Primary Sequence of Wheat Mitochondrial 5S Ribosomal Ribonucleic Acid: Functional and Evolutionary Implications†

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ABSTRACT: Using the procedures of Donis-Keller et al. [Donis-Keller, H., Maxam, A. M., & Gilbert, W. (1977) *Nucleic Acids Res. 4*, 2527–2538 (1977)] and Peattie [Peattie, D. A. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1760–1764], we have determined the nucleotide sequence of wheat mitochondrial 5S ribosomal ribonucleic acid (rRNA). This sequence

(A)AACCGGGCACUACGGUGAGACGUGAAAA-
CACCCGAUCCCAUUC*CGACC*UCGAUAUAUA-
UGUGGAAUCGUCUUGCGCCAUAUGUACUGA-
AAUUGUUCGGGAGACAUGGUCAAAGCCCGGAA(A)

is the first to be reported for a plant mitochondrial RNA. A highly conserved region (underlined) readily identifies the molecule as a structural homologue of other 5S rRNAs, as do potential base-paired regions which are characteristic of all known (prokaryotic, chloroplast, eukaryotic cytosol) 5S rRNA sequences. However, when assessed in terms of those structural features which distinguish prokaryotic from eukaryotic 5S rRNAs, wheat mitochondrial 5S rRNA cannot be classified readily as one or the other but instead displays characteristics of both types. In addition, the mitochondrial 5S rRNA has several unusual features, including (i) a variable number (two to three) of A residues at both the 5′ and 3′ ends, (ii) a unique sequence (CGACC, italic) in place of the prokaryotic sequence (CGAAC) which has been postulated to interact with aminoacyl-tRNA during translation, and (iii) a novel sequence, AUAUAUAU, immediately following the highly conserved sequence. In terms of overall primary sequence, wheat mitochondrial and cytosol 5S rRNAs seem to be slightly more divergent from each other than either is from *Escherichia coli* 5S rRNA, with which they are about equally homologous. From these observations, we propose that wheat mitochondrial 5S rRNA represents a distinct class of 5S rRNA. Our observations raise a number of questions about the evolutionary origin and functional role(s) of plant mitochondrial 5S rRNA.

Although 5S rRNA[1] is a ubiquitous constituent of prokaryotic, chloroplast, and eukaryotic cytosol ribosomes, it is not a common mitochondrial component, having been found so far only in the mitochondrial ribosomes of higher plants (Leaver & Harmey, 1976; Cunningham et al., 1976). By oligonucleotide cataloging, we previously demonstrated that the cytosol and mitochondrial 5S rRNAs of wheat have different primary sequences (Cunningham et al., 1976). More recently, we showed that wheat mitochondrial 5S rRNA hybridizes specifically with wheat mtDNA and is, therefore, a mitochondrial gene product (Bonen & Gray, 1980).

Knowledge of the primary sequence of a number of prokaryotic and eukaryotic 5S rRNAs has been useful in evaluating structure–function relationships for this RNA (Fox & Woese, 1975b; Erdmann, 1976; Weidner et al., 1977; Jagadeeswaran & Cherayil, 1980), in assessing the validity of various proposed secondary structure models (Nishikawa & Takemura, 1974a,b; Fox & Woese, 1975a,b; Erdmann, 1976; Hori, 1976; Vigne & Jordan, 1977; Barber & Nichols, 1978; Sankoff et al., 1978; Luoma & Marshall, 1978; Hori & Osawa, 1979), and in constructing phylogenetic trees (Kimura & Ohta, 1973; Hori, 1975, 1976; Schwartz & Dayhoff, 1978; Hori & Osawa, 1979). The existence of a mitochondrial 5S rRNA is of considerable interest in all of these contexts. In this report, we present the complete nucleotide sequence of wheat mitochondrial 5S rRNA, along with a possible model of its secondary structure. We also discuss some of the functional and evolutionary implications of this sequence, the first to be reported for a higher plant mitochondrial RNA.

Experimental Procedures

Mitochondria were prepared (Cunningham & Gray, 1977; Bonen & Gray, 1980) from viable wheat embryos (*Triticum vulgare* Vill., *Triticum aestivum* L. var. Thatcher) germinated for 24 h in the dark. Polyacrylamide gel electrophoresis was used to isolate 5S rRNA from the 1 M NaCl-soluble fraction of total mitochondrial RNA (Bonen & Gray, 1980). Alternatively, RNA from large mitoribosomal subunits (Bonen & Gray, 1980) was fractionated in 2.4:10% composite gels and the 5S rRNA recovered. The isolated RNA was labeled at its 5′ terminus by using polynucleotide kinase and [γ-^{32}P]ATP (Donis-Keller et al., 1977) or at its 3′ terminus by using RNA ligase and [5′-^{32}P]pCp (Peattie, 1979). Before 5′ end labeling, the RNA was treated with calf intestinal alkaline phosphatase to remove any monoester phosphate groups. After labeling, it was electrophoresed in 6% polyacrylamide gels [19:1 acrylamide/bis(acrylamide)] containing TBE buffer (Donis-Keller et al., 1977) and 7 M urea. These gels (40 × 33 × 0.05 cm) were run warm, at 1500–1700 V. RNA was dissolved in loading buffer (Peattie, 1979) and was applied to the gel at a maximum of 2.5 μg of total RNA/mm^2 of well surface area. When the xylene cyanol marker dye reached 35.5 cm

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[1] Abbreviations used: mtDNA, mitochondrial DNA; nDNA, nuclear DNA; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; TBE, 50 mM Tris–50 mM boric acid–1 mM NaEDTA (final pH 8.3); rRNA, ribosomal ribonucleic acid.

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Table I: Terminal Nucleotide Analysis of 3′ and 5′ End Labeled Wheat Mitochondrial 5S rRNA Species

chain length[a]	band[b]	5′ end labeled (phosphodiesterase hydrolysis) (%) pA	pC	pG	pU	band[b]	3′ end labeled (alkaline hydrolysis) (%) Ap	Cp[c]	Gp	Up[c]
123	5a	79.6	20.0	0.3	0.1	3a[d]	79.0	15.3	2.0	3.7
122	5b	96.1	3.5	0.3	0.1	3b	91.8	5.1	1.2	1.9
121	5c	96.9	1.0	1.9	0.1	3c	74.3	15.8	5.7	4.3
120	5d	63.9	1.3	34.5	0.3	3d	53.8	33.1	3.0	10.0
119	5e	72.6	0.6	25.5	1.2					
weighted average:		88.3	3.8	7.7	0.2		79.0	13.7	3.2	4.0

[a] Assigned with reference to the major and minor species of cytosol 5S rRNA (known chain lengths of 120 and 119 nucleotides, respectively; MacKay et al., 1980), either present in the isolated mitochondrial 5S rRNA (see text) and thereby serving as internal mobility standards during gel electrophoresis or run in parallel with individual mitochondrial 5S rRNA species recovered from polyacrylamide gels (see Figure 1). [b] Refers to individual species of 5′ or 3′ end labeled RNA separated by polyacrylamide gel electrophoresis (parts A and C, respectively, of Figure 1). [c] Corrected for deamination of C residues occurring during alkaline hydrolysis (Gray & Lane, 1967). [d] Values corrected for ~50% contamination by band 3b (see Figure 1D).

from the origin (3–3.5 h), the 5S RNA was located ~31 cm from the origin. By this procedure, both 5′ and 3′ end labeled 5S rRNAs were resolved into several discrete bands which could be detected by autoradiography (Figure 1); these were well enough separated to allow their individual recovery by electrophoretic elution (MacKay et al., 1980).

Individual bands of end-labeled 5S rRNA were each subjected to end-group analysis, Southern (1975) hybridization, and sequence analysis. For end-group analysis, labeled RNA was completely hydrolyzed either with 1 M NaOH at room temperature for 90 h (3′ end labeling) or with purified snake venom phosphodiesterase at 37 °C and pH 9.2 for 18 h (5′ end labeling). After separation of the resulting 2′(3′)-mononucleotides (alkaline hydrolysis) or 5′-mononucleotides (phosphodiesterase hydrolysis) by two-dimensional thin-layer chromatography, marker nucleotides were visualized under ultraviolet light, and radioactivity was detected by autoradiography and subsequently quantified by liquid scintillation counting. Details of the end-group analysis procedure are given elsewhere (MacKay et al., 1980). End-labeled RNAs were hybridized to restriction endonuclease fragments of wheat mtDNA and nDNA, as described (Bonen & Gray, 1980). Sequence analysis (MacKay et al., 1980) was carried out by the "rapid readout" techniques of Donis-Keller et al. (1977) and Peattie (1979) using 40 × 33 × 0.05 cm gels containing TBE buffer and 7 M urea. Gels were run at 1700 V. Autoradiography was performed at −70 °C by using Kodak X-Omat R X-ray film with Du Pont Cronex Lightning-Plus intensifying screens.

Results

Size Heterogeneity of Wheat Mitochondrial 5S rRNA. When wheat mitochondrial 5S rRNA, either 3′ or 5′ end labeled, was subjected to prolonged electrophoresis in large-pore (6%) denaturing polyacrylamide gels, four to five distinct bands were resolved (Figure 1A–C), each differing in length by one nucleotide from bands immediately above and below (Figure 1D). This apparent size heterogeneity was exhibited by 5S rRNA either directly extracted from mitochondria or prepared from isolated large mitoribosomal subunits. Southern (1975) hybridization patterns with *Sal*I-restricted mtDNA were identical for all resolved RNA bands, both 5′ end labeled (Figure 2A) and 3′ end labeled (Figure 2B), and the same as that previously observed by us for bulk mitochondrial 5S rRNA (Bonen & Gray, 1980). These results indicated the existence of multiple molecular species of mtDNA-encoded 5S rRNA.

End-group analysis (Table I) showed that A was the predominant 5′- and 3′-terminal nucleoside of all resolved species.

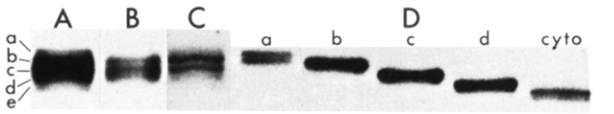

FIGURE 1: Size heterogeneity of wheat mitochondrial 5S rRNA. RNA was resolved on long 6% polyacrylamide gels as described in the text. (A) 5′ end labeled 5S rRNA isolated from total mitochondrial RNA; (B) 3′ end labeled 5S rRNA isolated from total mitochondrial RNA; (C) 3′ end labeled 5S rRNA isolated from large mitoribosomal subunits. Lower-case letters (a–e) refer to the individual bands of 5S rRNA resolved under these conditions; (D) rerun of individual species of 3′ end labeled mitochondrial 5S rRNA from (C) along with a marker of 5′ end labeled wheat cytosol 5S rRNA ("cyto"). The same electrophoretic conditions were used as for the initial fractionation. Note the resolution of the cytosol 5S rRNA marker into major and minor species (120 and 119 nucleotides long, respectively; MacKay et al., 1980).

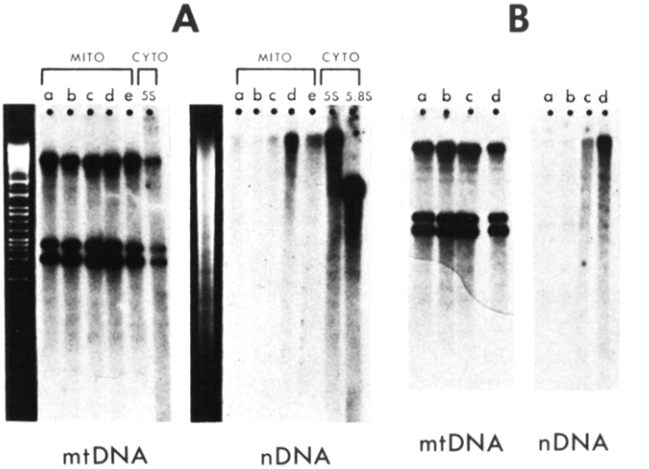

FIGURE 2: Genetic origin of wheat mitochondrial 5S rRNA species. Individual species of 5′ or 3′ end labeled RNA were used as probes against restriction endonuclease digested wheat mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) in Southern (1975) hybridization experiments. (A) Hybridization of 5′ end labeled mitochondrial 5S rRNA species (a–e; Figure 1A) and 5′-labeled wheat cytosol 5S and 5.8S rRNAs with *Sal*I-digested mtDNA (left) and *Eco*RI-digested nDNA (right). The corresponding restriction profiles (ultraviolet photographs of ethidium bromide stained restriction fragments) are shown to the left of each Southern pattern. Cytosol 5S rRNA (which was prepared from total wheat embryo RNA) hybridized weakly with mtDNA, and this probably reflects the presence of trace amounts of mtDNA-specific 5S rRNA in this particular probe. Note that cytosol 5S and 5.8S rRNAs hybridized at different positions in the nDNA restriction profile. (B) Hybridization of 3′ end labeled mitochondrial 5S rRNA species (a–d; Figure 1C) with *Sal*I-digested mtDNA (left) and *Eco*RI-digested nDNA (right).

Separate sequence analysis of the individual bands suggested that our preparations of wheat mitochondrial 5S rRNA were

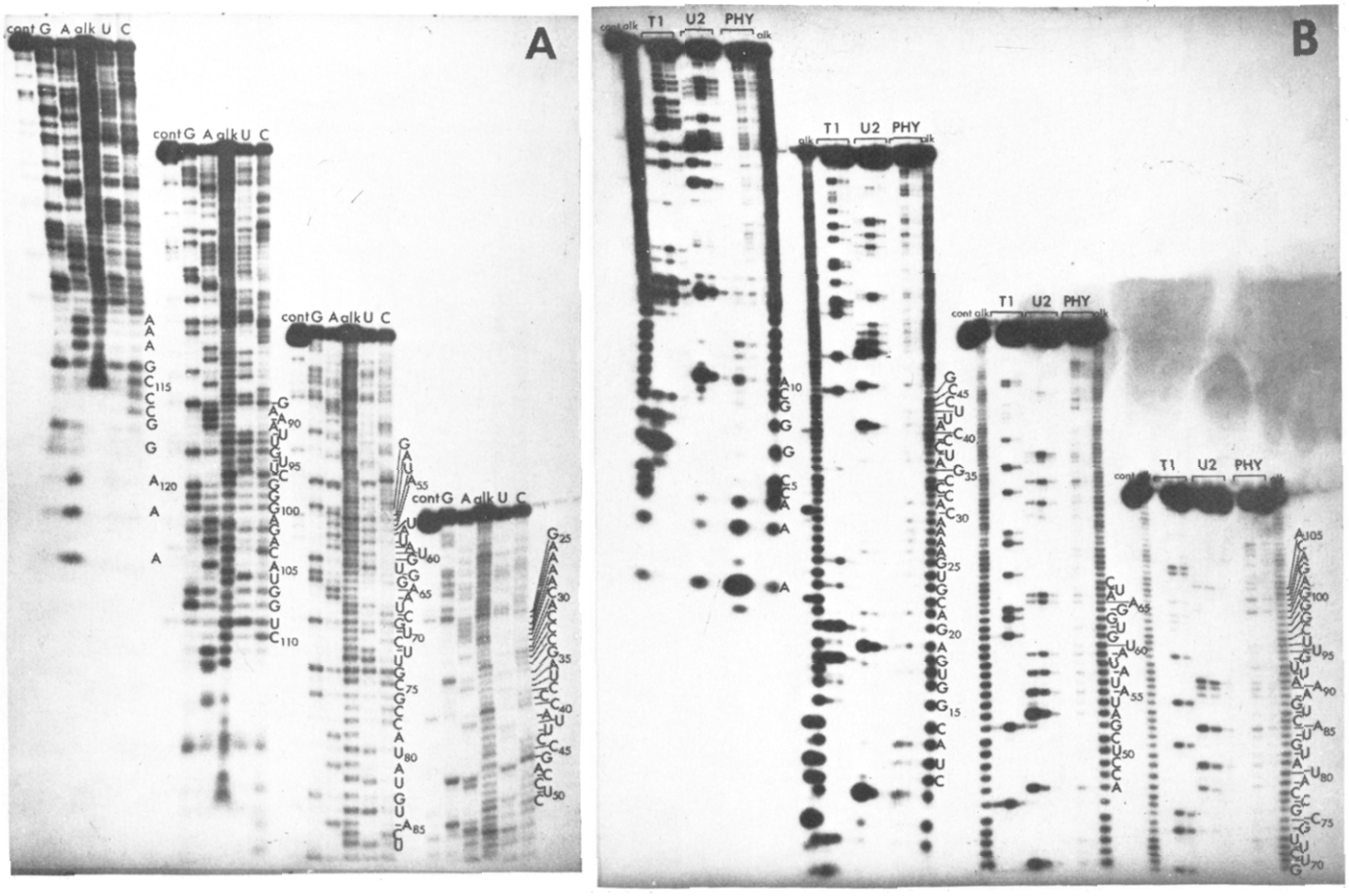

FIGURE 3: (A) Autoradiogram of sequencing gel (10%) showing the resolution of limited chemical digests of 3′ end labeled wheat mitochondrial 5S rRNA. Partial alkaline hydrolysis of the RNA sample provided a "ladder". Running times were 1, 2, 4, and 6 h, respectively, for sequential loadings (left to right across the gel). (B) Autoradiogram of sequencing gel (10%) showing the resolution of limited enzyme digests of 5′ end labeled wheat mitochondrial 5S rRNA. Enzyme/RNA ratios (units per microgram) were 0.05, 0.01, and 0.002 for RNase T_1 and 0.5, 0.1, and 0.02 for RNase U_2. Stock RNase *Phy*I (supplied by H. Donis-Keller) was used in aliquots of 2.0, 0.4, and 0.08 μL/10-μL reaction volume containing 2.0 μg of total RNA (unlabeled *E. coli* tRNA added as carrier plus labeled mitochondrial 5S rRNA). Running times were 1, 2, 4, and 6 h, respectively, for sequential loadings (left to right across the gel).

heterogeneous at both ends, having a variable number (two to three) of 3′- and 5′-terminal A residues. Terminal heterogeneity of this type, which may reflect unusual features of transcription and/or processing in wheat mitochondria, has not been described for other 5S rRNAs except that of the thermophilic bacterium, *Thermus aquaticus* (Nazar & Matheson, 1977). Species containing three A residues at the 5′ end and either two or three at the 3′ end together comprised 70% of end-labeled mitochondrial-encoded 5S rRNA. Table II summarizes terminal sequences consistent with end-group and sequence analysis and with gel mobility data, which independently suggested the presence of minor terminal variants in each band.

Sequence analysis of wheat mitochondrial 5S rRNA was also complicated by the presence of appreciable levels of nuclear-coded (presumably cytosol) 5S rRNA in bulk mitochondrial 5S rRNA, as indicated by the following observations: (1) certain of the end-labeled RNA bands (e.g., 3d, 5d, 5e) hybridized not only with mtDNA but also with the same *Eco*RI restriction fragment(s) of nDNA with which authentic wheat cytosol 5S rRNA hybridized (Figure 2); (2) there was a correlation between hybridization with nDNA and enrichment for the 3′- and 5′-terminal residues (C and G, respectively) characteristic of wheat cytosol 5S rRNA (Table I); (3) presumptive cytosol 5S rRNA species had the same sequence as authentic wheat cytosol 5S rRNA (MacKay et al., 1980) as far as the analysis was carried out (to within 40 nucleotides of the 5′ end; data not shown). It is noteworthy that we had previously found cytosol 5S rRNA in wheat mitochondrial 5S rRNA preparations labeled in vivo with [^{32}P]P_i (Cunningham et al., 1976), at a level (as in the present study, also) not readily attributable to a simple contamination of mitochondria by cytosol ribosomes. At present, however, the significance of these observations is unclear.

Primary Sequence of Wheat Mitochondrial 5S rRNA. Individual species of wheat mitochondrial 5S rRNA were found by sequence analysis to be identical, except for termini. With 3′ end labeled RNA analyzed by the chemical degradation procedure of Peattie (1979), the sequence could be read with little or no ambiguity to within 10 nucleotides of the 5′ end (Figure 3A). The remaining sequence at the 5′ end was determined by the enzymatic degradation technique of Donis-Keller et al. (1977), using 5′ end labeled RNA (Figure 3B); this also provided independent confirmation of most of the sequence. Our enzyme sequencing procedure lacked a C-specific ("+C") cleavage, and consequently in this method C residues were deduced by the absence of any band in the gel at these positions. There have been no indications, either in previous cataloging studies (Cunningham et al., 1976) or in the present work, of modified nucleosides in wheat mitochondrial 5S rRNA, so it is unlikely that any of the enzyme-determined C positions are instead occupied by RNase-resistant modified nucleosides.

The primary sequence of wheat mitochondrial 5S rRNA is presented in Figure 4, along with the predicted RNase T_1 ("T") and RNase A ("P") oligonucleotides, numbered in

Table II: Terminal Sequences of End-Labeled Mitochondrial 5S rRNA Species

band(s) analyzed[a]	chain length[b]	terminal sequence[c] 5′	terminal sequence[c] 3′	relative abundance	comments
5a	123	AAACC. . .	(. . .GGAAAA)[d]	minor	end-group analysis (Table I) suggests the presence of additional minor species having 5′- and/or 3′-terminal C
5b, 3b	122	AAACC. . .	. . .GGAAA	major	
5c, 3c	121	AAACC. . .	. . .GGAA	major	may also contain minor variant in which one A residue is shifted from 5′ to 3′ terminus (i.e., AACC. . .GGAAA)
5d, 3d	120	AACC. . .	. . .GGAA	minor	may contain minor variant in which one A residue is shifted from 3′ to 5′ terminus (i.e., AAACC. . .GGA); major cytosol 5S rRNA species (GGA. . .UCCC) present in this band (see Table I)
5e	119	AACC. . .	(. . .GGA)[d]	minor	minor cytosol 5S rRNA species (GGA. . .UCC) present in this band (see Table I)

[a] Refers to individual species of 3′ or 5′ end labeled RNA separated by polyacrylamide gel electrophoresis (see Figure 1 and Table I). [b] See Table I. [c] Determined by enzyme (5′ end labeled RNA) or chemical (3′ end labeled RNA) sequencing. [d] Sequence not directly determined but inferred from 5′-terminal sequence, chain length, and end-group analysis.

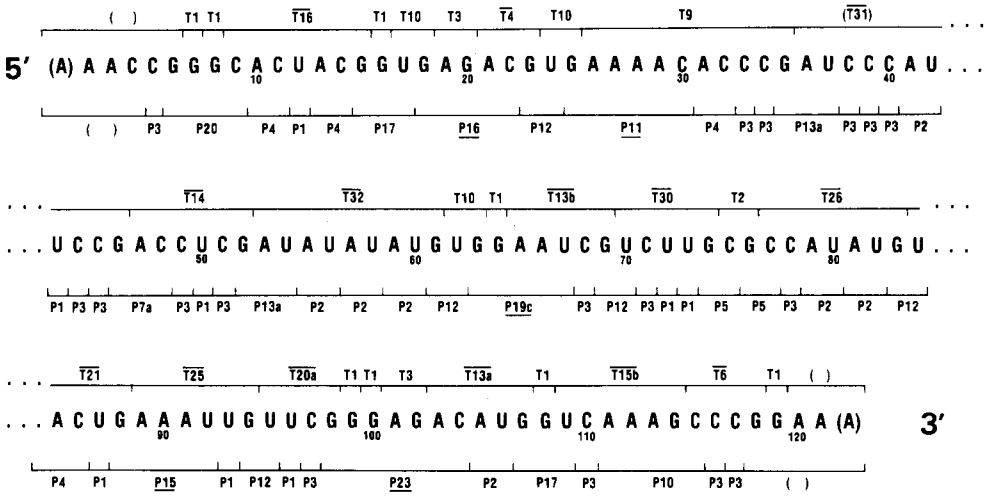

FIGURE 4: Primary sequence of wheat mitochondrial 5S rRNA. Bracketed residues at the 5′ and 3′ ends denote terminal heterogeneity, as discussed in the text. Also shown are the alignments of the T_1 (T) and pancreatic (P) oligonucleotides. Unique oligonucleotides previously attributed (Cunningham et al., 1976) to mitochondrial-specific 5S rRNA are indicated by overlining (T) and underlining (P). See text for discussion of the structure of oligonucleotide T31.

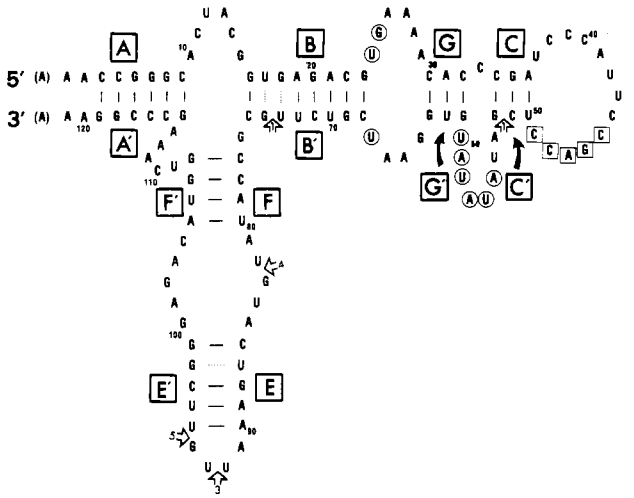

FIGURE 5: Model of a possible secondary structure for wheat mitochondrial 5S rRNA. Potential base-paired regions (AA′, BB′, etc.) have been labeled in accordance with published conventions (Fox & Woese, 1975a,b; Hori & Osawa, 1979). Circled residues are those assumed to be insertions in the wheat mitochondrial 5S rRNA sequence when compared to other 5S rRNA sequences (see Figure 6). Open arrows indicate the position and number of deleted residues assumed for the mitochondrial 5S rRNA sequence (Figure 6). Boxed residues denote the position of the postulated tRNA binding site in prokaryotic 5S rRNA.

accordance with our previous cataloging studies (Cunningham et al., 1976). There is excellent agreement between the sequence and the catalog, the only major inconsistency relating to oligonucleotide T31. This was previously assigned the sequence AUCCCAU(C,U)G, but the present analysis suggests that it is AUCCCAUUCCG, a difference of one additional C residue. This probably reflects a failure to distinguish between UUCCG and UUCG in secondary analysis of this oligonucleotide during the cataloging studies.

Model of the Secondary Structure of Wheat Mitochondrial 5S rRNA. As shown in Figure 5, the sequence of wheat mitochondrial 5S rRNA can be readily be arranged in a "Y-shaped" configuration (Sankoff et al., 1978). The resulting secondary structure (in which maximal base pairing is shown) possesses the three helices (AA′, BB′, CC′) present in the Fox & Woese (1975a,b) and other (Erdmann, 1976) 5S rRNA models, although there are some differences in detail. For example, helix AA′ contains six base pairs (all G·C) instead of the usual 8–11; the 5′-terminal residue is unpaired (as is also the case in *Anacystis nidulans* and *Thermus aquaticus* 5S rRNAs); and helix CC′ consists of only three base pairs, instead of the usual four. In all other sequenced 5S rRNAs, helix BB′ has five or six base pairs; the analogous mitochondrial helix could have as many as eight, but since two of these would be adjacent G·U pairs, it is more likely that only five base pairs (residues 19–23 and 68–72) are actually present.

A fourth helix, called DD′ (Hori & Osawa, 1979) and characteristic of prokaryotic 5S rRNA, appears to be absent in wheat mitochondrial 5S rRNA (as it is in eukaryotic 5S rRNAs). However, this part of the molecule does contain two

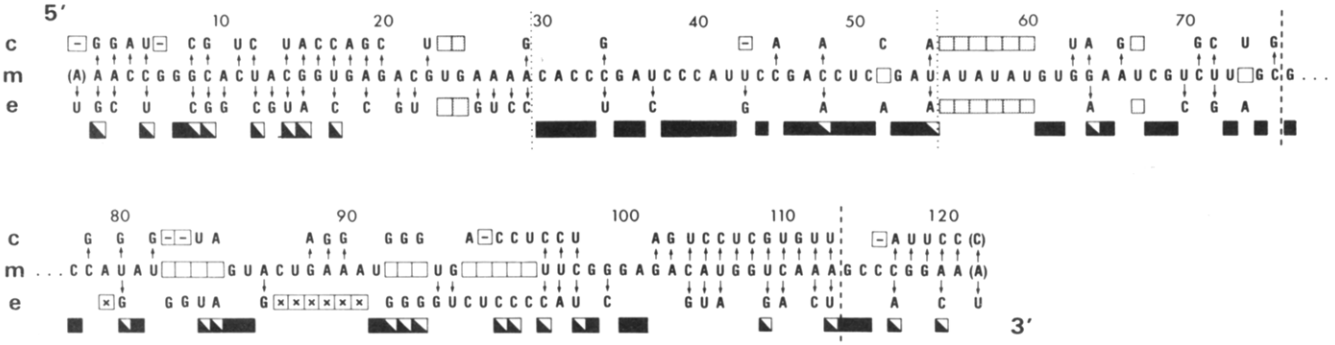

FIGURE 6: Alignment of primary sequences of wheat cytosol (c), wheat mitochondrial (m), and *E. coli* (e) 5S rRNAs. The alignment between c and e is based on that of Hori & Osawa (1979). Blank positions in the sequences of c and e denote identity with the same position in m; residues which are different are denoted by arrows. The dotted lines delineate the most highly conserved region of 5S rRNA, whereas the dashed lines enclose the region which is most variable between prokaryotic and eukaryotic 5S rRNAs. Symbols used are as follows: open squares, deletion in m; dashed squares, deletion in c; x-ed squares, deletion in e; dotted squares, insertion in m; half-filled squares, identical residue in c and e; filled squares, identical residue in c, m, and e at this position.

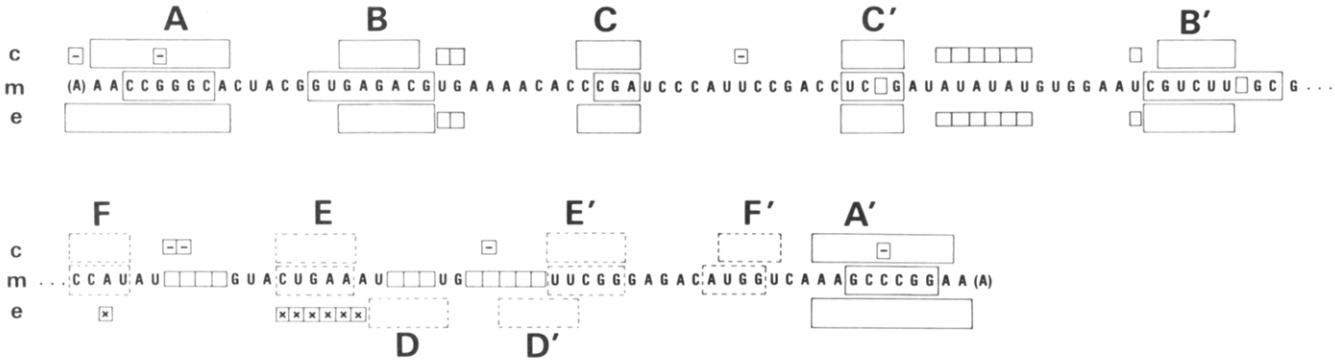

FIGURE 7: Alignment of potential base-paired regions in primary sequences of wheat cytosol (c), wheat mitochondrial (m), and *E. coli* (e) 5S rRNAs. This figure shows the extent of correspondence (based on the overall primary sequence alignment of Figure 6) between sections of primary sequence which may be involved in secondary structure (Figure 5). For clarity, only the sequence of wheat mitochondrial 5S rRNA is shown, although the positions of assumed insertions and deletions are noted for all three RNAs (symbols as in Figure 6). Continuous rectangles denote "universal" helices (AA′, BB′, CC′) whereas dotted rectangles delineate helices which are different between prokaryotic (DD′) and eukaryotic (EE′, FF′) 5S rRNAs.

other potential helices (EE′ and FF′ in Figure 5) which can be identified in wheat and other *eukaryotic* cytosol 5S rRNAs. Although DD′ and EE′ occupy roughly the same spatial position in secondary structure models of prokaryotic and eukaryotic 5S rRNAs, sequence alignment (Hori & Osawa, 1979) suggests that they actually involve nonhomologous regions of primary sequence. In this area we find a better alignment of wheat mitochondrial 5S rRNA with wheat cytosol than with *Escherichia coli* 5S rRNA (Figure 6), resulting in excellent correspondence between helices EE′ and FF′ in the two wheat 5S sequences (Figure 7). Helix DD′ is distinguished from EE′ by its high content of G·C base pairs and by the fact that it encloses a loop of only three to four nucleotides instead of the 11 to 12 enclosed by EE′. The mitochondrial 5S rRNA contains a *eukaryotic-like* helix EE′ enclosing a *prokaryotic-like* small (four-nucleotide) loop.

A sixth possible base-paired region (GG′) in wheat mitochondrial 5S rRNA is also potentially present in a number of prokaryotic 5S rRNAs, including that of *E. coli*, and is in fact a feature of some generalized 5S secondary structure models (Nishikawa & Takemura, 1974b; Sankoff et al., 1978). Additional work, including physicochemical studies and nucleotide accessibility measurements, will be necessary to define how accurately the structure we propose here (Figure 5) reflects the actual secondary structure of wheat mitochondrial 5S rRNA under biological conditions.

So far, all prokaryotic 5S rRNAs have been found to contain the sequence CGAAC in the single-stranded loop closed by helix CC′ (Hori & Osawa, 1979; Erdmann, 1981). Higher plant 5S rRNAs contain the sequence AGAAC while all other eukaryotes have PyGAUPy in this position (Hori & Osawa, 1979; Erdmann, 1981). Wheat mitochondrial 5S rRNA is unique in containing CGACC at this site (residues 45–49).

Primary Sequence Homology between Wheat Mitochondrial and Other 5S rRNAs. For evaluation of homology, we have aligned the sequence of wheat mitochondrial 5S rRNA with examples of prokaryotic (*E. coli*; Brownlee et al., 1967) and eukaryotic cytosol (wheat; MacKay et al., 1980) 5S sequences (Figure 6). Alignment was carried out by (i) identifying and matching any stretches of primary sequence which are obviously homologous (e.g., the 25-nucleotide stretch from residues 30–54 in the mitochondrial sequence), (ii) matching any homologous base-paired regions, and (iii) allowing the minimal number of additions and deletions (indicated in Figure 5 and 6) necessary to obtain the "best-match alignment" for the remaining, non-base-paired regions of the molecule. Figure 7 highlights sections of potential secondary structure in the three 5S rRNAs and indicates how well these correspond when the overall primary sequence alignment of Figure 6 is used.

On the basis of this alignment, pairwise comparison shows that the number of identical residues at equivalent positions is 61 [wheat mitochondria (m) vs. *E. coli* (e)], 55 (wheat mitochondria vs. wheat cytosol (c)], and 63 (wheat cytosol vs. *E. coli*). Expressed as "percent homology", these values become 45% (m vs. e), 40% (m vs. c), and 46% (c vs. e) (calculated on the basis of 136 positions in total, which includes the gaps introduced to bring about the alignment shown in Figure 6, but by assuming that a "blank vs. blank" is a non-

identity). If the most variable region (dashed lines encompassing residues 76–113 in wheat mitochondrial 5S rRNA; Figure 6) is eliminated from consideration, as suggested by Fox & Woese (1975b), then the corresponding number of identical positions becomes 45 (m vs. e), 38 (m vs. c), and 42 (c vs. e). Either way, the results suggest that in overall primary sequence, wheat embryo mitochondrial and cytosol 5S rRNAs are slightly more divergent from each other than either is from *E. coli* 5S rRNA, with which they are about equally homologous. In the highly conserved region (residues 30–54 of the mitochondrial sequence, delineated by the dotted lines in Figure 6), wheat mitochondrial 5S rRNA is 77% homologous (20 identical positions out of 26) with both *E. coli* and wheat cytosol 5S rRNAs, which are themselves 81% (21 identical positions out of 26) homologous in this region. The three RNAs are identical at 19 out of the 26 positions (73%) within this region.

Discussion

Attempts to discern the evolutionary history of the mitochondrion are complicated by the great structural diversity of the mitochondrial genome and translation apparatus [see Mahler & Perlman (1979)]. In the specific case of wheat, we had previously demonstrated by T_1 oligonucleotide cataloging that the mitochondrial 18S rRNA is prokaryotic in nature and had argued that this supports an endosymbiotic origin for mitochondria (Bonen et al., 1977; Cunningham et al., 1977). It was therefore surprising to find that wheat mitochondrial 5S rRNA shares only marginally more sequence homology with *E. coli* 5S rRNA than with wheat cytosol 5S rRNA. In terms of potential secondary structure, wheat mitochondrial 5S rRNA appears to display features of both prokaryotic and eukaryotic 5S rRNAs. By the same criteria, chloroplast 5S rRNA is decidedly prokaryotic (Dyer & Bowman, 1979). In fact, because wheat mitochondrial 5S rRNA possesses some novel features which have not been observed in other sequenced 5S rRNAs, we consider that it represents a distinct class of 5S rRNA. It is intriguing that a similar pattern seems to be emerging in the case of mitochondrial transfer RNAs [e.g., Heckman et al. (1978), Bos et al. (1979), Canaday et al. (1980), and Crews & Attardi (1980)].

Although the findings reported here do not allow use to draw any definitive conclusions about the phylogenetic origin of wheat mitochondrial 5S rRNA, we would argue that they are not necessarily inconsistent with our previous demonstration of the prokaryotic nature of wheat mitochondrial 18S rRNA (Bonen et al., 1977; Cunningham et al., 1977). A basic premise governing primary sequence comparisons is that the rate of evolution of the compared molecules has been similar. However, because the mitochondrial translation system must decode only a limited number of mRNAs by using tRNAs which show an expanded codon recognition pattern (Heckman et al., 1980; Barrell et al., 1980; Bonitz et al., 1980), there may be less selective pressure to prevent alterations in the component rRNAs than in other translation systems. If so, the sequence of 5S rRNA in wheat mitochondria could have diverged to such an extent that its phylogenetic ancestry is now obscure. Recent evidence suggests, in fact, that mtDNA may be characterized by an unusually rapid rate of evolution (Brown et al., 1979). This does not, however, answer the question of why wheat mitochondrial 18S rRNA should still retain clear indications of a prokaryotic ancestry. At present, there is no obvious explanation for this anomaly, but possibly evolution of the mitochondrial 18S rRNA is constrained not only by its functional activities in the mature mitoribosome but also by the role it plays during biosynthesis and assembly of the small mitoribosomal subunit.

It has been proposed that 5S rRNA may (i) provide a binding site for aminoacyl-tRNA during translation (Erdmann, 1976), (ii) play a role during the translocation step by "switching" between two different conformations (Fox & Woese, 1975a,b; Weidner et al., 1977), and (iii) act as a "bridge" to mediate reversible association between the small and large ribosomal subunits (Azad & Lane, 1973; Azad, 1979). These proposed functions are based in part on primary sequence data for 5S rRNA, and we have therefore examined the sequence of wheat mitochondrial 5S rRNA to determine whether it could partake in these same interactions.

The proposed tRNA binding site (CGAAC) in prokaryotic 5S rRNA is replaced by CGACC in wheat mitochondrial 5S rRNA; thus, interaction with the sequence GTψCPu in wheat mitochondrial tRNA would appear to be precluded unless this latter sequence has undergone a reciprocal change. Except in higher plants, eukaryotic cytosol 5S rRNA contains GAUPy in place of GAAC at the position of the putative tRNA binding site; in these cases it has been suggested that the 5S rRNA only interacts with initiator tRNA, while 5.8S rRNA is the functional analogue of prokaryotic 5S rRNA in binding elongator tRNAs (Nishikawa & Takemura, 1974a; Erdmann, 1976; Wrede & Erdmann, 1977). This alternative, eukaryotic scheme is not possible in plant mitochondria, which do not possess 5.8S rRNA (Leaver & Harmey, 1976) and whose 5S rRNA does not contain GAUPy at the proposed tRNA binding site.

In secondary structure models proposed to explain the transition between different conformational forms of 5S rRNA (Weidner et al., 1977; Jagadeeswaran & Cherayil, 1980), disruption of one or more of the helical regions of the Fox and Woese model is followed by formation of a new helix. The primary sequence we have obtained for wheat mitochondrial 5S rRNA does not appear to be compatible with either of these "conformational switch" models. We have, however, noted the possibility of other base-paired interactions which could give rise to secondary structures substantially different from that shown in Figure 5. Examples of such alternative helices are residues 32–36 (CCCGA)/96–100 (UCGGG), 42–47 (UUCCGA)/96–101 (UCGGGA), and 56–64 (UAUAUGUGG)/77–85 (CCAUAUGUA). The last of these involves the unusual $(AU)_4$ sequence identified in wheat mitochondrial 5S rRNA.

The third proposed 5S function (Azad & Lane, 1973) is based on the observation that 5S rRNA complexes efficiently and preferentially with small ribosomal subunit RNA (Azad & Lane, 1975). For technical reasons, we have not yet been able to test directly whether such a complex can be formed between wheat mitochondrial 5S and 18S rRNAs. Potential base pairing between wheat mitochondrial 5S rRNA and the 3′-terminal region of wheat mitochondrial 18S rRNA, suggested by preliminary sequence data for the latter (M. N. Schnare and M. W. Gray, unpublished results), is considerably less extensive than that demonstrated between wheat cytosol 18S and 5S rRNAs (Nichols & Wijesinghe, 1978).

In spite of considerable effort to elucidate the biological function of 5S rRNA, this is still uncertain, and it remains an open question whether functional significance can be ascribed to the intra- and intermolecular interactions which have been observed or postulated by others for this molecule. Our data suggest that if comparable interactions occur in wheat mitochondrial 5S rRNA, their precise molecular details would have to be somewhat different.

It is puzzling that a 5S-sized RNA species is not present in other than plant mitochondria. It has been suggested that in these cases there may still be a functional analogue of 5S rRNA, either smaller in size or covalently contiguous with the large mitoribosomal subunit rRNA (Lizardi & Luck, 1971), but there is at present no direct evidence for such proposals. The "$3S_E$" RNA of hamster cell mitochondria, which Baer & Dubin (1980) suggested may be a 5S rRNA equivalent, instead appears to be an unusual $tRNA^{Ser}$ (Arcari & Brownlee, 1980; de Bruijn et al., 1980). It may be significant that the plant mitochondrial ribosome, when compared to prokaryotic and eukaryotic cytosol ribosomes, is less "atypical" in its physicochemical properties and those of its component rRNAs than any other mitoribosome studied to date (Leaver & Harmey, 1973; Cunningham & Gray, 1977). This suggests that in spite of evidence of rapid evolution of mtDNA, plant mitoribosomes may still be evolutionarily more conservative than other mitoribosomes, which may in part explain the requirement for a 5S rRNA in plant but not other mitoribosomes.

In view of the novel structure of wheat mitochondrial 5S rRNA, it would be desirable to extend the studies reported here to other mitochondrial 5S rRNAs from plant species closely and distantly related to wheat. In this regard, sequence data (virtually complete) for the mitochondrial and cytosol 5S rRNAs of rye suggest that both these RNAs are identical with their wheat counterparts (D. F. Spencer and M. W. Gray, unpublished results).

Added in Proof

While this paper was in press, Küntzel et al. (1981) published an extensive discussion of the wheat mitochondrial 5S rRNA sequence determined by us, citing as their source of this sequence an abstract of a poster presented by us at the 12th International Bari Conference on Mitochondria (Martina Franca, Italy, June 23–28, 1980). However, the abstract which Küntzel et al. cite does not contain the sequence of wheat mitochondrial 5S rRNA. In fact, the sequence used by Küntzel et al. and attributed to us differs in several places from that presented by us at the Bari Conference and documented in full here. As a result, Küntzel et al. draw incorrect conclusions about potential secondary structure and its phylogenetic significance, since the base pairing which they postulate between L3 and L5 (Figure 1 of their paper) cannot exist when the correct sequence is used.

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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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