UNIQUE SPECIES OF 5 S, 18 S, and 26 S RIBOSOMAL RNA IN WHEAT MITOCHONDRIA

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1. Introduction

Mitoribosomes from higher plants have been shown to contain high-molecular-weight RNA species that differ only slightly in physicochemical properties from the corresponding cytosol rRNA components [1–4]. Plant mitoribosomes also contain a 5 S RNA component [5] but not, apparently, the '5.8' S RNA species which is hydrogen-bonded to the large (26 S) rRNA of plant cytoribosomes [6]. The sedimentation coefficient of higher-plant mitoribosomes is 77–78 S [2,3], very similar to that of plant cytoribosomes (80 S), Nevertheless, protein synthesis in plant mitochondria is sensitive to many of the antibiotics which differentially inhibit protein synthesis in prokaryotes, as well as in chloroplasts and in mitochondria of other organisms [7].

Recent studies of the RNA species extracted from purified wheat embryo mitochondria ([8] Gray, in preparation) are in accord with the results obtained with other higher-plant mitochondrial systems. During polyacrylamide gel electrophoresis, the '26' S, '18' S, and '5' S wheat mitochondrial RNA species have mobilities very similar to those of their cytosol counterparts; in fact, only the two 18 S species are partially resolved under the usual, non-denaturing conditions. In addition, the G + C content of the bulk, high-molecular weight, ribosomal-type RNA from wheat embryo mitochondria is quite close to that of the same RNA fraction isolated from the cytosol ([8] Cunningham and Gray, in preparation).

Because of these gross physicochemical similarities between the wheat mitochondrial and cytosol rRNA species, we have sought additional evidence of the uniqueness of the mitochondrial rRNA components. The ability of germinating wheat embryos to incorporate [^{32}P]orthophosphate into mitochondrial and cytosol RNA components has allowed us to prepare (Cunningham and Gray, in preparation) specimens of 26 S, 18 S, and 5 S mitochondrial and cytosol [^{32}P] RNA which are suitable for oligonucleotide mapping [9,10]. We present here oligonucleotide 'fingerprints' of wheat mitochondrial and cytosol 26 S, 18 S, and 5 S RNA (as well as catalogs of the T1 and pancreatic ribonuclease digests of the 5 S species) which demonstrate that the three mitochondrial rRNA species are distinct in sequence from their cytosol counterparts.

Abbreviations: mitoribosome, mitochondrial ribosome; cytoribosome, (eukaryotic) cytosol ribosome.

2. Materials and methods

2.1. *Preparation of the individual 26 S, 18 S, and 5 S [^{32}P]rRNA species from wheat mitochondria and cytosol*

Viable wheat embryos [11] were imbibed for 24 h in a medium containing [^{32}P]orthophosphate. Conditions of labeling, preparation of mitochondria, and extraction, purification, and characterization of ^{32}P-labeled mitochondrial and cytosol nucleic acids are to be described in detail elsewhere (Cunningham and Gray, in preparation). Control experiments eliminated the possibility that contaminating fungi or bacteria could have contributed significantly to the [^{32}P]RNA isolated from the purified mitochondrial fraction. Total nucleic acids were separated into a fraction insoluble in 3 M NaCl (and containing the 26 S and 18 S rRNA components), and a fraction soluble under the same conditions (and containing 5 S rRNA and tRNA, as well as DNA). The salt-insoluble

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RNA was further resolved on linear 5–25% sucrose density gradients, to give the individual 26 S and 18 S species. The salt-soluble RNA was recovered by ethanol precipitation and further resolved by chromatography on Sephadex G-100 [12,13]. Fractions containing 5 S rRNA (which was completely separated from the much greater amount of tRNA, as well as from DNA) were mixed with an equal volume of cold isopropanol and the precipitated RNA was recovered by high-speed centrifugation. Residual salt was removed by repeated washing of the alcohol-precipitated RNA with minimal volumes of cold 70% ethanol or isopropanol, after which the RNA samples were lyophilized.

2.2. *Oligonucleotide mapping*

Purified cytosol and mitochondrial 26 S and 18 S RNAs were digested to completion with T1 ribonuclease and the products were resolved by two-dimensional paper ionophoresis [9,10] to yield 'T1 fingerprints' (fig.1A and B; fig.2A and B). Cytosol and mitochondrial 5 S RNA preparations were separately digested with T1 and pancreatic ribonuclease to yield both T1 fingerprints (fig.3A and B) and 'pancreatic fingerprints' (fig.4A and B).

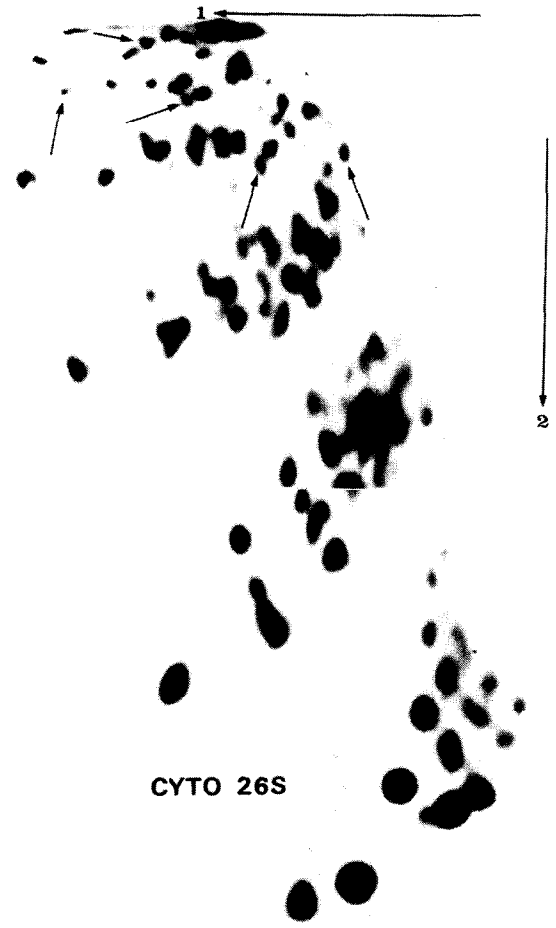

Fig.1A

Fig.1B

Fig.1. T1 fingerprints of wheat embryo cytosol (A) and mitochondrial (B) 26 S RNA. Since the cytosol 26 S RNA was prepared from undenatured ribosomal RNA, its fingerprint includes oligonucleotides derived from the '5.8' S RNA species.

3. Results and discussion

Wheat embryo cytosol and mitochondrial 26 S rRNAs are distinct molecular species. Although the T1 fingerprints of these large RNAs are complex (fig.1A and B), each contains several unique oligonucleotides not present in the other (a few such oligonucleotides are indicated by arrows). Other (similarly-migrating) oligonucleotides showed significant quantitative differences in molar yield (as determined by their radioactivity). In spite of these differences, low-level (15–20%) contamination of mitochondrial 26 S rRNA by the cytosol species was apparent, both from the fingerprints and from separate studies of the content of characteristic modified nucleosides (Gray, unpublished results).

Wheat embryo cytosol and mitochondrial 18 S rRNAs are also distinct molecular species, showing readily distinguishable fingerprints (fig.2A and B; again, a few unique oligonucleotides are indicated by arrows). There was in this case no significant (< 5%) contamination of either (18 S) species by the other.

The presence of a 5 S RNA component in higher-plant mitochondrial ribosomes [5] is of interest in view of the failure to demonstrate a similar-sized molecule in other mitoribosomes [14]. However, there is as yet no information to indicate whether higher-plant mitochondrial and cytosol 5 S RNAs are distinct molecular entities (indeed, the two species cannot be distinguished by electrophoresis in 10% polyacrylamide gels [5]). The results presented in figs.3 and 4 and table 1 allow us to conclude that (at least for wheat)

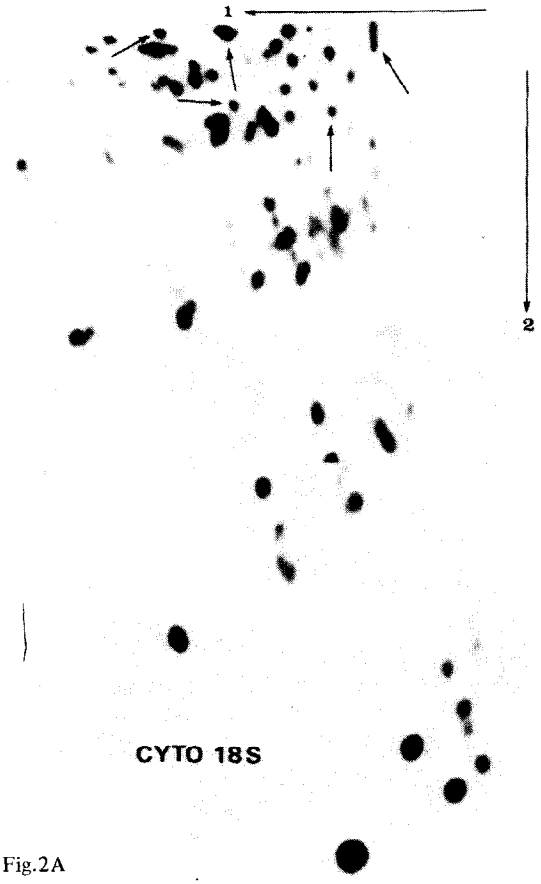

Fig.2A

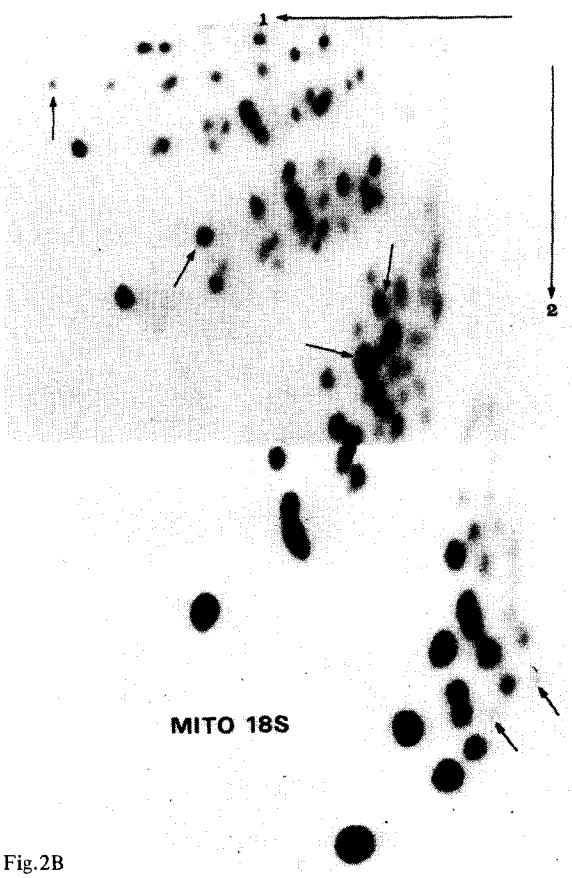

Fig.2B

Fig.2. T1 fingerprints of wheat embryo cytosol (A) and mitochondrial (B) 18 S RNA.

Table 1
T1 and pancreatic ribonuclease catalogs of wheat embryo cytosol and mitochondrial 5 S RNA preparations

T1 RNase products				Pancreatic RNase products			
Spot	Sequence	Molar yield Cytosol	Mitochondrial	Spot	Sequence	Molar yield Cytosol	Mitochondrial
1	G	8–10	13–11	1	U	10–11	12–11
2	CG	3–4	~2	2	AU	3	9
3	AG	2–3	4–3	3	C	16	19
4	ACG	0	1–2	4	AC	4	4
5	AAG	2	0–1	5	GC	3	3
6	CCCG	0	1	6	AAU	0	0–1
7	CAU(C_{1-2}, U_{0-1})X_{OH}	1	0	7	GAC	1	2
8	CACCG	1	0		AGC	1	
9	AAAACACCCG	0	1	8	AAAU?	0	1
10	UG	3–4	4–3	9	AAGC	1	0
11	UAG	1–2	0	10	AGAAC	1	1–2
12	AUG	2	0		AAAGC	1	
13	(C,A)AUG	0	1	11	GAAAAC	0	1
	AAUCG	0	1	12	GU	4	7
14	ACCUCG	0	1	13	GAU	2–3	3–2
15	CAAAUG	0	1–0 [a]		AGU		
	(U,C)AAAG	0	1–2	14	GGC	0	0–1
16	CA,CUA,CG	0	1–0 [b]	15	GAAAU	0	1 [d]
17	AAC(C,U)CG	1	0–1	16	AG,G,AC	0	1
18	CACUAAAG	1	0	17	GGU	0–1	3–2
19	UUG	1	0	18	GGAU	1	0
20	UUCG?	0	1	19	AG,G,AU	1	0
	CUUG	1	0		AAG,G,U	1	0
21	UACUG	0	1		GGAAU	0	1
22	UUAAG	1	0	20	GGGC	0–1	1
23	UCCUCG	1	0	21	GGGU	1	0
24	UACUAG	1	0	22	AG,AG,G,U	1	0
25	AAAUUG	0	1	23	AG,G_3,AC	0	1
26	CCAUAUG	0	1	24	AAG,G_3,U	1	0
27	ACCUCCUG [c]	1	0				
28	A(UCCCA,UCA)G	1	0				
29	A(CCA,UA,UCA)G	1	0				
30	UCUUG	0	1				
31	AUCCCAU(C,U)G	0	1				
32	AUAUAUAUG	0	1				
33	pG	½	0				

This table lists sequences of oligonucleotides in the numbered spots on the fingerprints shown in figs.3 and 4. Cytosol molar yields were determined directly by measurement of radioactivity in spots. Mitochondrial molar yields were similarly determined after subtraction of the contribution of contaminating cytosol oligonucleotides. Sequences unique to the mitochondrial 5 S species are underlined.

[a] A total of two mitochondrial-specific oligonucleotides in this spot.
[b] A total of one mitochondrial-specific oligonucleotide in this spot.
[c] Sequence probable but not certain.
[d] Quantitation suggests more than one copy; 3′- or 5′-terminus may migrate in this position.

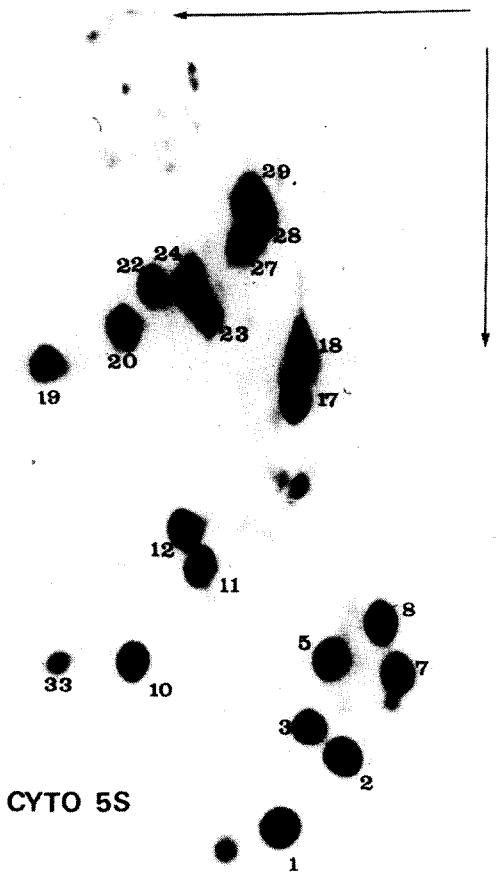

Fig.3A

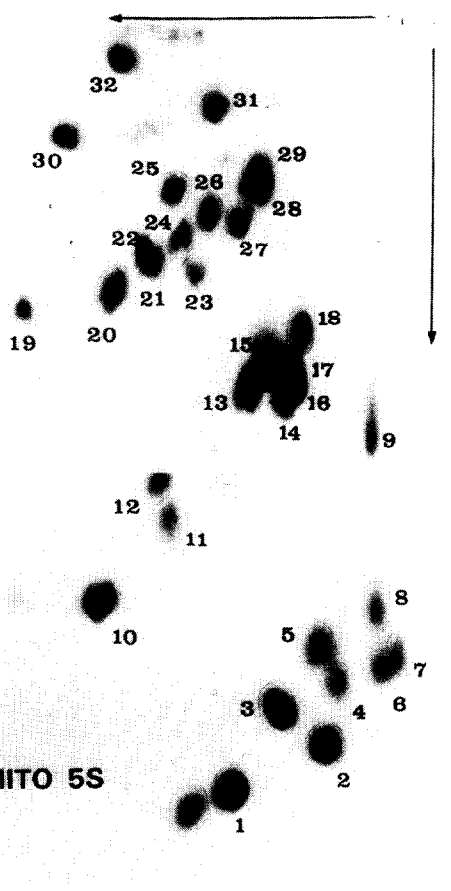

Fig.3B

Fig.3. T1 fingerprints of wheat embryo cytosol (A) and mitochondrial (B) 5 S RNA.

this is so. The T1 and pancreatic fingerprints of wheat mitochondrial 5 S RNA show, respectively, 7 and 13 oligonucleotide spots not present in the corresponding fingerprints of the cytosol 5 S RNA. In addition, the mitochondrial fingerprints contain all of the oligonucleotides found in the corresponding cytosol fingerprints. The wheat mitochondrial 5 S RNA preparation analyzed here thus appears to contain a mixture (ca. 1:1) of a unique mitochondrial 5 S RNA species and 'contaminating' cytosol 5 S rRNA. This conclusion was confirmed by secondary and, in the case of cytosol 5 S RNA, tertiary nuclease digestion [9,10] of all oligonucleotides. The T1 and pancreatic oligonucleotide catalogs of wheat cytosol 5 S rRNA and the unique mitochondrial 5 S RNA species are shown in table 1 (the catalogs for the latter were determined by subtracting, from the catalogs of the mitochondrial 5 S RNA preparation, oligonucleotides contributed by 'contaminating' cytosol 5 S rRNA). The computed length of the unique mitochondrial species, which contains no detectable post-transcriptionally modified residues, is 125–145 nucleotides (exclusive of termini). The molecule does contain the sequence GAUCCCAU (C,U)G, a closely-related variant of which occurs in the highly-conserved region (positions 28–59) of all 5 S rRNA molecules [15]. This oligonucleotide differs by a single residue from the wheat cytosol 5 S sequence, GA(UCCCA,UCA)G.

Not surprisingly, the wheat cytosol 5 S rRNA shows very strong homology with the 5 S rRNA of rye [16]. The T1 catalogs of the two show only two differences (A(UCCCA,UCA)G in wheat, AUCCAUCAG in rye, and the anomalous [16] presence in rye 5 S rRNA of (C_3U)G, while pancreatic catalogs show only one

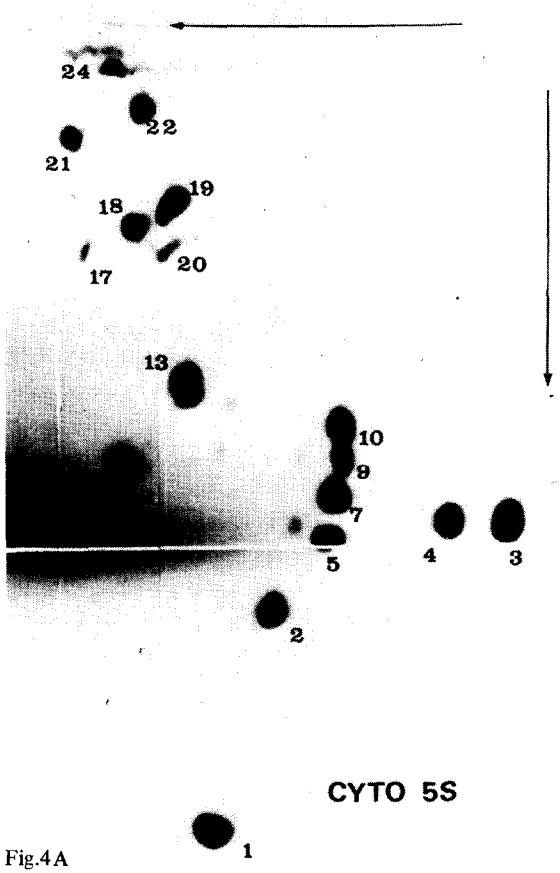

Fig.4A

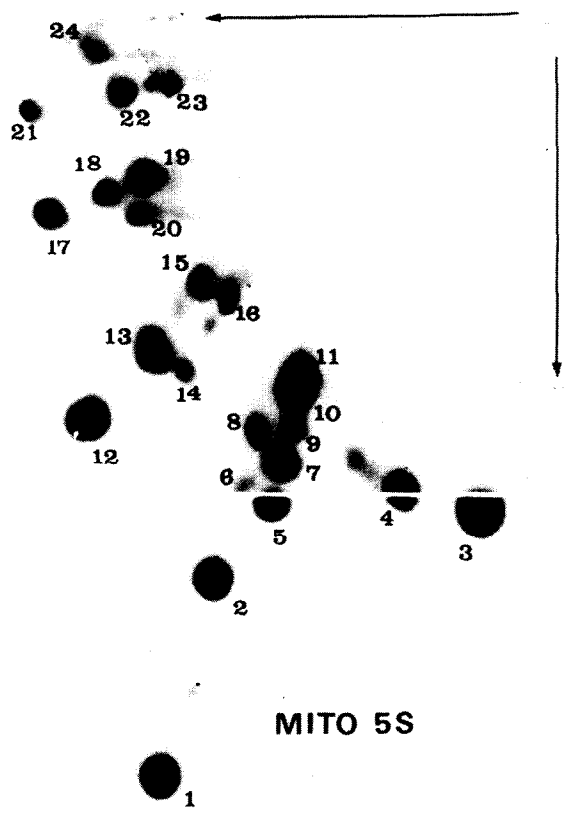

Fig.4B

Fig.4. Pancreatic fingerprints of wheat embryo cytosol (A) and mitochondrial (B) 5 S RNA.

difference (GGGU in wheat, GGU in rye).

Although T1 ribonuclease fingerprints of the two high-molecular-weight rRNA species from *Aspergillus nidulans* [17] and mouse liver [18] have been published, the fingerprints presented here represent the first ones obtained for mitochondrial rRNA species from a higher plant. Together with the T1 and pancreatic oligonucleotide catalogs of 5 S RNA, they clearly demonstrate the existence of different species of 26 S, 18 S, and 5 S RNA in the mitochondria and cytosol of wheat.

Acknowledgements

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