of 5.8S rRNA, the binding of 5.8S rRNA to 28S rRNA, and the heterogeneity of the rDNA genes.

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Base Pairing in Wheat Germ Ribosomal 5S RNA As Measured by Ultraviolet Absorption, Circular Dichroism, and Fourier-Transform Infrared Spectrometry†

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ABSTRACT: Ultraviolet absorption (UV) and circular dichroism (CD) spectra of wheat germ 5S RNA, when compared to $tRNA^{Phe}$, indicate a largely base-paired and base-stacked helical structure, containing up to 36 base pairs. Fourier-transform infrared (FT-IR) spectra of $tRNA^{Phe}$ and wheat germ ribosomal 5S RNA have been acquired at 30 and 90 °C. From the difference of the FT-IR spectra between 90 and 30 °C, the number of base pairs in both RNAs was determined by modification of a previously published procedure [Burkey, K. O., Marshall, A. G., & Alben, J. O. (1983) *Biochemistry 22*, 4223-4229]. The base-pair composition and total base-pair number from FT-IR data are now consistent for the first time with optical (UV, CD, Raman) and NMR results for ribosomal 5S RNA. Without added Mg^{2+}, $tRNA^{Phe}$ gave 18 ± 2 base pairs [7 A-U and 11 G-C], in good agreement with the number of secondary base pairs from X-ray crystallography [8 A-U, 12 G-C, and 1 G-U]. Within the 10% precision of the FT-IR method, wheat germ 5S RNA exhibits essentially the same number of base pairs [14 A-U, 17 G-C, and 5 G-U; for a total of 36] in the absence of Mg^{2+} as in the presence of Mg^{2+} [14 A-U, 18 G-C, and 3 G-U; for a total of 35], in agreement with the UV hyperchromism estimate of G-C/(A-U + G-C) = 0.58. Addition of Mg^{2+} increases the melting midpoint (T_m) for both A-U and G-C pairs in wheat germ 5S RNA. The average T_m for both types of base pairs increases from 48 (CD) or 54 °C (UV) in the absence of Mg^{2+} to 63 (CD) or 69 °C (UV) in the presence of Mg^{2+}. From FT-IR, addition of Mg^{2+} increases the average base pair melting midpoint by 11 °C for G-C pairs (56 to 67 °C) but only by 4 °C for A-U pairs (54 to 58 °C). The above results are consistent with three generalized eukaryotic 5S RNA secondary base-pairing schemes, of which the cloverleaf model gives the closest match.

5S RNA appears to be present and essential for protein synthesis in virtually all 70-odd species from which the molecule has been isolated and base sequenced (Erdmann, 1976; Singhal & Shaw, 1983). It is therefore widely speculated that a universal secondary structure exists, and several models have been proposed (Fox & Woese, 1975; Nishikawa & Takemura, 1978; Luoma & Marshall, 1978a,b; Hori & Osawa, 1979; Luehrsen & Fox, 1981; Studnicka et al., 1981; Kuntzel et al., 1983; Pieler & Erdmann, 1983; Singhal & Shaw, 1983).

These models necessarily differ in both the *total* number and the *relative* numbers of the three main base-pair types (A-U, G-C, and G-U). Choosing between the existing models or devising a new model therefore requires techniques that can discriminate between the four bases with respect to base

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stacking and/or base pairing. Although a variety of spectroscopic probes has been directed at prokaryotic 5S RNA (principally *Escherichia coli*), relatively less spectroscopy has been performed on eukaryotic 5S RNA (and 5.8S RNA). Raman spectra of *Saccharomyces cerevisiae* (bakers' yeast) 5S RNA provided some of the earliest evidence for a highly base-stacked and base-paired secondary structure (Luoma & Marshall, 1978b), as confirmed by subsequent UV, circular dichroism, (CD), and ^{1}H Fourier-transform nuclear magnetic resonance (FT-NMR) (Luoma et al., 1980), electron spin resonance (ESR) spin-labeling (Luoma et al., 1982), UV differential thermal melting (Ohta et al., 1983), Fourier-transform infrared (FT-IR) (Burkey et al., 1983), and ^{31}P FT-NMR (K. O. Burkey and A. G. Marshall, unpublished results).

In the present paper, we analyze UV absorption, CD, and FT-IR spectra of wheat germ (eukaryotic) 5S RNA, in order to discover its number and types of base pairs. The protocol consists of comparing 5S RNA spectra to spectra of yeast tRNA^{Phe}, a molecule whose secondary base-pairing pattern is completely known in the solid from X-ray diffraction (Ladner et al., 1975; Sussman & Kim, 1976) and in solution from proton nuclear Overhauser enhancements (NOE) (Heerschap et al., 1982; Roy & Redfield, 1983; Haasnoot et al., 1983). Preliminary X-ray diffraction (Morikawa et al., 1982) and ^{1}H NOE experiments (Kime & Moore, 1983a,b) for prokaryotic 5S RNA have proved difficult to analyze. Therefore, it is important to make maximal use of all information available from optical spectra, particularly in the case of FT-IR spectra, which exhibit the best resolution and thus the highest information content.

The first serious attempts to determine RNA base pairing from IR hyperchromism were based on comparing a single IR spectrum of native 5S RNA with the best fit linear combination of reference spectra of poly(rA)·poly(rU) and poly(rG)·poly(rC) for base-paired segments and 5′–3′ ApA, 5′–3′ UpU, 5′–3′ CpC, and 5′-GMP for unpaired segments (Appel et al., 1979; Stultz et al., 1981). In both cases, the deduced total base-pair number turned out to be too large by at least 50% when compared to other available measures: UV absorbance and CD (Luoma et al., 1980), Raman (Luoma & Marshall, 1978a), ^{1}H NMR, (Burns et al., 1980), and ^{19}F NMR (Marshall & Smith, 1980). Much more reasonable base-pair numbers were obtained for *E. coli* 5S RNA by fitting the RNA *difference* spectrum for unpaired minus paired bases (90 minus 20 °C) with the reference difference spectra between paired and unpaired poly(rA)·poly(rU) and poly(rG)·poly(rC) (Böhm et al., 1981). Most recently, in a study of yeast 5S RNA, the precision of the base-pair estimates was further improved with the replacement of the dispersive IR spectrometer by a Fourier-transform instrument and the introduction of a graphical data reduction based on IR absorption at or near the wavelengths for which the RNA difference spectrum (90 minus 20 °C) has maximum intensity (Burkey et al., 1983).

Although published procedures proved adequate for analyzing the UV absorption and CD spectra, we found it necessary to modify existing FT-IR data reduction to account for the presence of G-U pairs, which are rare in tRNAs but may be more common in 5S RNAs (see below). A dividend of the new analysis is that the base-pair composition and total base-pair number from FT-IR data are now consistent for the first time with optical (UV absorption, CD, Raman) and NMR results for ribosomal 5S RNA.

Table I: Comparison of Optical Parameters for Wheat Germ 5S RNA and Yeast tRNA^{Phe}

Mg^{2+}	A_{260} hyperchromism (UV)	λ_{max} (CD) (nm)	$(\Delta A/A)_{260}$ (CD)
5S RNA absent	0.19	265	9.6×10^{-4}
present	0.20	263	9.2×10^{-4}
tRNA^{Phe} absent	0.16		
present	0.22	260.5	9.3×10^{-4}

Materials and Methods

Isolation of RNA. Wheat germ 5S RNA for the UV and CD work was isolated from commercial wheat germ tRNA (Sigma), which contains substantial amounts of ribosomal RNAs. For the FT-IR experiments, wheat germ was obtained as a gift from International Multifoods, Columbus, OH 43208, and wheat germ 5S RNA was isolated as previously described (Li et al., 1984). Yeast tRNA^{Phe} was isolated from whole brewers' yeast (*Saccharomyces carlsbergensis*) cells (UV and CD experiments) as previously described (Luoma et al., 1982) or purchased from Boehringer-Mannheim (FT-IR experiments). tRNA^{Phe} had [^{14}C]phenylalanine acceptance > 1600 pmol/A_{260} unit and is thus 98% pure.

Preparation of RNA Samples. Mg^{2+}-deficient samples of 5S RNA and tRNA were prepared by dissolving lyophilized RNA in 10 mM phosphate (UV and CD) or 10 mM sodium cacodylate (FT-IR), pH 7.0, containing 10–15 mM ethylenediaminetetraacetic acid (EDTA) and 100 mM NaCl. The UV and CD samples were heated to 65 °C for 5 min, cooled, and passed through a Sephadex G-25 column (1 × 40 cm) preequilibrated with the same buffer containing 1 mM EDTA. The FT-IR samples were heated to 65 °C for 2 min, cooled, dialyzed against 10 mM EDTA buffer, and then dialyzed against buffer to remove excess EDTA. D_2O rather than H_2O was used for the FT-IR buffers at a pH meter reading of 7.0. Mg^{2+}-containing 5S RNA and tRNA samples were prepared by dissolving lyophilized RNA in 10 mM phosphate (UV and CD) or 10 mM sodium cacodylate (FT-IR), pH 7, containing 10 mM $MgCl_2$ and 100 mM NaCl.

Ultraviolet Absorption and Circular Dichroism Measurements. All UV-melting experiments were performed with a Cary-15 spectrophotometer equipped with a hollow cell holder. The temperature was controlled with a Haake temperature bath and the sample temperature monitored by a YSI telethermometer with a thermistor probe inserted directly into the sample cuvette through a hole in the cap. A_{260} and A_{280} measurements were recorded at 2 °C intervals, and the rate of heating was maintained at 1 °C/min. CD measurements were performed on a Jasco J-20 spectrometer equipped with a temperature-regulated cell block. Samples were heated at a rate of 10 °C/h, and spectra were recorded every 5 °C. RNA concentrations for both the UV and CD experiments were adjusted to give $0.7 < A_{260} < 0.8$ at 20 °C.

Fourier-Transform Infrared Spectra. FT-IR spectra were acquired at 1-cm^{-1} resolution (and later smoothed to 2-cm^{-1} resolution to suppress artifacts from imperfect subtraction of water vapor) in cells with CaF_2 windows and 0.044-mm path length on a Digilab FTS-14D interferometer fitted with a liquid nitrogen cooled HgCdTe photoconductive detector. Sample preparation, data acquisition, RNA concentration determination (±2%), and correction for differences in D_2O concentration, sample thickness, and water vapor were as previously described (Burkey et al., 1983). Virtually all the bases in an RNA are unpaired and unstacked at 90 °C in the presence or absence of Mg^{2+}. Therefore, the FT-IR extinction coefficient at 1505 cm^{-1} of an RNA at 90 °C in the presence

Table II: Number of Secondary Base Pairs in Wheat Germ 5S RNA and Yeast tRNA^{Phe}: Precision of Experimental Values Is ±10%

species	source	A-U	G-C	G-U	total
yeast tRNA^{Phe}	X-ray and ^{1}H NMR	8	12	1	21
	FT-IR	7	11		18
wheat germ 5S RNA	UV hyperchromism	15	21 (G-C + G-U)		36
	FT-IR				
	$-Mg^{2+}$	14	17	5[a]	31 (36)[a]
	$+Mg^{2+}$	14	18	3[a]	32 (35)[a]
	cloverleaf model	12	17	6	35
	revised Foz-Woese model	9	17	5	31
	Studnicka et al.-Nishikawa-Takemura model	10	20	4	34

[a] Based on assumptions discussed in the text.

Table III: Melting Midpoints (T_m) (°C) of Wheat Germ 5S RNA

technique	type of base pair	$-Mg^{2+}$	$+Mg^{2+}$
UV hyperchromism	A-U, G-C, G-U	54	69
circular dichroism	A-U, G-C, G-U	48	63
FT-IR	A-U	54	58
	G-C	56	67

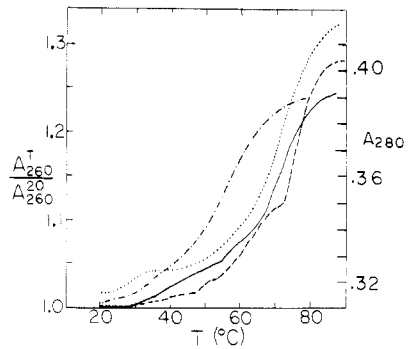

FIGURE 1: UV thermal melting curves: (--) tRNA^{Phe} (normalized A_{260}; 10 mM Mg^{2+}); (—) wheat germ 5S RNA (normalized A_{260}; 10 mM Mg^{2+}); (-·-) wheat germ 5S RNA (normalized A_{260}; no Mg^{2+}); (···) wheat germ 5S RNA (A_{280}; 10 mM Mg^{2+}).

of Mg^{2+} was normalized to the height of the same peak for the same RNA at 90 °C in the absence of Mg^{2+}. FT-IR RNA sample concentration was 35.7 ($-Mg^{2+}$) or 25.5 mg/mL ($+Mg^{2+}$). The proton FT-NMR spectrum shows no evidence of aggregation at such concentrations.

Results

A_{260} UV Hyperchromism. Figure 1 shows the UV melting profiles for both wheat germ 5S RNA and yeast tRNA^{Phe}, from which the UV hyperchromism = A_{260}(90 °C) - A_{260}(20 °C) can be computed (see Table I). Comparison of wheat germ 5S RNA (hyperchromism = 0.20) and yeast tRNA^{Phe} [hyperchromism = 0.22 (Luoma, 1978)] indicates that wheat germ 5S RNA has proportionately 10% fewer stacked bases than tRNA^{Phe}. If one uses a value of 0.30 for the hyperchromism expected from maximal double-stranded stacking (i.e., maximum base pairing) and a 10% minimum contribution to hyperchromism from single-stranded stacking (Boedtker & Kelling, 1967), an upper-limit base-pair number is (0.20 - 0.02)/0.30 × 100 = 60% of the 120 bases or about *36 base pairs* (Table II).

The A_{260} melting midpoint (T_m) for wheat germ 5S RNA (Table III) is 4–5 °C lower than that for yeast tRNA^{Phe}, indicating slightly less stable base pairing in the 5S RNA. Nevertheless, a T_m of 70 °C in the presence of Mg^{2+} still represents a very stable secondary structure. The melting range (between 0.25 and 0.75 of the total change in A_{260}) is an indicator of the relative extents of single- and double-stranded stacking (Boedtker & Kelling, 1967) and is 12% higher for wheat germ 5S RNA than for yeast tRNA^{Phe}, further corroborating our interpretation of the A_{260} total hyperchromism.

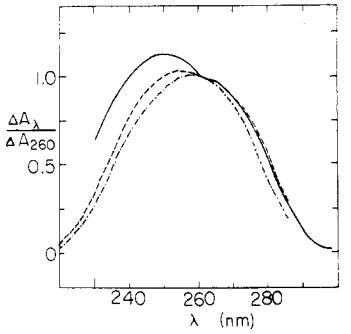

FIGURE 2: UV hyperchromism spectrum for wheat germ 5S RNA (—) compared to curves calculated from standard spectra for 50% G-C pairs (-·-) or 60% G-C pairs (--).

Although melting of both RNAs is highly cooperative, the A_{260} melting profile reveals detectable multiphasic character for wheat germ 5S RNA. There appear to be at least three distinct melting stages, two of which are small helical segments and one (at highest temperature) of which produces about 60% of the total hyperchromism.

A_{280} Hyperchromism. Figure 1 also compares the A_{280} and normalized A_{280} UV melting profiles. Previous studies have shown that hyperchromism at 280 nm is due almost entirely to the unstacking of G-C pairs (Fresco et al., 1963; Van et al., 1977). Most of the A_{280} hyperchromism for wheat germ 5S RNA results from a small but sudden increase near 30 °C and a large increase near 75 °C. Thus, the most stable helical region is relatively G-C-rich, but a small, less stable G-C-rich segment is also present (perhaps with loops and bulges that reduce its stability).

Effect of Mg^{2+} on UV Hyperchromism. Finally, the normalized experimental A_{260} melting profiles for wheat germ 5S RNA in the presence and absence of Mg^{2+} can be compared in Figure 1. The hyperchromism (Table I) and melting range are very similar. Thus, base stacking in wheat germ 5S RNA increases only slightly in the presence of Mg^{2+}. However, the average T_m increases from 54 to 69 °C (Table III), much as for the recently reported differential A_{260} thermal melting of yeast 5S RNA (Ohta et al., 1983).

Hyperchromism vs. Wavelengths—G-C/A-U Ratio. Since the individual normalized hyperchromism spectra for poly(rG)·poly(rC) and poly(rA)·poly(rU) are markedly different in the 260–290-nm range (Fresco et al., 1963), linear combinations of the two curves may be used to estimate the relative proportions of G-C and A-U pairs in wheat germ 5S RNA. The solid line in Figure 2 is the experimental 5S RNA UV hyperchromism spectrum. The curves that bracket the ex-

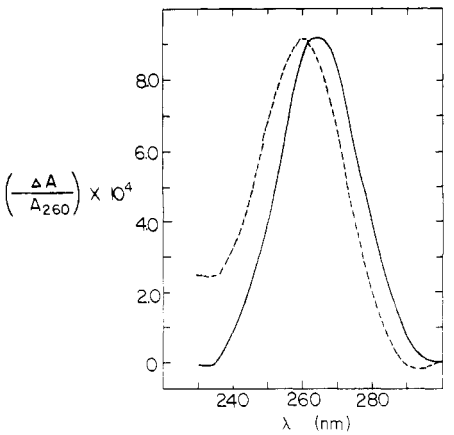

FIGURE 3: Normalized circular dichroism spectra at 260 nm for $tRNA^{Phe}$ (--) and wheat germ 5S RNA (—) at 25 °C in the presence of 10 mM Mg^{2+}.

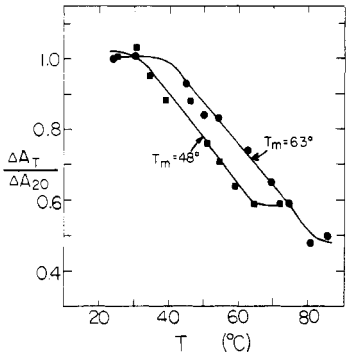

FIGURE 4: Normalized circular dichroism melting profiles for wheat germ 5S RNA in the absence (■) or presence (●) of 10 mM Mg^{2+}.

perimental spectrum have been computed from 50:50 and 60:40 weighted sums of the hyperchromism spectra for poly(rG)·poly(rC) and poly(rA)·poly(rU). As evident from the figure, the best match for the experimental curve is found to be 58 ± 5% G-C pairs. In this estimate, single-stranded stacking, G-U pairs, nearest-neighbor effects, and end-of-helix effects are not considered.

CD Spectra. Figure 3 consists of normalized CD spectra of Mg^{2+}-containing wheat germ 5S RNA and tRNA, from which λ_{max} (wavelength at maximum peak height) and $(\Delta A/A)_{260}$ (normalized maximum dissymmetry ratio) are listed in Table I. Previous work has shown that an increase in base stacking leads to a blue shift in λ_{max} and an increase in $(\Delta A/A_{260}$ (Gratzner & Richards, 1971). The value of λ_{max} for wheat germ 5S RNA is slightly higher than that for tRNA, whereas the $(\Delta A/A)_{260}$ values are identical. Thus, the two RNAs exhibit very similar total helical content, although the 5S RNA structure may be less tightly stacked.

Effect of Mg^{2+} on CD Spectrum. Figure 4 shows normalized CD melting profiles for wheat germ 5S RNA in the presence and absence of Mg^{2+}. As for the UV absorption data, the removal of Mg^{2+} causes little or no change in the CD parameters of wheat germ 5S RNA [λ_{max} and $(\Delta A/A)_{260}$ in Table I]. Both parameters change slightly in the direction of a loosening of the helixes on removal of Mg^{2+}. The presence of Mg^{2+} does increase the CD T_m by the same amount as the UV absorption T_m (i.e., 15 °C, as shown in Table III).

FT-IR Spectra. FT-IR extinction coefficient spectra of the four ribonucleotide 5′-monophosphates are shown in Figure 5. The reason for including these reference spectra is to point out that only 5′-CMP exhibits significant absorption at 1505 cm^{-1}, as discussed further below. FT-IR spectra for wheat germ 5S RNA in the presence and absence of Mg^{2+} are reported in Figures 6 and 7, in a previously presented format (Burkey et al., 1983). FT-IR spectra of yeast $tRNA^{Phe}$ closely resemble those previously reported from dispersive IR (Schernau & Ackermann, 1977) and are not shown.

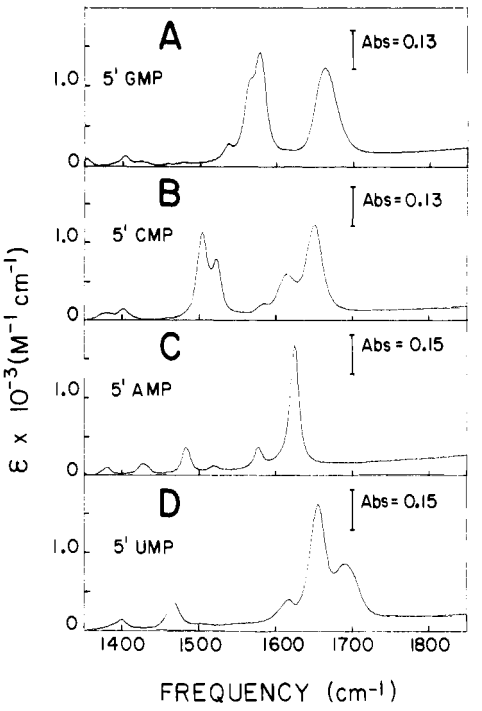

FIGURE 5: FT-IR reference spectra of the four ribonucleotide 5′-monophosphates.

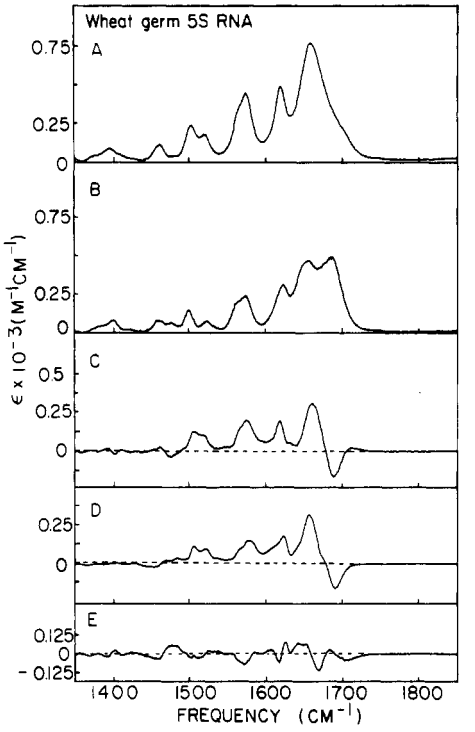

FIGURE 6: FT-IR spectra of wheat germ 5S RNA in the absence of Mg^{2+}: (A) FT-IR spectrum of wheat germ 5S RNA at 90 °C; (B) FT-IR spectrum of wheat germ 5S RNA at 30 °C; (C) difference spectrum, or base-pair spectrum, (A) − (B), representing the infrared intensity due to base pairing of the RNA bases; (D) simulation of (C), computed from the reference A-U and G-C base-pair spectra with the base-pair numbers listed in Table I (14 A-U and 17 G-C pairs); (E) difference spectrum, (D) − (C), representing the difference between experimental and simulated base-pair content.

Determination of RNA Base-Pair Composition from FT-IR Absorbance. All methods for determining RNA base pairing

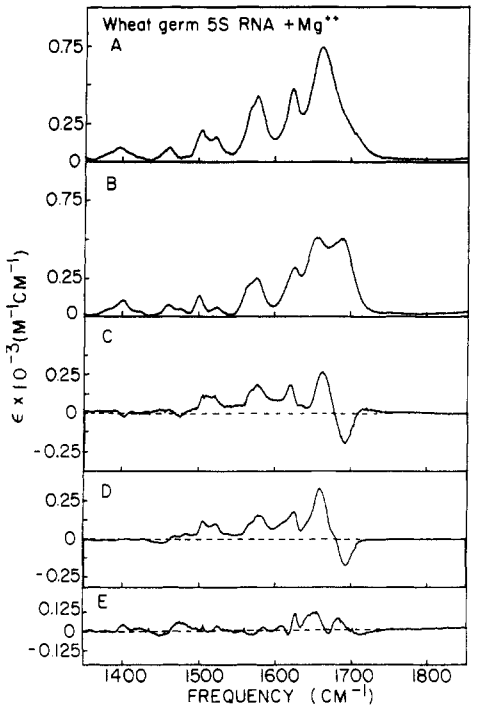

FIGURE 7: FT-IR spectra of wheat germ 5S RNA in the presence of Mg^{2+}: (A)–(C) and (E) as in Figure 6; (D) simulation of (C), computed from the reference A-U and G-C base-pair spectra with the base-pair numbers listed in Table II (14 A-U and 18 G-C pairs).

from IR spectra are based on the increase in IR molar extinction coefficient, $\epsilon(\nu)$, resulting from unstacking of the bases (as by heating the RNA). Extension of previous treatments (Thomas, 1969; Schernau & Ackermann, 1977; Burkey et al., 1983) to include all three types of base pairs leads immediately to

$$\frac{\epsilon(\nu)_{\mathrm{RNA,90°C}} - \epsilon(\nu)_{\mathrm{RNA,30°C}}}{2} = \left[\frac{\epsilon(\nu)_{\mathrm{A,u}} + \epsilon(\nu)_{\mathrm{U,u}}}{2} - \epsilon(\nu)_{\mathrm{A\text{-}U}}\right] f_{\mathrm{A\text{-}U}} + \left[\frac{\epsilon(\nu)_{\mathrm{G,u}} + \epsilon(\nu)_{\mathrm{C,u}}}{2} - \epsilon(\nu)_{\mathrm{G\text{-}C}}\right] f_{\mathrm{G\text{-}C}} + \left[\frac{\epsilon(\nu)_{\mathrm{G,u}} + \epsilon(\nu)_{\mathrm{U,u}}}{2} - \epsilon(\nu)_{\mathrm{G\text{-}U}}\right] f_{\mathrm{G\text{-}U}} \quad (1)$$

In eq 1, $\epsilon(\nu)_{\mathrm{A,u}}$ represents the molar extinction coefficient for unstacked A residues and $\epsilon(\nu)_{\mathrm{A\text{-}U}}$ the molar extinction coefficient for base-paired A residues. Each bracketed expression on the right represents the increase in molar extinction coefficient produced by unstacking of the corresponding base pair. The fraction of paired A's is $f_{\mathrm{A\text{-}U}}$—for example, the number of paired A's is obtained by multiplying $f_{\mathrm{A\text{-}U}}$ by the total number of bases in the RNA (e.g., 120 for wheat germ 5S RNA).

The left-hand side of eq 1 is directly measurable. If the various bracketed expressions on the right were available from suitable reference compounds, then accurate measurement of the extinction coefficients at three IR wavelengths would suffice to determine the desired fractions of each base pair present. Although self-consistent values for the extinction coefficients associated with paired and unpaired A-U and G-C can be assigned from reference spectra of the two corresponding poly(rA)·poly(rU) and poly(rG)·poly(rC) duplexes and the four ribonucleoside monophosphates, 5′-AMP, 5′-UMP, 5′-GMP, and 5′-CMP (Burkey et al., 1983), there is as yet no reliable estimate for the IR hyperchromism of a G-U base pair because poly(rG) does not pair up with poly(rU) when the two homopolymers are mixed.

There are two possible approaches to the G-U problem. The simplest (Burkey et al., 1983) is to assume that the $\epsilon(\nu)$ at every wavelength changes by the same amount for a G-U pair as for a G-C pair. In that case, the second and third terms in brackets on the right of eq 1 are equal, leading to

$$\frac{\epsilon(\nu)_{\mathrm{RNA,90°C}} - \epsilon(\nu)_{\mathrm{RNA,30°C}}}{2} = \left[\frac{\epsilon(\nu)_{\mathrm{A,u}} + \epsilon(\nu)_{\mathrm{U,u}}}{2} - \epsilon(\nu)_{\mathrm{A\text{-}U}}\right] f_{\mathrm{A\text{-}U}} + \left[\frac{\epsilon(\nu)_{\mathrm{G,u}} + \epsilon(\nu)_{\mathrm{C,u}}}{2} - \epsilon(\nu)_{\mathrm{G\text{-}C}}\right] (f_{\mathrm{G\text{-}C}} + f_{\mathrm{G\text{-}U}}) \quad (2)$$

Use of a graphical method based on eq 2 (Burkey et al., 1983) gave 16 G-C and 8 A-U base pairs for yeast $tRNA^{Phe}$, in marked disagreement with the 12 G-C, 8 A-U, and 1 G-U from the X-ray structure (Sussman & Kim, 1976; Ladner et al., 1975). Scrutiny of the data indicated that the spectral intensity values (left-hand side of eq 2) near 1688 cm^{-1} introduced most of the error, because the FT-IR spectra of unfolded RNA and the component mononucleotides are least similar in that region.

A better approach is suggested by close inspection of the spectra of the four ribonucleotide monophosphates in Figure 5. Since only 5′-CMP exhibits significant IR absorbance at 1505 cm^{-1}, $\epsilon(1505)$ should be affected by G-C stacking but not by A-U or G-U stacking. Thus, the fraction of G-C pairs can be deduced from the RNA hyperchromism at 1505 cm^{-1} alone. The hyperchromism at 1620 cm^{-1} is due to mainly to A-U stacking with some G-C stacking contribution (Burkey et al., 1983). Since $f_{\mathrm{G\text{-}C}}$ is now known, as is the G-C contribution to hyperchromism at 1620 cm^{-1} (middle term in brackets on the right of eq 1), $f_{\mathrm{A\text{-}U}}$ can be computed from the 1620-cm^{-1} hyperchromism. Finally, all three base-pair types (A-U, G-C, and G-U) are expected to contribute to the hyperchromism at 1575 cm^{-1}. Since $f_{\mathrm{G\text{-}C}}$ and $f_{\mathrm{A\text{-}U}}$ are now known, $f_{\mathrm{G\text{-}U}}$ can be obtained from $(f_{\mathrm{G\text{-}C}} + f_{\mathrm{G\text{-}U}})$ computed from hyperchromism at 1575 cm^{-1} with eq 2, under the assumption that $\epsilon(\nu)_{\mathrm{G\text{-}C}} = \epsilon(\nu)_{\mathrm{G\text{-}U}}$ at 1575 cm^{-1} only. The base-pair numbers resulting from these calculations are listed in Table II, and the melting midpoints (T_m) deduced from their temperature dependence (Figure 8) are listed in Table III. Finally, the new data reduction gives essentially the same base-pair numbers for yeast 5S RNA as were previously obtained by Burkey et al. (1983).

Discussion

UV and CD Spectra. UV absorption data suggest that wheat germ 5S RNA contains up to 36 base pairs of which approximately 60% are G-C and 40% are A-U, when only these two types of pairs are considered (Table II). The UV melting profiles (Figure 1, Table III) also show that the wheat germ 5S RNA structure is extremely stable (T_m = 69 °C in the presence of Mg^{2+}) and appears to contain at least three segments of different stability. The G-C-rich regions account for most of the stable helices. One of the G-C-rich helical segments must be the "stem" region, because ESR spin-label results for wheat germ 5S RNA found that the 3′-terminal end of the stem is stable up to >55 °C (Luoma et al., 1982). Although addition of Mg^{2+} increases T_m for both UV absorption and CD dissymetry ratio by 15 °C, Mg^{2+} introduces little change in the room-temperature helicity and base-stacking and base-pair number.

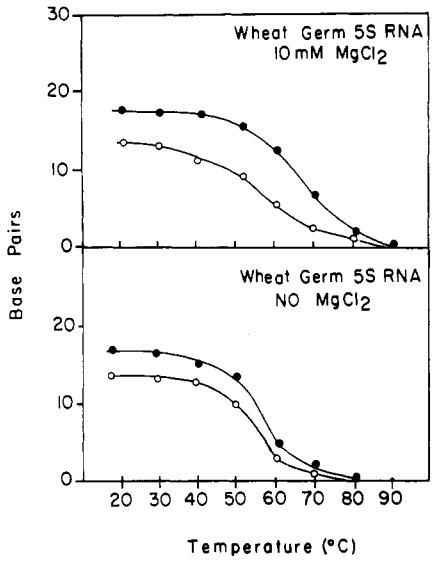

FIGURE 8: Number of A-U (○) and G-C (●) base pairs of wheat germ 5S RNA as a function of temperature: (top) in the presence of Mg^{2+}; (bottom) in the absence of Mg^{2+}.

FT-IR Spectra. The principal results of the FT-IR analysis are listed in Tables II–III. Figures 6 and 7 illustrate the good agreement between the experimental RNA hyperchromism and that simulated from linear combination of reference spectra in the proportion listed in Table II. The reliability of the data reduction is further evidenced by the close agreement between the relative and absolute numbers of yeast $tRNA^{Phe}$ A-U and G-C base pairs determined by FT-IR and by high-field proton FT-NMR (Table II). In fact, $tRNA^{Phe}$ is a severe test case, because of the large number of non Watson–Crick base pairs and derivatized bases: there are 9 "normal" G-C and 7 "normal" A-U pairs, 3 G-C base pairs with methylated G or C, and 1 A-Ψ base pair. Further evidence for self-consistency is the close agreement between the base-pair ratio: G-C/(G-C + A-U) = 0.58 (UV absorbance) vs. 0.56 (FT-IR) for wheat germ 5S RNA in the presence of Mg^{2+}.

Because FT-IR can distinguish between A-U and G-C base pairs, it is possible to monitor independently the melting of these two types of pairs (Figure 8). It is interesting to find that the stability of A-U pairs increases only slightly on addition of Mg^{2+} (T_m increases from 54 to 58 °C), whereas the G-C stability increases by much more (T_m increases from 56 to 67 °C).

Secondary Structural Models. Figure 9 shows three popular secondary base-pairing schemes for eukaryotic 5S RNA, each adapted to the wheat germ 5S RNA sequence. Single-stranded segments of wheat germ 5S RNA have been identified from enzymatic cleavage experiments. T_1 RNase digestion (Barber & Nichols, 1978) suggests that G_{26}, G_{55}, G_{86}, G_{87}, and G_{89} are unpaired and exposed whereas S_1 nuclease digestion (Payne & Dyer, 1976) indicates that bases 8–17 and 32–40 must be single-stranded, as noted on the models of Figure 9.

The base-pair content for each model is listed in Table II, for comparison to experimental UV, CD, and FT-IR results. The experimental results give base-pair totals slightly higher than predicted by the models of Figure 9 but with similar distribution among A-U and G-C pairs. Since the models do not include tertiary pairs, one would expect to find more pairs experimentally than in the models. The secondary base-pairing model that most closely matches the experimental base-pair totals and base-pair ratios is the cloverleaf (Luoma & Marshall, 1978a,b), although experimental imprecision does not rule out the modified Fox–Woese model (Nishikawa & Takemura, 1978; Studnicka et al., 1981).

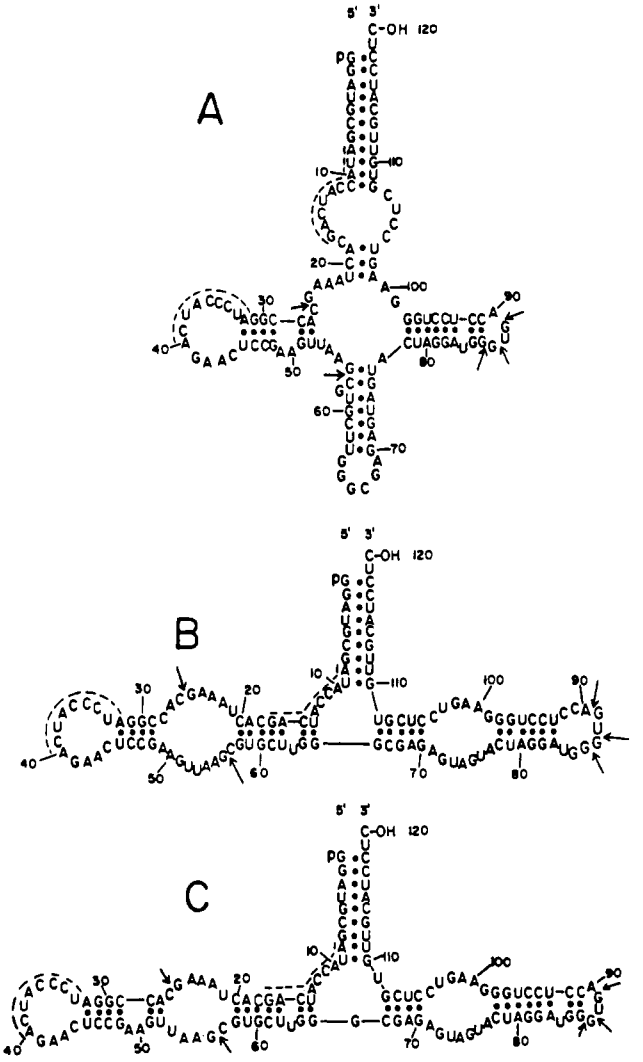

FIGURE 9: Proposed secondary structure models of wheat germ 5S RNA. Sites of cleavage by T_1 RNase (→) and S_1 nuclease (- - -) are indicated. (A) Cloverleaf (Luoma–Marshall) model; (B) revised Fox–Woese model; (C) Studnicka et al.–Nishikawa–Takemura model.

Other assumptions underlying the FT-IR data reduction have been given previously (Burkey et al., 1983). In addition, the dependence of IR hyperchromism on sequence (Tsuboi, 1969) contributes to the total ± 10% error in Table II. Because all three optical techniques reflect base stacking and/or helicity and because the X-ray structure of $tRNA^{Phe}$ proves that single-stranded bases can be stacked without being paired, the experimental base-pair estimates in Table II should be taken as upper limits. Proton FT-NMR and proton intramolecular NOE experiments now in progress at 500 MHz should establish lower limits, since base-pair hydrogens that exchange rapidly with H_2O may be broadened beyond detection. Combination of the optical and ^1H NMR results should go far toward establishing the correct secondary structure for ribosomal 5S RNAs in solution.

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Registry No. Mg, 7439-95-4; 5′-GMP, 85-32-5; 5′-CMP, 63-37-6; 5′-AMP, 61-19-8; 5′-UMP, 58-97-9; guanine, 73-40-5; uracil, 66-22-8.

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