Physicochemical Studies on the 5S Ribonucleic Acid–Protein Complex from a Eucaryote, *Saccharomyces cerevisiae*†

Gordon E. Willick,* Ross N. Nazar,‡ and Nguyen T. Van

ABSTRACT: A physicochemical study of the 5S ribonucleic acid–protein complex from a eucaryote, *Saccharomyces cerevisiae*, has been made with sedimentation velocity ultracentrifugation, gel electrophoresis, circular dichroism spectroscopy, thermal denaturation, and fluorescence enhancement of ethidium bromide as probes of the structure of the complex and its components. The 5S RNA–protein complex isolated from *S. cerevisiae* contains, in addition to 5S RNA, one acidic protein with a molecular weight of 38 000 [Nazar, R. N., Yaguchi, M., Willick, G. E., Rollin, C. F., & Roy, C. (1979) *Eur. J. Biochem. 102*, 573–582]. Sedimentation velocity and gel electrophoresis studies as a function of potassium chloride concentration showed that this complex was stable at low ionic strength but was dissociated by ionic strengths of this salt greater than about 150 mM. At low ionic strength (10 mM KCl) circular dichroism studies showed that the 5S RNA had a greater secondary structure in the complex than when free in solution. Difference circular dichroism studies between the complex and free 5S RNA in 10 mM KCl and 500 mM KCl implied the protein also gained some secondary structure on complexing with the 5S RNA. The protein had little secondary structure in either case, with an apparent increase from 10 to 14% α helix having occurred. Thermal denaturation curves were almost monophasic for free, but clearly biphasic for complexed, 5S RNA at low ionic strengths, and were biphasic for both at the highest ionic strength (100 mM KCl). The data were interpreted in terms of a model in which the protein stabilizes a structure of the 5S RNA in the complex which is probably similar but not equivalent to that of free 5S RNA in moderate concentrations of salt. In clear contrast to the procaryote complexes previously studied, the complex bound almost as much ethidium bromide as free 5S RNA, as evidenced by the fluorescence enhancement as ethidium bromide was added. This implied the protein had little effect on the accessibility of this dye to its sites on 5S RNA.

The 5S RNA–protein complexes have been isolated from the ribosomes of both procaryotic and eucaryotic organisms and appear to be universal in existence. In general, the isolation procedure simply consists of depleting the large ribosomes of Mg^{2+}, which liberates a complex consisting of 5S RNA plus two to three proteins in procaryotes, or one protein in eucaryotes. The size of the 5S RNA is highly conserved, with a molecular weight of about 38 000. In addition, the total mass of the protein appears to be also highly conserved, with a molecular weight also of about 38 000, and thus the complex has a molecular weight of 78 000–80 000. The actual function of this complex is unknown, but the very fact that it appears to have been rather highly conserved through evolution suggests that its function is likely to be an important one.

We have been carrying out a general study of 5S RNA–protein complexes, not only to establish as much as possible just how real the apparent similarities are but also because we believe these complexes, easy to isolate in large quantities, serve as simple prototypes of the much more difficult general problem of ribosomal RNA–protein interaction. Considerable previous work has been carried out on the procaryotic complexes derived from *Escherichia coli* (Monier, 1974; Erdmann, 1976) and the extreme halophile, *Halobacterium cutirubrum* (Smith et al., 1978). More recently, we have isolated (Nazar et al., 1979b), and initiated a detailed study of, the complex from *Saccharomyces cerevisiae*. To this point we have carried out studies on the yeast complex compatible with those previously carried out on the procaryotes in order that a consistent comparison be made with the two procaryote complexes. This has included partial information on the sequence of the protein component, YL3[1] (Yaguchi et al., 1978; Nazar et al., 1979b) and on the region of 5S RNA binding to YL3, as inferred from partial nuclease digestion studies (Nazar, 1979). In this paper we present some physicochemical studies for comparison with the procaryote data and will show that, while these properties are broadly similar, there are some important differences between the complexes that, in turn, imply differences at the structural level.

Materials and Methods

Ribosomal 50S Subunits. *Saccharomyces cerevisiae*, strain 5288C, was grown aerobically in 0.3% yeast extract, 0.5% Bacto-peptone, and 2% glucose at 28 °C and harvested in late log phase. Ribosomes and ribosomal subunits were prepared essentially by the method of Torano et al. (1974), as described by Nazar (1979).

5S RNA–Protein Complex. All procedures were carried out at 4 °C. The 60S subunits (10 000–20 000 A_{260} units) were suspended in 72 mL of water, and 8 mL of EDTA (250 mM, pH 7.0) was added to the suspension. The ribosomes were then centrifuged at 165 000*g* for 18 h. Essentially the only material left in the supernatant was the 5S RNA–protein complex (RNP) (Nazar et al., 1979b).

Gel Electrophoresis. RNA and RNP were fractionated on 8% slab gels in a manner similar to that previously described (Nazar et al., 1975). In experiments with ethidium bromide (British Drug House), the drug was incorporated into both the gel components before polymerization and the running buffer. The experiment in 200 mM KCl was run for 16 h at 3 V/cm. The electrophoresis in 500 mM KCl was carried out

† From the Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6 (G.E.W. and R.N.N.), and the Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030 (N.T.V.). *Received September 14, 1979.*

‡ Present address: Department of Botany and Genetics, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

[1] Abbreviations used: RNP, *S. cerevisiae* 5S RNA–YL3 complex; EthBr, ethidium bromide; YL3, 5S RNA binding protein from *S. cerevisiae*; EDTA, ethylenediaminetetraacetic acid.

0006-2960/80/0419-2738$01.00/0

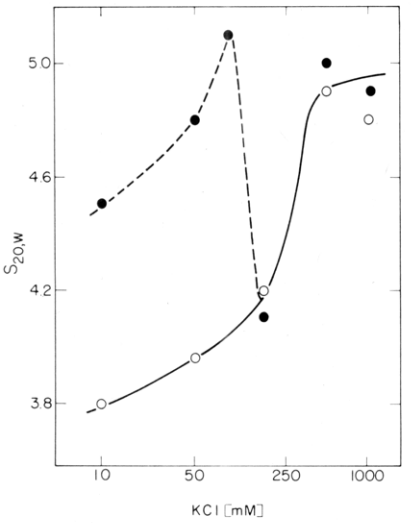

FIGURE 1: Sedimentation coefficients of yeast RNA (○) and RNP (●) as a function of potassium chloride concentration. The remaining solvent was 10 mM Tris-HCl and 1 mM EDTA, pH 7.8. All runs were at 20 °C, and the concentration of the samples was 1–1.5 A_{260} units/mL.

for 24 h at 3 V/cm, with one change of reservoir buffer, and was cooled with 2 °C water.

Fluorescence Spectroscopy. The fluorescence enhancement of ethidium bromide on binding to free or bound 5S RNA was measured at 21 °C using a Hitachi Perkin-Elmer spectrophotofluorometer. Excitation and emission wavelengths were 500 and 605 nm, respectively.

Circular Dichroic Spectroscopy. Circular dichroic spectra were obtained on a Cary 61 circular dichrograph operated at 27 °C. RNA concentrations were determined by using a value of 23.4 $cm^{-1}\cdot(mg/mL)^{-1}$ for the extinction coefficient at 260 nm. Spectra are reported in units of $deg\cdot cm^2\cdot(decimean\ residue\ weight)^{-1}$. A value of 320 was used as the mean residue weight for the RNA and a value of 120 for the binding protein calculated from its amino acid composition (Nazar et al., 1979a). The spectrum of the protein in the complex was calculated as previously described (Nazar et al., 1979a).

Thermal Melts. Circular dichroic melts were carried out in the Cary 61 instrument externally thermostated with a Haake water bath. The samples (1 mL) were contained in a jacketted 1 cm path length cell (Hellma, Inc.) with the outer jacket filled with silicone oil (Beckmann Instruments). The temperature, which was increased in increments of about 2 °C, was monitored with a thermistor probe (Yellow Springs Instrument Co., Yellow Springs, OH) placed on the cell block beside the cuvette. Light absorption thermal melts at 260 nm were performed as previously described (Van et al., 1976).

Ultracentrifugation. Sedimentation velocity experiments were performed in a Beckmann Model E ultracentrifuge using the photoelectric scanner at 280 nm.

Results

Salt-Induced Dissociation of RNP. The RNP is normally isolated in a very low ionic strength solvent, in which it is quite stable. Because many of the experiments to be described below were carried out in solvents of higher ionic strength, we decided to investigate the stability of this RNP as a function of increasing potassium chloride concentration (Figure 1). At low ionic strengths (0–100 mM KCl), the RNP has a sedimentation coefficient about 20% greater than that of the 5S RNA (Figure 1). At about 150 mM potassium chloride, the complex apparently dissociates, since its sedimentation coefficient becomes virtually identical with that of the 5S RNA.

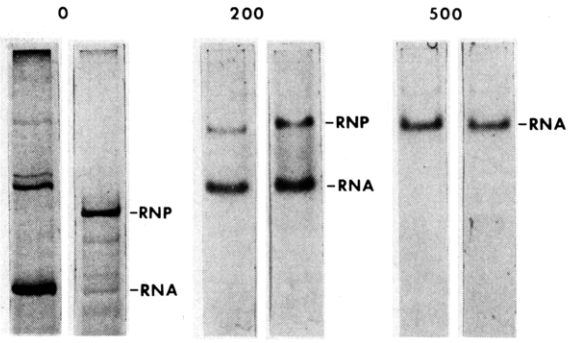

FIGURE 2: Slab gels of RNA and RNP with 0, 200, and 500 mM potassium chloride added to the gel buffer. The running temperature of the gels was in the range 2–10 °C.

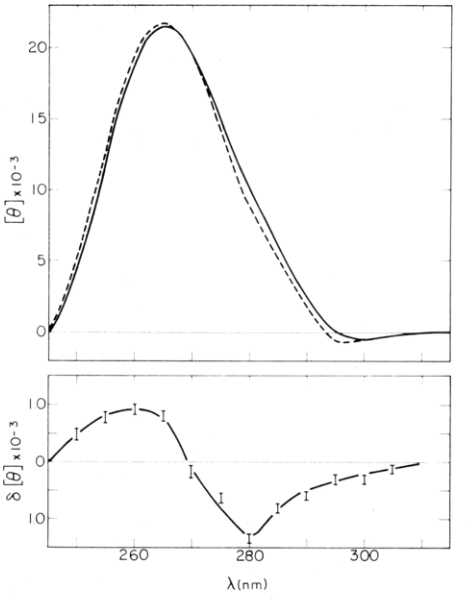

FIGURE 3: The CD spectra of yeast RNP (- - -) and 5S RNA (—) with the calculated difference spectrum in the lower panel. The solvent was 10 mM potassium chloride, 10 mM Tris-HCl, and 1 mM EDTA, pH 7.8. The runs are at the instrument ambient temperature, 27 °C.

This conclusion was confirmed by running the RNP and free 5S RNA in slab gels at increasing ionic strengths (Figure 2). With no added potassium chloride, almost all of the 5S RNA is in the form of the complex, whereas with 200 mM added salt, most of the sample is seen as free 5S RNA. In 500 mM salt, only the RNA band is observed.[2]

Circular Dichroism in the 250–320-nm Region. The circular dichroism spectrum in this region is a good measure of conformational changes that may occur when the protein becomes complexed to the 5S RNA, since the protein contribution is negligible here in comparison to the RNA. In Figure 3, the circular dichroism spectra of the RNP and the free 5S RNA in the 10 mM KCl solvent are shown. The large positive maximum undergoes a small increase in magnitude and a shift of 1 nm (from 265.5 to 264.5 nm) when the 5S RNA is complexed as opposed to free in solution. Similarly, the small negative maximum is increased slightly and blue shifted by about 2 nm. This result indicates the 5S RNA is more ordered in the complex than when free in solution.

Circular Dichroism in the 200–240-nm Region. The difference spectrum between the RNP and the free 5S RNA in

[2] The trailing band seen in the 5S RNA samples is not nucleic acid, although it stains with methylene blue (R. N. Nazar, unpublished experiments). This material is not isolated with the RNP under the present conditions of isolation.

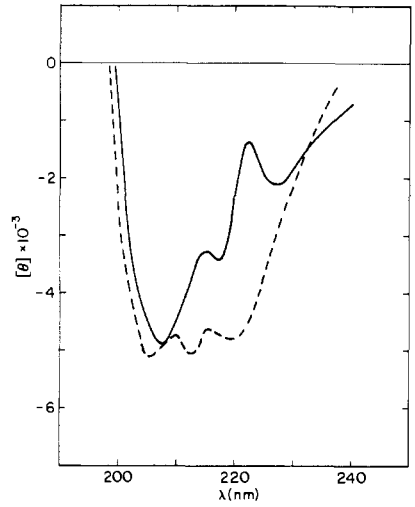

FIGURE 4: CD spectra of protein YL3 calculated from the complex minus 5S RNA difference spectrum in 10 mM potassium chloride (---) or 500 mM potassium chloride (—). The remaining solvent was 10 mM Tris-HCl and 1 mM EDTA, pH 7.8.

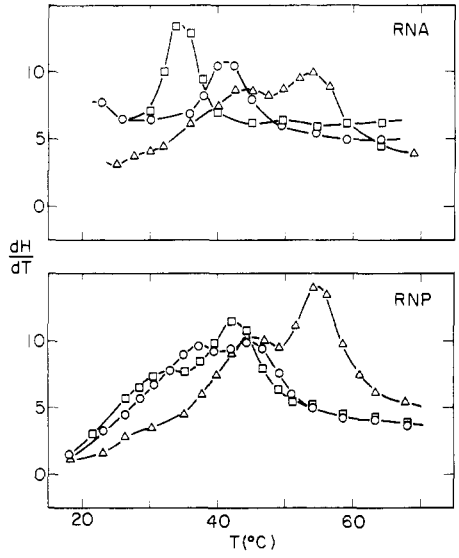

FIGURE 5: Thermal melt curves for yeast 5S RNA (upper) and RNP (lower) at potassium chloride concentrations of 0 mM (□), 10 mM (○), and 100 mM (△) in 2.5 mM EDTA, pH 7.0.

this region is a measure of the secondary structure of the protein (Willick et al., 1979). The difference spectra are shown in Figure 4 for both 10 mM potassium chloride and 500 mM potassium chloride (where the complex is largely dissociated). The difference spectra in both cases were relatively weak, suggesting the protein had little secondary structure. Analysis of the data using a linear least-squares approach (Willick & Zuker, 1977) indicated an α-helical content of 14% in 10 mM KCl and 10% in 500 mM KCl. The β structure was estimated to be 12% for both solvent conditions. Protein YL3 is apparently denatured under the conditions of isolation and somewhat insoluble in nondenaturing solvents, and no reasonably reliable spectrum could be obtained of the protein alone in solution.

Thermal Denaturation Studies. Derivative absorption thermal melt profiles were obtained for both the free 5S RNA and the RNP in increasing concentrations of potassium chloride (Figure 5). In both 0 and 10 mM potassium chloride, biphasic curves were obtained for the RNP, whereas the curves were almost monophasic for the 5S RNA. In these low ionic strength examples, the binding protein appears to have both

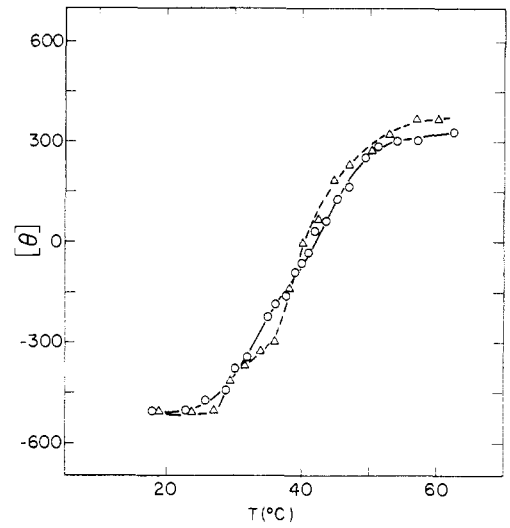

FIGURE 6: CD thermal melt at 300 nm of yeast 5S RNA (△) and RNP (○). The remaining solvent was 1 mM Tris-HCl and 1 mM EDTA, pH 7.8.

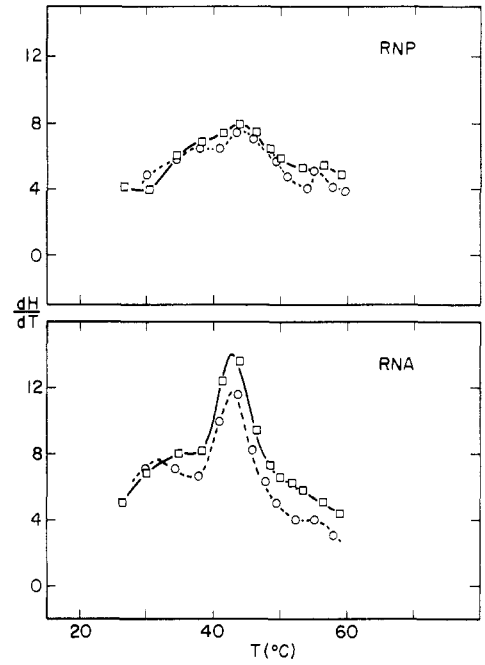

FIGURE 7: Thermal denaturation profiles of yeast 5S RNA and RNP at 260 nm (○) and 278 nm (□). The solvent was 10 mM KCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 7.8.

stabilized and destabilized the RNA with respect to its thermal melt properties. At a higher ionic strength, 100 mM potassium chloride, the RNP and RNA have similar biphasic curves. An example of a CD melt, monitored at 300 nm, is shown in Figure 6. This result, in 10 mM potassium chloride, was consistent with the results obtained when the change in absorption was used as a monitor. The RNP gave a clearly biphasic curve, and the RNA was monophasic except for a slight inflection occurring at about 30 °C.

It has been shown previously (Van et al., 1976) that comparison of melt curves at 260 and 278 nm provides an indication of the relative proportion of A·U and G·C base pairs giving rise to the thermal transitions. An experiment in 10 mM potassium chloride is shown in Figure 7. The principal transition at 43 °C seen in 5S RNA alone has a substantially greater hyperchromicity when measured at 278 nm than at 260 nm, indicating the melting of a relatively G + C rich structure. In contrast, the curves for the RNP at the two

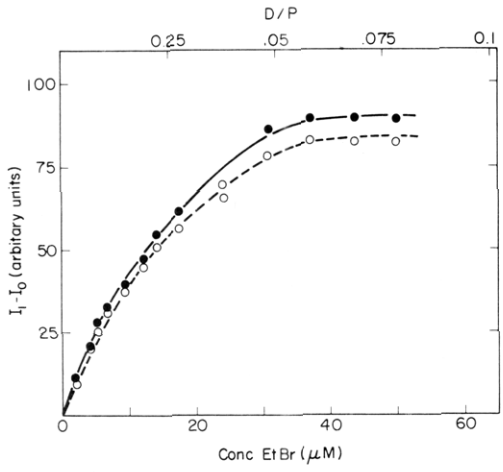

FIGURE 8: Fluorescence enhancement of EthBr on addition to yeast 5S RNA (●) or RNP (○). The solvent was that of the slab gel in Figure 9. The temperature was 20 °C, and the concentration of RNA or RNP was 2.5 μM.

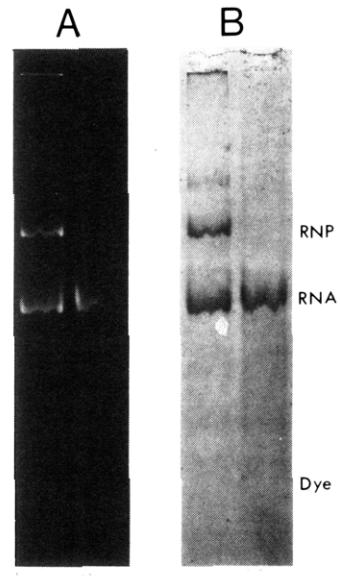

FIGURE 9: Slab gel of yeast RNP and 5S RNA in 15 μm ethidium bromide. In A the fluorescence of the EthBr was photographed; B is the same gel after staining with methylene blue. The conditions of the gel are equivalent to the 0 mM potassium chloride experiment in Figure 2.

wavelengths are almost identical and are suggestive of a less G + C rich structure.

Experiments were also carried out to determine whether dissociation of the RNP was associated with the thermal denaturation of the 5S RNA. Aliquots were removed from a RNP sample subjected to an increasing temperature gradient. After the aliquots were quick-cooled on ice, they were applied to a polyacrylamide gel as in Figure 2 (left). The results showed that the RNP dissociated at 43–45 °C in 100 mM KCl under the solvent conditions shown in Figure 6 and at approximately 50 °C in 10 mM KCl under the solvent conditions of Figure 7. Therefore, the 100 mM KCl RNP thermal denaturation curve is simply that of the 5S RNA, whereas in 10 mM KCl the RNP does not begin to dissociate until the thermal denaturation is almost complete. The corresponding thermal denaturation profiles shown in Figures 6 and 7 represent those due to the intact RNP.

Binding of Ethidium Bromide. The 5S RNA–protein complexes of both *E. coli* (Feunteun et al., 1975) and *H. cutirubrum* (Nazar et al., 1979a) have been found to bind much less EthBr, as measured by the fluorescence enhancement, than the corresponding free 5S RNAs. The corresponding data in the case of this eucaryotic complex showed that the binding of EthBr to the complex was only slightly less than to that of the free 5S RNA (Figure 8). There was a possibility that this negative finding was a result of the EthBr simply dissociating the complex. A sample of RNP and 5S RNA was electrophoresed under the standard conditions, with 15 μM EthBr added to the gel and running buffer (Figure 9). The RNP clearly binds EthBr, although comparison of Figures 2 (middle) and 9 suggests there has been some dissociation of the RNP. This implies a competition of some of the EthBr for the protein binding site has occurred.

Discussion

The eucaryote complex of yeast studied here has some general properties which are roughly comparable in many respects to the procaryote complexes previously reported. Formation of the complex results in a conformational change in both the 5S RNA and the protein. Similar conformational changes in the 5S RNA have been reported for both the *E. coli* (Bear et al., 1977; Fox & Wong, 1978; Spierer et al., 1978) and *H. cutirubrum* (Nazar et al., 1979a; Willick et al., 1979) complexes. We have also shown a similar conformational change in the principal binding protein of *H. cutirubrum*, but the *E. coli* complex has not been studied in this detail. However, Allen & Wong (1978) have reported overall conformational changes of the *E. coli* ribosomal proteins occurring as a result of ribosome formation. We therefore suggest that ribosomal protein–RNA interactions commonly involve an induced fit mechanism.

The thermal denaturation data also suggest that the protein stabilizes enhanced secondary and tertiary structures of the 5S RNA in the complex. The melt curve is almost monophasic for the free 5S RNA at low ionic strength, but becomes biphasic in the complex (Figures 6 and 7). This implies that a relatively uniform secondary structure of the 5S RNA at low ionic strength (for example, a hairpin structure) is replaced by a more complicated secondary structure. Neither of the thermal denaturation curves shown for RNP and RNA in 10 mM KCl (Figure 7) is consistent with proposed models (Nazar, 1979), which may simply reflect the presence of a tertiary structure not included in the models. Analysis of the hyperchromicity data of Figure 7 in terms of a linear combination of poly(A-U) and poly(G-C) hyperchromicities (Coutts, 1971; Riesner & Römer, 1973) indicated that all transitions were relatively rich in G·C base pairs. The low-temperature region (28–38 °C) was calculated to have 75–80% G·C base pairs, whereas the high-temperature transition was calculated to have 80% G·C for the RNP and 87% for the RNA. This is in contrast with G·C estimates of the order of 50–60% based on current models.

The presence of the protein in the complex had little effect on the extent of EthBr binding as shown by the fluorescence enhancement curve (Figure 8), and this was not due to a dissociation of the RNP (Figure 9). This result was somewhat surprising since both the *E. coli* (Feunteun et al., 1975) and *H. cutirubrum* (Nazar et al., 1979a; Willick et al., 1979) complexes bind EthBr much less than the free 5S RNAs. However, it agrees with the partial nuclease digestion studies on the yeast complex (Nazar, 1979). There it was found that the protein gave little protection to the 5S RNA against either T_1 or pancreatic RNase attack. The protein binding site was inferred from the sequence of two fragments that remained bound to YL3 after the nuclease digestion. Both sets of data support the view that the YL3 binding site on the 5S RNA

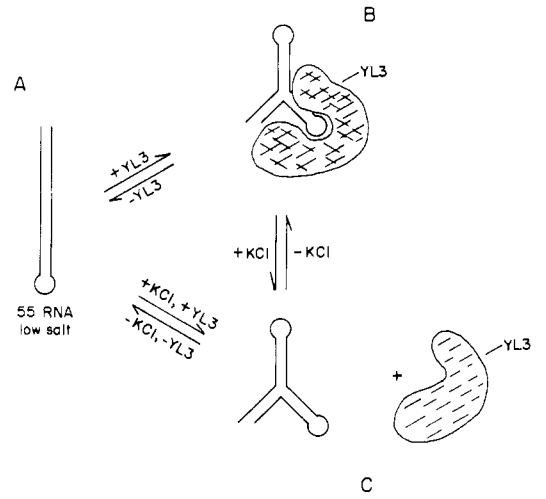

FIGURE 10: A schematic showing the conformational changes in *S. cerevisiae* 5S RNA and binding protein YL3 as a result of complex formation or addition of salt. Form B of the 5S RNA is suggested to be similar, but not identical, to form C.

of yeast is probably physically somewhat smaller than the sites of the two procaryotes previously studied, although all of the sites are in the same general region of the 5S RNA molecule. Despite the reduction in size of the site, the equilibrium constant of YL3 binding is of the same order of magnitude as that of either HL13 from *H. cutirubrum* or EL18 from *E. coli* for their cognate 5S RNAs. Although we have not been able to directly measure the equilibrium constant for the *S. cerevisiae* complex, an estimate can be made based on the gel data. The sample applied to the gel in Figure 2 with 0 mM added KCl had a total concentration of about 2×10^{-6} M, and the sample was at least 90% in the form of the complex, thus giving a minimal estimate for the association constant in the gel buffer of 5×10^{7} M^{-1}. Values for the association of EL18 or HL13 to their 5S RNAs have been reported to be about 10^{8} M^{-1} (Spierer et al., 1978; Willick et al., 1979).

The outline in Figure 10 summarizes our interpretations of the data presented above. This includes the salt effect on the stability of the complex. It suggests that 5S RNA in the complex in low salt has a similar, but not necessarily identical, conformation to 5S RNA in moderately high salt concentrations. The CD curve of 5S RNA in these two cases was found to be very similar (G. E. Willick, unpublished experiments).

To date, three 5S RNA–protein complexes have been studied in detail. They include the procaryote complexes from *E. coli* and *H. cutirubrum* and the eucaryote complex from *S. cerevisiae*, which is the subject of this paper. Much of the structure of the complex has been retained throughout evolution. However, if the *S. cerevisiae* complex proves to be representative of eucaryote complexes, then there appears to have been a lessening of the protein–nucleic acid contact area, leaving a larger remaining mass of protein free for an as yet unknown function. These 5S RNA–protein complexes should continue to provide a fruitful system for obtaining a detailed understanding of ribosomal protein–RNA interactions, and most especially in following the evolution of such an interaction.

Acknowledgments

The authors thank Dr. Makoto Yaguchi for his support and C. F. Rollin for valuable technical assistance.

References

Allen, S. H., & Wong, K.-P. (1978) *Biochemistry 17*, 3791–3798.

Bear, D. G., Schleich, T., Noller, H. F., & Garrett, R. A. (1977) *Nucleic Acids Res. 4*, 2511–2526.

Coutts, S. M. (1971) *Biochim. Biophys. Acta 232*, 94–106.

Erdmann, V. A. (1976) *Prog. Nucleic Acid Res. Mol. Biol. 18*, 45–90.

Feunteun, J., Monier, R., Garrett, R., LeBret, M., & LePecq, J. B. (1975) *J. Mol. Biol. 93*, 535–541.

Fox, J. W., & Wong, K.-P. (1978) *J. Biol. Chem. 253*, 18–20.

Monier, R. (1974) in *Ribosomes* (Nomura, M., Tissieres, A., & Lengyel, P., Eds.) pp 141–168, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Nazar, R. N. (1978) *J. Biol. Chem. 253*, 4505–4507.

Nazar, R. N. (1979) *J. Biol. Chem. 254*, 7724–7729.

Nazar, R. N., Sitz, T. O., & Busch, H. (1975) *J. Biol. Chem. 250*, 8591–8597.

Nazar, R. N., Matheson, A. T., & Bellemare, G. (1978) *J. Biol. Chem. 253*, 5464–5469.

Nazar, R. N., Willick, G. E., & Matheson, A. T. (1979a) *J. Biol. Chem. 254*, 1506–1512.

Nazar, R. N., Yaguchi, M., Willick, G. E., Rollin, C. F., & Roy, C. (1979b) *Eur. J. Biochem. 102*, 573–582.

Riesner, D., & Römer, R. (1973) in *Physico-Chemical Properties of Nucleic Acids* (Duchesne, J., Ed.) p 237, Academic Press, New York.

Smith, N., Matheson, A. T., Yaguchi, M., Willick, G. E., & Nazar, R. N. (1978) *Eur. J. Biochem. 89*, 501–509.

Spierer, P., Bogdanov, A. A., & Zimmermann, R. A. (1978) *Biochemistry 17*, 5394–5398.

Torano, A., Sandoval, A., & Heredia, C. F. (1974) *Methods Enzymol. 30*, 254–261.

Van, N. T., Holder, J. W., Woo, S. L. C., Means, A. R., & O'Malley, B. W. (1976) *Biochemistry 15*, 2054–2062.

Willick, G. E., & Zuker, M. (1977) *Biophys. Chem. 7*, 223–227.

Willick, G. E., Nazar, R. N., & Matheson, A. T. (1979) *Biochemistry 18*, 2855–2859.

Yaguchi, M., Nazar, R. N., Willick, G. E., Rollin, F., & Roy, C. (1978) *Int. Conf. Yeast Genet. Mol. Biol. 9*, 106.