourea under conditions which favour the formation of the complex; Un, free RNA or complex incubated in the absence of ethylnitrosourea.

Fig.2. (Left) Additional site of reduced phosphate alkylation in the protein-associated *S. cerevisiae* 5 S rRNA. The samples were treated and analyzed by electrophoresis on a 12% polyacrylamide gel at 1000 V for 20 h as described in fig.1.

of the RNA but are not located in either of the three helical domains which constitute the protein binding site [10]. In view of this the second explanation appears more likely with the protein-RNA interaction drawing the RNA structure tighter [24] allowing the additional phosphates to interact in the folding process. Indeed, the region of sequence containing A_{27} and G_{30} is predicted to fold against the opposite arm in the lollipop model of the tertiary structure and earlier, residue U_{48} was found to be subject to a very slight and variable reduction in reactivity in the free RNA molecule [15].

While the present results, therefore, do not conclusively define the higher order structure of the protein bound 5 S RNA they add to our understanding of the structure in two ways; they indicate that much of the tertiary structure in the free RNA is largely retained within the ribonucleoprotein complex and they underline further regions of the 5 S RNA sequence which are affected by the RNA-protein interaction. The studies also demonstrate

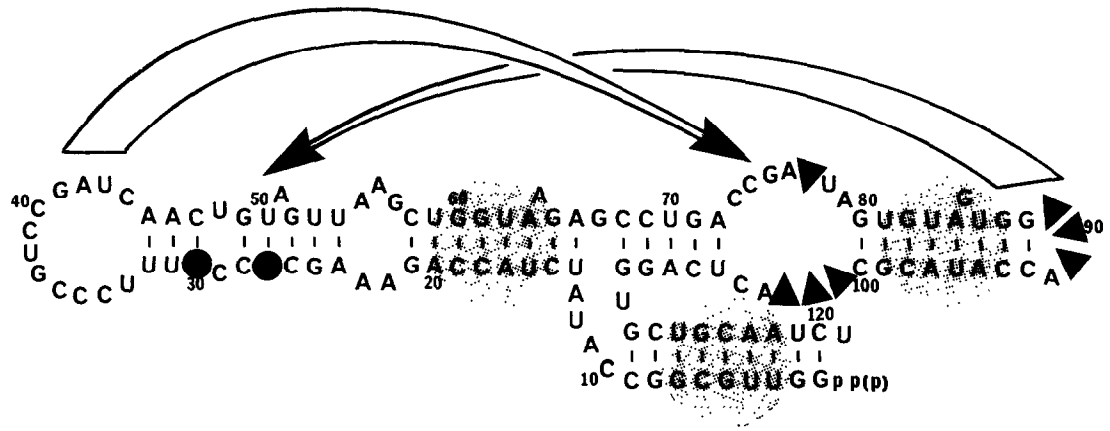


Fig.3. Ethylnitrosourea-resistant phosphodiester bonds in the *S. cerevisiae* ribosomal 5 S RNA-protein complex. The secondary structure is estimated according to the base-pairing scheme of Nishikawa and Takemura [25]. The solid triangles cover residues which are partially resistant to ethylnitrosourea modification in both the free and protein-associated molecule; the solid circles cover residues which are partially resistant only in the protein-associated molecule. The shaded areas indicate the three helical domains which previously have been shown to constitute the ribosomal protein binding site [10,25] and the open arrows indicate regions of tertiary structure interaction as suggested in the lollipop model [15].

the potential utility of ethylnitrosourea reactivity as a probe for important phosphate interactions or sterically restricted phosphate groups within ribonucleoprotein complexes, in general.

ACKNOWLEDGEMENT

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

[1] Gray, P.H., Bellemare, G., Monier, R., Garrett, R.A. and Stoffler, G. (1973) J. Mol. Biol. 77, 133–152.
[2] Zimmermann, R.A., Mackie, G.A., Muto, A., Garrett, R.A., Ungewickell, E., Ehresmann, C., Stiegler, P., Ebel, J.-P. and Fellner, P. (1975) Nucleic Acids Res. 2, 279–302.
[3] Dahlberg, A.E. and Dahlberg, J.E. (1975) Proc. Natl. Acad. Sci. USA 72, 2940–2944.
[4] Branlant, C., Scri Wilada, J., Krol, A. and Ebel, J.P. (1977) Eur. J. Biochem. 74, 155–170.
[5] Zimmerman, J. and Erdmann, V.A. (1978) Nucleic Acids Res. 5, 2267–2288.
[6] Nazar, R.N., Willick, G.E. and Mathesen, A.T. (1979) J. Biol. Chem. 254, 1506–1512.
[7] Garrett, R.A., Douthwaite, S. and Noller, H.F. (1981) Trends Biochem. Sci. 6, 137–141.
[8] Wower, I., Wower, J., Meinke, M. and Brimacombe, R. (1981) Nucleic Acids Res. 9, 4285–4302.
[9] Wower, I. and Brimacombe, R. (1983) Nucleic Acids Res. 11, 1419–1437.
[10] Nazar, R.N. and Wildeman, A.G. (1983) Nucleic Acids Res. 11, 3155–3168.
[11] Racke, I.D. (1971) Proc. Natl. Acad. Sci. USA 68, 2357–2360.
[12] Erdmann, V.A. (1976) Prog. Nucleic Acid Res. Mol. Biol. 18, 45–90.
[13] Weidner, H., Yaun, R. and Brothers, D.M. (1977) Nature 266, 193–194.
[14] Azad, A.A. (1979) Nucleic Acids Res. 7, 1913–1929.
[15] McDougall, J. and Nazar, R.N. (1983) J. Biol. Chem. 258, 5256–5259.
[16] Wagner, R. and Garrett, R.A. (1978) Nucleic Acids Res. 5, 4065–4075.
[17] Hancock, J. and Wagner, R. (1982) Nucleic Acids Res. 10, 1257–1269.
[18] Muller, J.J., Damashun, G., Bohn, S., Fabian, H. and Welfle, H. (1982) Stud. Biophys. 87, 11–14.
[19] Pieler, T. and Erman, V.A. (1982) Proc. Natl. Acad. Sci. USA 79, 4599–4603.

[20] McDougall, J. (1982) PhD Thesis, University of Guelph, Guelph, Canada.
[21] Vlaasov, V.V., Giege, R. and Ebel, J.P. (1980) FEBS Lett. 120, 12–16.
[22] Nazar, R.N., Yaguchi, M., Willick, G.E., Rollin, C.F. and Roy, C. (1979) Eur. J. Biochem. 102, 573–582.
[23] Peattie, D.A. (1979) Proc. Natl. Acad. Sci. USA 76, 1760–1764.
[24] Willick, G.E., Nazar, R.N. and Van, N.T. (1980) Biochemistry 19, 2738–2742.
[25] Nishikawa, K. and Takemura, S. (1974) FEBS Lett. 40, 106–109.
[26] Nazar, R.N. (1979) J. Biol. Chem. 254, 7724–7729.