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Effect of Point Mutations on 5.8S Ribosomal Ribonucleic Acid Secondary Structure and the 5.8S–28S Ribosomal Ribonucleic Acid Junction†

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ABSTRACT: Naturally occurring differences in the nucleotide sequences of 5.8S ribosomal ribonucleic acids (rRNAs) from a variety of organisms have been used to study the role of specific nucleotides in the secondary structure and intermolecular interactions of this RNA. Significant differences in the electrophoretic mobilities of free 5.8S RNAs and the thermal stabilities of 5.8S–28S rRNA complexes were observed even in such closely related sequences as those of man, rat, turtle, and chicken. A single base transition from a guanylic acid residue in position 2 in mammalian 5.8S rRNA to an adenylic acid residue in turtle and chicken 5.8S rRNA results both in a more open molecular conformation and in a 5.8S–28S rRNA junction which is 3.5 °C more stable to thermal denaturation. Other changes such as the deletion of single nucleotides from either the 5′ or the 3′ terminals have no detectable effect on these features. The results support secondary structure models for free 5.8S rRNA in which the termini interact to various degrees and 5.8S–28S rRNA junctions in which both termini of the 5.8S molecule interact with the cognate high molecular weight RNA component.

5.8S ribosomal ribonucleic acid (rRNA), a constituent of eukaryotic cytoplasmic ribosomes, exists as a hydrogen-bonded complex with the large RNA component of 60S ribosomal subunits (Pene et al., 1968; Weinberg & Penman, 1968). This RNA complex can be dissociated by heat or by denaturing agents such as urea or formamide (Pene et al., 1968; Weinberg & Penman, 1968; Oakden & Lane, 1973; Nazar et al., 1975) and, under appropriate conditions of salt concentration and temperature, can be easily re-formed [see Oakden & Lane (1973) and Nazar & Sitz (1980)]. Isolated 5.8S rRNA also forms multimers with itself (dimers, trimers, and tetramers) apparently through interactions between its 5′- and 3′-terminal sequences (Sitz et al., 1978). The ability of the 5.8S rRNA to re-form 5.8S–28S rRNA complexes and various 5.8S rRNA multimers at relatively low RNA concentrations indicates a high propensity for RNA interactions, which may be important in how this molecule functions during protein synthesis. Experimentally, it also offers a simple model for studying RNA–RNA interactions in general.

Although a universal model ("burp gun" model) for 5.8S rRNA secondary structure (Figure 1) has been proposed (Nazar et al., 1975) and the primary sequence of a number of 5.8S rRNA species is now known [see Erdmann (1980)], the interactions between 5.8S rRNA and its cognate 25S–28S rRNA have not been completely elucidated. Pace et al. (1977) have shown that the 3′-terminal region of mouse 5.8S RNA is associated with 28S rRNA and have suggested that other regions of the molecule may also be involved. Pavlakis et al. (1979), working with *Drosophila*, have shown that both pieces of the split 5.8S rRNA will bind to the 26S molecule, indicating that regions of the sequence other than just the 3′ end are involved in complex formation. Recently, we have shown that the 5′ end of yeast 5.8S RNA is important in forming this complex, suggesting that both the 5′- and 3′-terminal sequences are important in binding to the 25S rRNA (Nazar & Sitz, 1980). It would appear that the secondary structure of the 5.8S rRNA may be important in bringing the two distant termini together to form the binding site that allows the 5.8S component to associate with its cognate 25S–28S rRNA.

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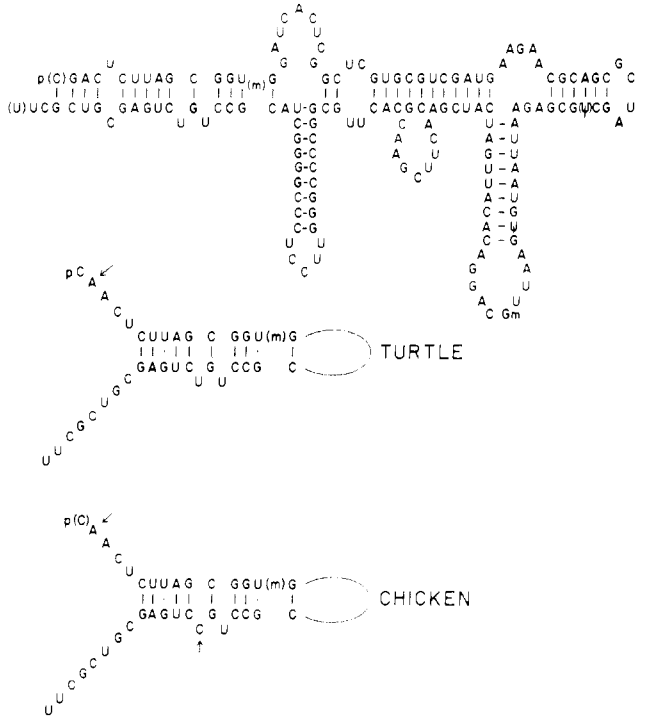

FIGURE 1: Comparison of eukaryotic 5.8S rRNAs from man, rat, chicken, and turtle. The 3′-uridylic acid residue in parentheses is present in the human (HeLa cell) RNA sequence (Nazar et al., 1976) but absent from various 5.8S RNAs of rat origin (Nazar et al., 1975, 1976). The arrows indicate nucleotide substitutions at position 2 in turtle RNA (Nazar & Roy, 1976) and at positions 2 and 146 in chicken RNA (Khan & Maden, 1977).

The primary nucleotide sequences of rat (Nazar et al., 1975, 1976), human (Nazar et al., 1976), turtle (Nazar & Roy, 1976), and chicken (Khan & Maden, 1977) 5.8S rRNAs are identical except for a limited number of single base changes in either their 5′- or their 3′-terminal regions (Figure 1), essentially point mutations in a common nucleotide sequence. In the present study, we have used these naturally occurring variations to compare the stabilities of homologous and heterologous 5.8S–28S rRNA complexes in order to specifically identify residues in 5.8S RNA which are involved in 28S rRNA binding.

Materials and Methods

Unlabeled ribosomal 5.8S RNA and the 5.8S–28S rRNA complex were isolated from whole cell RNA extracted from either tissue culture cells or liver tissue with a phenol–sodium dodecyl sulfate ($NaDodSO_4$) buffer at 25 or 65 °C (Nazar et al., 1975). After a 25 °C extraction, the native complex was purified on a 5–25% sucrose gradient (Sitz et al., 1973). Heat-dissociated 5.8S rRNA was purified on 10% polyacrylamide slab gels (Nazar et al., 1975). For preparation of radioactive RNA, tissue culture cells from normal rat kidney (NRK) cells (Duc-Nguyen et al., 1966), HeLa cells, turtle (*Terrapine carolina*) heart cells (CCL50), and chick embryo fibroblast (CEO) were incubated with 2–5 mCi of [^{32}P]-orthophosphate, and the RNA was extracted and purified as described above.

For clarification of the nucleotide sequence of chick 5.8S RNA, ^{32}P-labeled RNA isolated from chick embryo cells was completely digested with T_1 ribonuclease and alkaline phosphatase, and the resulting oligonucleotides were fractionated by electrophoresis on cellulose acetate at pH 3.5 and then on diethylaminoethyl (DEAE) paper in 7% formic acid (Brownlee, 1972). The individual oligonucleotides were identified by their nucleotide compositions, and their sequences were deduced by further digestion with pancreatic or U_2 ribonuclease (Nazar et al., 1975). Digestion products were separated by electrophoresis on DEAE paper at pH 3.5 and, where required, were further identified by their nucleotide compositions (Brownlee, 1972).

The thermal stability of the native 5.8S–28S rRNA complexes from various sources was determined by dissolving RNA in 0.15 M salt buffer [0.15 M NaCl, 1 mM EDTA, and 1 mM 3-(*N*-morpholino)propanesulfonic acid (Mops), pH 7.2] at an RNA concentration of 1 mg/mL. Aliquots of 100 μL were sealed in glass capillary tubes, heated in a water bath for 2 min at the appropriate temperatures, and rapidly cooled in an ice bath. The samples were then analyzed by electrophoresis on 10% polyacrylamide disc gels; the release of 5.8S RNA was quantitated by staining the gels with methylene blue and scanning them in a Beckman Model 25 spectrophotometer at 600 nm.

Homologous and heterologous complexes of 5.8S–28S rRNA from various tissues were reconstituted by using a procedure similar to that of Pace et al. (1977). Either ^{32}P-labeled 5.8S rRNA or ^{32}P-labeled 5.8S–28S rRNA complex at a final concentration of 0.1 and 5 μg/mL, respectively, was mixed with a large amount (2–3 mg/mL) of unlabeled complex in 0.4 M salt buffer (0.4 M NaCl, 2.6 mM EDTA, and 2.6 mM Mops, pH 7.2). The samples were heated in a boiling water bath for 2 min, quickly transferred to a 58 °C water bath for 15 min to allow association of the complex, and chilled rapidly in an ice bath. After being chilled, the associated complexes were diluted with autoclaved water to a salt concentration of 0.15 M and an RNA concentration of about 1 mg/mL. The thermal stabilities of the annealed complexes were determined as described above for the native complexes. The gels were stained and scanned to quantitate the release of unlabeled 5.8S rRNA and then were sliced, and the radioactivity was determined by using the Cerenkov method to quantitate the release of ^{32}P-labeled RNA. Both the homologous and heterologous complexes could be formed in the same reaction tube by mixing a small amount of radioactive RNA with a large excess of unlabeled RNA from another animal species. Under these conditions, the large amount of unlabeled RNA drove the reaction so that the radioactive 5.8S rRNA formed a complex almost exclusively with the unlabeled 28S rRNA (^{32}P-labeled 5.8S rRNA–unlabeled 28S rRNA) while the unlabeled 5.8S rRNA re-formed a complex with unlabeled 28S rRNA (unlabeled 5.8S rRNA–unlabeled 28S rRNA).

Results

As shown in Figure 2, when RNA from a variety of species was separated on a 10% polyacrylamide slab gel, rat 5.8S rRNA had the fastest migration rate, turtle and chicken RNA migrated next, and yeast 5.8S RNA showed the slowest electrophoretic mobility. In contrast, all the 5S rRNA molecules were essentially equal in mobility (Figure 2). Other mammalian 5.8S rRNA species (human, calf, hamster, and the Novikoff hepatoma) had rates which were essentially identical with that of rat RNA (results not shown). Since all of these species are very similar in molecular weight and charge, it appears that the mammalian 5.8S rRNA molecules have a more compact hydrodynamic shape than those of chicken or turtle, and yeast 5.8S rRNA has the most open conformation.

As shown in Figure 1, the reported nucleotide sequences of rat (or human), chick, and turtle 5.8S RNAs appear to be identical except for two nucleotide substitutions at residues 2 and 146 in the 5′ and 3′ ends, respectively, and additional

Table I: Analysis of Longer Oligonucleotides Obtained by Combined T_1 Ribonuclease and Alkaline Phosphatase Digestion of Chicken 5.8S rRNA[a]

spot	nucleotide composition[b]	molar yield	pancreatic ribonuclease digestion products	sequence[c]
15	C_2AU_2G	0.8 (1)	AC, C, *U*	C-A-C-U-U-Gp
16	$C_2A_2U_2G$	0.7 (1)	*AU, C*	A-U-C-A-U-C-Gp
17a	$C_3A_2U_2G$	0.9 (1)	AU, AC, *C*, U	A-U-C-A-C-U-C-Gp
17b	$C_3A_2U_2G$	0.9 (1)	*AC*, C, *U*	A-C-A-C-U-U-C-Gp
18	$C_2A_3U_2G$	0.9 (1)	AU, *AC*, U	A-C-A-C-A-U-U-Gp
19	C_5U_3G	1.0 (1)		U-U-C-C-U-C-C-C-Gp
20	CA_3U_2GmG	1.0 (1)	A_2U, $AG_{(OH)}$, GmC, U	A-A-U-U-Gm-C-A-Gp
22	A_4U_3G	0.9 (1)	*A_2U*, U	A-A-U-U-A-A-U-Gp
5′a	$C_2A_3U_3G$	0.6 (0.6)	A_2C, $AG_{(OH)}$, C, U	pA-A-C-U-C-U-U-A-C-Gp[d]
5′b	$C_3A_2U_3G$	0.4 (0.4)	A_2C, $AG_{(OH)}$, *C*, U	pC-A-A-C-U-C-U-U-A-C-Gp[d]

[a] The digests of ^{32}P-labeled RNA were fractionated by two-dimensional electrophoresis, the radioactivity of each spot was determined, and each spot was eluted and identified by its nucleotide composition and by further digestion with pancreatic ribonuclease. The experimental molar yields are averages of three determinations based on a 158 nucleotide chain length; the predicted yields are noted in parentheses. The notation for the relative molar yields of products is lightface, italic, and boldface for molar yields of 1, 2, and 3, respectively. [b] The presence of Gp was confirmed by analyses of T_1 digests without alkaline phosphatase. [c] These sequences are based on the nucleotide compositions, the pancreatic ribonuclease digestion products, and on analogous fragments in mammalian 5.8S rRNA. [d] pAp and pCp were confirmed by the presence of pA-Cp and pCp in pancreatic ribonuclease digest of whole chicken 5.8S rRNA.

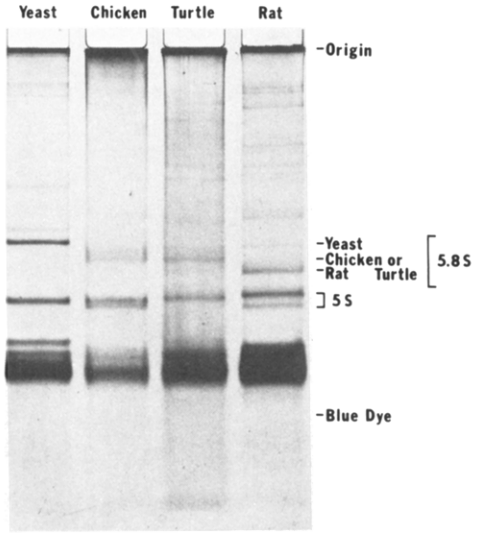

FIGURE 2: Polyacrylamide gel fractionation of whole cell RNA from rat, chicken, and turtle livers and yeast cells. Electrophoresis was from top to bottom at pH 8.3; the positions of RNA species and the bromphenol blue dye marker are indicated in the margin.

nucleotides at the 5′ or 3′ ends of some of the species. Because previously the 5′-terminal sequence of chick 5.8S RNA was not unambiguously established (Khan & Maden, 1977), we have further examined this RNA after digestion with T_1 RNase and alkaline phosphatase. Such an approach has been successfully used to resolve heterogeneous termini in trout 5.8S rRNA (Nazar & Roy, 1978). As shown in Table I, when the products were analyzed by two-dimensional electrophoresis, two major termini, pA-A-U-C-U-U-A-Gp and pC-A-A-U-C-U-U-A-Gp, were present. As observed with yeast (Rubin, 1974) and Novikoff hepatoma (Nazar et al., 1975) 5.8S rRNA, the chicken RNA had a heterogeneous 5′ terminal in which some of the molecules contain an additional cytidylic acid residue. This longer form was not resolved by the conditions of electrophoresis used in Figure 2 and, therefore, was not a factor in the variations in electrophoretic mobility.

Because the differences between rat, human, turtle, and chicken molecules are essentially point mutations in a common nucleotide sequence (Figure 1) and one of these had a rather striking effect on molecular conformation (Figure 2), we decided to examine their effect on the 5.8S–28S rRNA interaction. The thermal stabilities of native 5.8S rRNA complexes from rat, human, turtle, and chicken were determined by

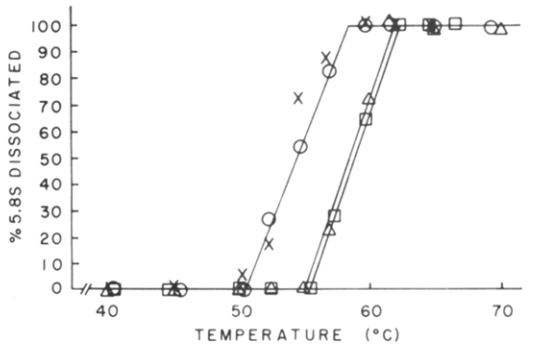

FIGURE 3: Dissociation of native 5.8S–28S rRNA complexes by thermal denaturation in 0.15 M salt buffer. Aliquots of 100 μL of complex (1 mg/mL) were sealed in glass capillaries and incubated at the indicated temperatures as described under Materials and Methods. The samples were analyzed by gel electrophoresis, and the amount of free 5.8S rRNA was determined by scanning the gels after staining with methylene blue. Denaturation profiles are shown in human (×), rat (○), turtle (□), and chicken (△) 5.8S–28S rRNA complexes.

Table II: Thermal Stability of Native 5.8S–28S rRNA Complexes[a]

source of complex	T_m (°C)
rat	53.9 ± 0.6
human	54.0 ± 0.7
turtle	57.6 ± 0.6
chicken	57.4 ± 0.4

[a] The T_m or temperature at which 50% of the 5.8S rRNA was released was determined as described in Figure 3. The T_m values for rat and chicken complexes are the average of five experiments while the values for human and turtle are the average of two experiments; standard deviations are included in each instance.

heating in 0.15 M salt buffer. The rat complex was heated at 55 °C for different times to establish a reasonable heat-treatment period. All of the 5.8S rRNA that would dissociate had separated in 1 min; heating for as long as 5 min did not release more RNA. Accordingly, in the thermal denaturation experiments, the complex samples were routinely incubated for 2 min. An example of the melting profiles is shown in Figure 3. All the complexes showed a sharp cooperative melting curve, with the rat and human 5.8S–28S rRNA complex melting about 3.5 °C lower than the chicken or turtle complex. A summary of these T_m values with standard deviations is presented in Table II.

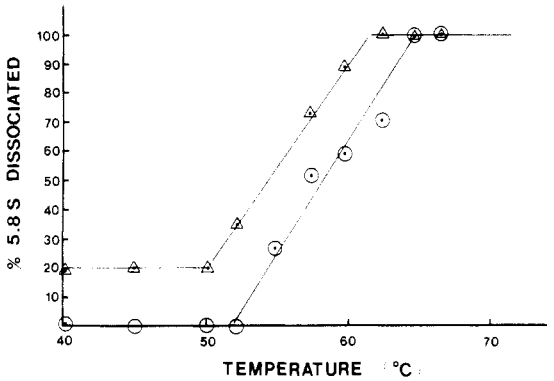

FIGURE 4: Thermal denaturation profiles for reconstituted complexes between ^{32}P-labeled rat 5.8S and turtle 28S rRNAs (△) and between turtle 5.8S and turtle 28S rRNAs (○). Native ^{32}P-labeled rat complex was heated in the presence of a large excess of unlabeled turtle complex, and complexes were reconstituted as described under Materials and Methods. The samples were diluted with water to 0.15 M salt, and denaturation profiles were obtained as described in Figure 3.

To ensure that the differences in stability were not due to variations in endogenous salt in the RNA samples or composition of the buffer, we mixed 0.1 μg of ^{32}P-labeled rat complex with 100 μg of chicken complex, and the T_m's were determined by following the release of both radioactive and unlabeled 5.8S rRNAs in the same capillary tubes. This experiment gave values identical with those listed in Table II; i.e., the rat complex was less stable.

The stability differences could be interpreted in two ways: they either were the result of differences in the 5.8S rRNAs or were due to sequence differences in the various 28S rRNAs. This question was resolved by examining the thermal stability of reconstituted complexes. Heterologous complexes such as ^{32}P-labeled rat 5.8S rRNA–chicken 28S rRNA were formed by mixing small amounts (5 μg/mL) of radioactive native rat complex with unlabeled chicken complex, heating them to release the 5.8S RNAs, and incubating them at appropriate temperatures before cooling. Because the concentration of labeled components was very low, virtually no native rat complex re-formed under these conditions, and only the heterologous rat–chicken complex and homologous chicken complex were present. This was verified when small amounts of rat complex (5–160 μg/mL) were heated at 100 °C and placed at 58 °C for 15 min; no association was observed. In contrast, when a large amount of unlabeled complex was added, a complex with radioactive 5.8S rRNA was formed. In all the reconstitution experiments, it was important to first heat the radioactive and unlabeled RNAs at 100 °C, or no annealed complex formed. When unheated ^{32}P-labeled 5.8S rRNA was added to previously heated complex, no association took place unless the 5.8S rRNA was heated at 100 °C first and quickly mixed with the unlabeled complex at 58 °C. Finally, to ensure that reassociation was limited to only one specific site, radioactive 5.8S rRNA was heated to 100 °C and mixed with native complex at 58 °C; no complex formed unless the native complex was also heated to 100 °C to release the 5.8S rRNA and make the 28S RNA binding site available.

Examples of thermal denaturation profiles for a heterologous ^{32}P-labeled rat 5.8S rRNA–turtle 28S rRNA complex and the homologous complex of turtle RNA (turtle 5.8S rRNA–turtle 28S rRNA) are shown in Figure 4. Two differences were observed: the heterologous complex was less stable by 3.5 °C, and the rat 5.8S RNA did not reassociate completely. (Generally in these experiments, only 70–80% of the rat or human 5.8S RNAs formed complexes while virtually 100% of the turtle and chicken RNAs could be reconstituted.) A summary of all the data obtained for the various complexes is presented in Table III. In each experiment, the unlabeled homologous complex served as an internal control to show that reconstituted complexes were equivalent to the native complexes described in Table II. The stability of the associated complexes was clearly dependent on the 5.8S molecule used and independent of the 28S molecules from various animal species.

Table III: Thermal Stability of Reconstituted 5.8S–28S rRNA Complexes[a]

	complex	T_m (°C)
rat	rat 5.8 S–rat 28 S	54.9 ± 0.5
	^{32}P-labeled rat 5.8 S–rat 28 S	54.4 ± 0.5
	turtle 5.8 S–turtle 28 S	58.7 ± 0.2
	^{32}P-labeled rat 5.8 S–turtle 28 S	54.9 ± 0.5
	chicken 5.8 S–chicken 28 S	57.4 ± 0.2
	^{32}P-labeled rat 5.8 S–chicken 28 S	54.5 ± 0.5
turtle	rat 5.8 S–rat 28 S	55.0
	^{32}P-labeled turtle 5.8 S–rat 28 S	58.0
	chicken 5.8 S–chicken 28 S	58.4
	^{32}P-labeled turtle 5.8 S–chicken 28 S	58.6
chicken	rat 5.8 S–rat 28 S	54.9
	^{32}P-labeled chicken 5.8 S–rat 28 S	57.3
	turtle 5.8 S–turtle 28 S	58.8
	^{32}P-labeled chicken 5.8 S–turtle 28 S	58.8
human	rat 5.8 S–rat 28 S	55.0
	^{32}P-labeled human 5.8 S–rat 28 S	54.5
	chicken 5.8 S–chicken 28 S	58.5
	^{32}P-labeled human 5.8 S–chicken 28 S	55.0

[a] The T_m values of the associated complexes were determined as described in Figures 3 and 4. The standard deviations that are given are the average of three experiments.

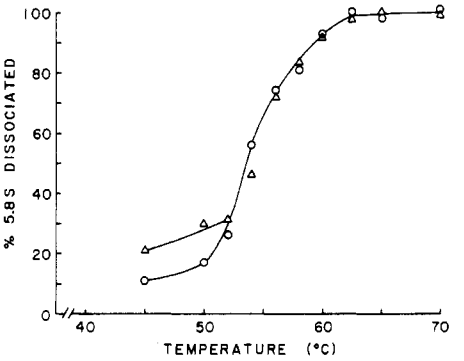

FIGURE 5: Release of 5.8S rRNA dimers from a reconstituted rat 5.8S–28S rRNA complex during thermal denaturation. Native rat complex was heated and reconstituted at a high RNA concentration (5 mg/mL) as described under Materials and Methods, and RNAs released after thermal denaturation were analyzed as described in Figure 3. The release of free 5.8S rRNA and dimers is indicated by open circles and open triangles, respectively.

The inability of mammalian 5.8S RNA to fully reconstitute into 5.8S–28S rRNA complexes was unexpected and, therefore, was examined further at higher concentrations of rat complex (up to 5 mg/mL). Still, only about 80% of the rat 5.8S rRNA was associated with 28S RNA, but interestingly, at the higher RNA concentrations 5.8S RNA dimer (Sitz et al., 1978) began forming. As shown in Figure 5, the appearance of dimer completely paralleled the release of free 5.8S rRNA during thermal denaturation in 0.15 M salt buffer with about half of the 5.8S RNA released as dimer. Since the temperature range and RNA concentration, at which the thermal denaturation profiles were obtained, were significantly below those previously shown to be optional for dimer formation (Sitz et al., 1978), it appears that the dimer was formed during the initial reconstitution experiment and that 5.8S rRNA dimers actually retain their ability to complex with 28S

rRNA analogous to the formation of higher multimer forms previously observed, such as trimers and tetramers.

Discussion

Although the use of point mutations is a very valuable tool for determining nucleic acid structure and function [see, e.g., Dickson et al. (1975)], it is relatively difficult to generate site-specific mutations [see, e.g., Gillam & Smith (1979)]. In the present study, we have taken advantage of naturally occurring differences in the sequences of 5.8S rRNA molecules from a variety of species to examine the role of specific nucleotides in the secondary structure and intermolecular interactions of this RNA. As shown in Figure 1, mammalian 5.8S rRNA sequences are generally identical except for extra nucleotides at the 5′ and 3′ ends of some of the species. Turtle 5.8S rRNA only differs from human RNA at residue 2, which is adenylic rather than guanylic acid, and chicken RNA differs at two positions, the adenylic acid in position 2 and also cytidylic rather than uridylic acid at residue 146. In total, therefore, among the sequences which were examined there were four possible differences from one basic sequence (human): one less C at the 5′ end, A rather than G in position 2, C rather than U in position 146, and one less U at the 3′ end. The experimental results indicate that only residue 2 at the 5′ end has a profound influence on the molecule's conformation and also affects the stability of the 5.8S–28S rRNA junction. The remaining changes, which, as suggested in Figure 1, are not essential for the secondary structure, appear not to influence these features, further underlining the importance of the second residue.

Generalized estimates of the secondary structure of free 5.8S rRNA (Figure 1) suggest that the 5′ and 3′ ends of this molecule interact, forming a relatively stable structure in mammals. As indicated for turtle and chicken 5.8S rRNA, the substitution of adenylic acid in place of the guanylate residue in position 2 might be expected to destabilize this interaction or reduce the free energy by about −5.4 kcal [calculated according to Tinoco et al. (1973)], resulting in a partially open structure. This is consistent with the slower electrophoretic mobility which was observed (Figure 2). The difference could further involve changes in the tertiary structure, but, as yet, this has not been determined. On the other hand, the very open structure of yeast 5.8S rRNA suggests that the strong interaction between the termini of free 5.8S rRNAs from higher eukaryotes may not be physiologically significant.

In an earlier study, mammalian 5.8S rRNA was shown to readily form multimer structures (dimers, trimers, etc.) at high RNA concentration, apparently through interactions between the 5′ and 3′ termini (Sitz et al., 1978). Chick and turtle 5.8S rRNAs also formed dimers but less efficiently, and yeast RNA dimers were extremely difficult to form. The changes in electrophoretic mobilities, observed in the present study, correlate well with the molecules' ability to form dimers. Furthermore, the ability of chicken and turtle RNAs to reassociate with the 28S rRNA to a greater degree than mammalian species also seems to correlate with the more open structure in the chicken and turtle RNAs. As previously suggested (Sitz et al., 1978), it appears that, especially with mammalian 5.8S rRNA, there is a strong interaction between the termini which enables dimer formation but which competes with complex formation. As a result, a higher temperature is required for dimer formation, and the 5.8S–28S rRNA complex is less efficiently formed in mammals than observed in chicken or turtle. Dimers could also be the result of an interaction of a "palindromic region" (Pavlakis et al., 1979), but this appears less likely in higher animals as it does not explain the differences in formation and stability of chicken dimer and rat dimer and could not result in the previously reported trimers and tetramers (Sitz et al., 1978).

The effect of residue 2 on the 5.8S–28S rRNA junction strongly supports previous arguments [see Nazar & Sitz (1980)] that the 5′-terminal sequence is involved in the 5.8S–28S rRNA junction and suggests that the immediate 5′-end sequence is interacting with 28S rRNA. An indirect tertiary sequence effect on another site cannot be entirely ruled out, but this is unlikely because, in yeast, a longer 5′-terminal fragment actually retained some binding capacity (Nazar & Sitz, 1980). The reduced stability in the 5.8S–28S rRNA junction in mammals, where guanylic acid is present in position 2, suggests that there is a uridylic acid residue in the cognate binding site of the 28S RNA molecule.

The fact that heterologous complexes are readily formed indicates that both sites were highly conserved in the course of evolution and that the site within 28S rRNA in mammals, chicken, and turtle may be identical. Further sequence analyses and other probes (e.g., chemical cross-linking) will be required to fully describe the 5.8S–28S rRNA interaction. For the moment, however, this and previous studies (Pace et al., 1977; Pavlakis et al., 1979; Nazar & Sitz, 1980) strongly indicate that the 5.8S rRNA interacts with its cognate high molecular weight RNA through two sites located in the immediate 5′- and 3′-terminal sequences.

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