Hypothesis

5 S RNA-like structures in large ribosomal subunit RNAs of fungal mitochondria

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Ribosomes from fungal mitochondria apparently lack free 5 S rRNA. However, secondary-structure models of nucleotide sequences within domain V of the large ribosomal subunit rRNAs (L-rRNAs) from the mitochondria of *Saccharomyces cerevisiae* and *Aspergillus nidulans* reveal the presence of inserted segments which can be folded into highly base-paired structures that resemble free 5 S rRNA. The occurrence of such structures within domain V, which contains a number of important functional sites as well as a region that in *Escherichia coli* 23 S rRNA mediates the attachment of 5 S rRNA and its associated proteins, lends support to the notion that the 5 S rRNA-like inserts in fungal mitochondrial L-rRNAs may carry out the activities performed by free 5 S rRNA in ribosomes from other organisms.

5 S ribosomal RNA *Mitochondrial ribosome* *Protein synthesis* Saccharomyces cerevisiae Aspergillus nidulans

The apparent lack of free 5 S rRNA in the ribosomes of fungal and mammalian mitochondria is puzzling in view of its presence in all prokaryotic, eukaryotic, chloroplast and plant mitochondrial ribosomes examined to date [1–3]. In prokaryotic ribosomes, the essentiality of 5 S rRNA has been demonstrated by reconstitution experiments in which it was shown to be required for the proper assembly of biologically active *Escherichia coli* 50 S subunits [4]. We assume as a working hypothesis that 5 S rRNA, or a functional equivalent thereof, is needed by all ribosomes and that where free 5 S rRNA is absent, there must be other ribosomal components present that carry out the same role. Specifically, we propose that unique regions within the large ribosomal subunit RNAs (L-rRNAs) of fungal mitochondria may constitute the functional equivalents of free 5 S rRNA.

In this paper, we focus our attention upon domain V of L-rRNA, a highly ordered structural unit which in *E. coli* 23 S rRNA encompasses nucleotides 2043–2625 ([5,6], see fig.1). This domain is of great interest as it contains: a region known to interact with 5 S rRNA and its associated proteins, L5, L18 and L25, in *E. coli* 23 S rRNA [7]; the binding site for *E. coli* protein L1, which is conserved in both prokaryotic and eukaryotic L-rRNAs [8,9]; several different post-transcriptionally modified bases in L-rRNAs from a number of organisms [4,10]; sites that mediate the action of antibiotics such as chloramphenicol and erythromycin in mitochondrial and bacterial L-rRNAs [11–14]; and, in certain cytoplasmic, chloroplast and mitochondrial L-rRNAs, large intervening sequences that are removed in the course of ribosome assembly [11,15–19]. The foregoing observations indicate that domain V plays an important role in the structure and function of L-rRNAs from all of the major phylogenetic lineages, and it has even been suggested that this region may be involved directly in peptidyl transferase activity [5].

By constructing secondary-structure models of nucleotide sequences within domain V of L-rRNAs

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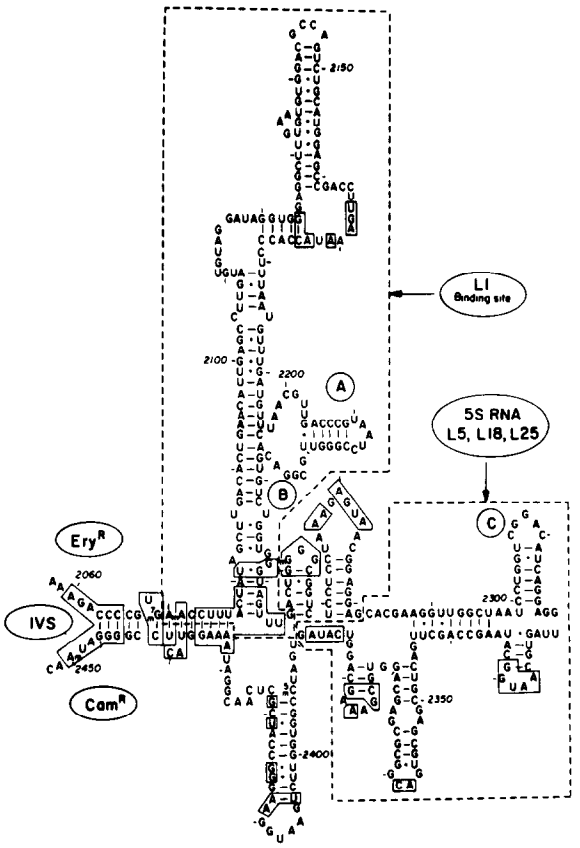

Fig.1. Secondary-structure model for nucleotides 2058–2452 from domain V of *E. coli* 23 S rRNA. The base-pairing scheme is adapted from [5]. Regions associated with ribosomal protein L1 [8,9] and a complex containing 5 S rRNA, L5, L18 and L25 [7] are enclosed by dashed lines. Sequences common to L-rRNAs from *E. coli* and fungal mitochondria are enclosed by solid lines. A–C denote 3 regions of high variability among L-rRNAs from organisms of different phylogenetic lineages. Locations of mutations in mitochondrial and bacterial L-rRNAs which confer resistance to erythromycin (Ery^R) and chloramphenicol (Cam^R) are indicated [11–13]. The sites of large intervening sequences in L-rRNAs from mitochondria of *S. cerevisiae* [20] and *A. nidulans* [19] are designated by IVS.

from the mitochondria of *Saccharomyces cerevisiae* [20] and *Aspergillus nidulans* [19], we have identified regions which have no counterparts in prokaryotic or eukaryotic L-rRNAs and which can be folded into highly base-paired structures that resemble free 5 S rRNA (see fig.2,3). We have assumed that sequences common to the L-rRNAs from *E. coli* and the two fungal mitochondria, which are boxed in fig.1, occur at analogous positions in each of the 3 models. This condition constrains the secondary structures that are available to neighboring segments. Certain portions of domain V are quite variable, however. The stem-loop structure (labeled A in fig.1), for instance, is almost completely absent in L-rRNAs from the fungal mitochondria, whereas the corresponding region of eukaryotic and archaebacterial L-rRNAs is either greatly expanded or characterized by an entirely different pattern of base pairing ([10,23]; D.L. Thurlow, P.B. Cahill, M.L. Zeller, A.T. Matheson and R.A. Zimmermann, in preparation). In addition, the fungal mitochondrial L-rRNAs contain large inserts relative to bacterial L-rRNAs at the positions labeled B and C in fig.1,2. In 21 S rRNA from *Saccharomyces* mitochondria, approx. 160 bases are added at position B, whereas in 20 S rRNA from *Aspergillus* mitochondria, some 200 bases are inserted at position C (see fig.1,2). It is these inserted sequences which can be folded into secondary structures that display a number of features characteristic of free 5 S rRNA.

The secondary structure of *E. coli* 5 S rRNA, adapted from the model of Fox and Woese [24], is presented in fig.3 along with our proposed secondary structures for the 5 S rRNA-like portions of the inserts from the mitochondrial L-rRNAs of *Saccharomyces* and *Aspergillus*. Similar features in the 3 models include helices II, III and IV as well as the terminal loops, L1 and L2. In all cases, helix II contains the single bulged nucleotide on the 3′-proximal side which has been cited as a general feature of 5 S rRNAs by Peattie et al. [25]. Variations in the length of helix II – from 7 base pairs in *Saccharomyces* to 10 base pairs in *Aspergillus* – do not exceed those found in authentic 5 S rRNAs. For instance, helix II can be as short as 4 base pairs in 5 S rRNA from *Prochloron* [26] and can extend to 10 base pairs in *E. coli* 5 S rRNA if G-A base pairing is allowed (fig.3). Differences in the number of base pairs and bulged nucleotides in helices III and IV from the fungal mitochondrial L-rRNAs are also within the range observed for free prokaryotic and eukaryotic 5 S rRNAs [3]. Finally, base pairs within the nominally single-stranded loop L1, which can be formed in the L-rRNA insert from *Aspergillus* (fig.3), are poten-

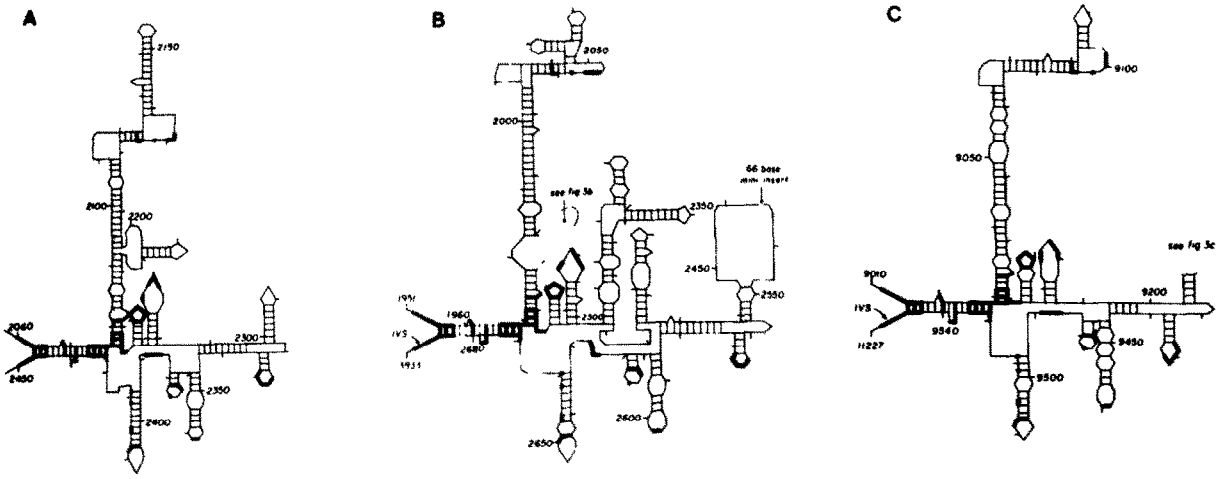

Fig.2. Secondary-structure diagrams for a portion of domain V from *E. coli, S. cerevisiae* and *A. nidulans* L-rRNAs. (A) *E. coli* 23 S rRNA, (B) *S. cerevisiae* 21 S rRNA, and (C) *A. nidulans* 20 S rRNA; nucleotide positions are numbered according to [5], [20] and [19], respectively. The *E. coli* model is shown in detail in fig.1. Thick lines represent sequences common to *E. coli* and both fungal mitochondrial L-rRNAs. Dashed lines indicate regions that contain the 5 S rRNA-like structures, which are presented in fig.3. A total of 8 G-A base pairs are included in the mitochondrial secondary structures. Our models for the fungal mitochondrial L-rRNAs are generally similar to those presented in [20,21], but differ substantially in the regions which contain the 5 S rRNA-like structures. In particular, a 191-base segment of *A. nidulans* mitochondrial L-rRNA, omitted for the sequence alignment presented in [21], is included in our model. The absence of looped-out structures near this region in electron micrographs of hybrids between *Aspergillus* mitochondrial L-rRNA and its DNA complement [22] supports our contention that these sequences are present in the mature 20 S rRNA molecule.

tially present in certain 5 S rRNAs as well [26,27]. While the similarities in secondary structure between free 5 S rRNAs, on the one hand, and inserts in the fungal mitochondrial L-rRNAs, on the other, are suggestive, they do not in themselves prove that these sequences are functionally analogous. However, the occurrence of the inserts within domain V, through which *E. coli* 23 S rRNA interacts within 5 S rRNA and its associated proteins [7], indicates that such regions could be involved in activities comparable to those of 5 S rRNA.

We note that the structural features shared by free 5 S rRNA and the 5 S rRNA-like segments of the mitochondrial L-rRNAs involve secondary structure only. There are no apparent homologies among them in nucleotide sequence. Furthermore, there is no structure analogous to helix I in the mitochondrial inserts, although this helix is universally present in free 5 S rRNA ([3], see fig.3). However, it seems reasonable to speculate that helix I may be required for the stabilization of 5 S rRNA as an independent molecule or for its proper integration into the ribosome. Neither of these properties would be needed by comparable structures located within the base sequences of the mitochondrial L-rRNAs. Alternatively, other helices present in the fungal mitochondrial L-rRNAs, somewhat displaced from helices 'II', 'III' and 'IV', might serve as the counterparts of helix I (see fig.3).

Although mammalian mitochondria also appear to lack free 5 S rRNA, there is no evidence for the existence of 5 S rRNA-like structures within domain V of mammalian mitochondrial L-rRNAs. The latter molecules, which contain only 1560–1580 nucleotides, are among the smallest L-rRNAs known [28–31]. Fig.4 shows that while several of the sequences and secondary-structure features characteristic of domain V are conserved in mammalian mitochondrial L-rRNAs (boxed structures), a number of other segments are either severely truncated or highly variable. The variable regions bear no similarity to the 5 S rRNA-like structures in the fungal mitochondrial L-rRNAs. A potential homology has been noted between a

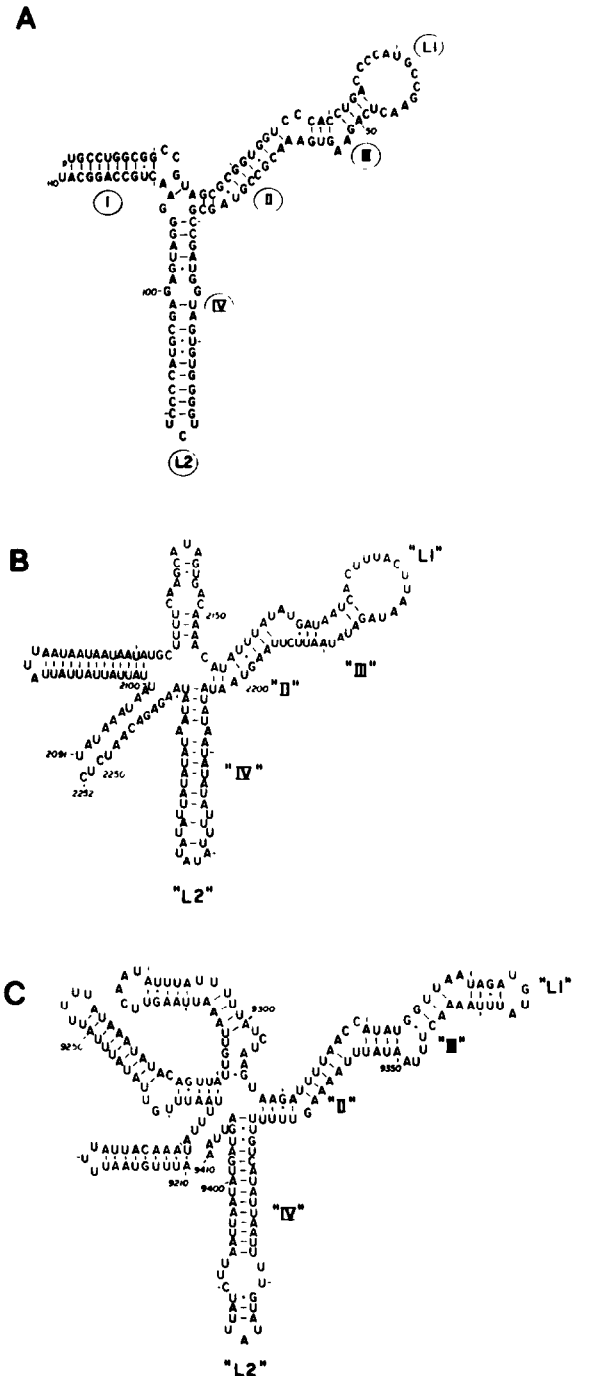

Fig.3. Secondary structures of the 5 S rRNA and 5 S rRNA-like portions of fungal mitochondrial L-rRNAs. (A) *E. coli* 5 S rRNA, (B) *S. cerevisiae* 21 S rRNA, and (C) *A. nidulans* 20 S rRNA. The 4 major helical regions of *E. coli* 5 S rRNA [24] are numbered I–IV and the two terminal loops are designated L1 and L2. Similar structures in fungal mitochondrial L-rRNAs are indicated in quotation marks. Bases in yeast and *Aspergillus* mitochondrial L-rRNAs are numbered according to [20] and [19], respectively.

23-base segment at the 3′-end of human mitochondrial L-rRNA and the portion of certain bacterial 5 S rRNAs that encompasses helix III and loop L1 ([32], see fig.3a). The suggestion that this segment represents the analog of 5 S rRNA in human mitochondria is not persuasive, however, because it can form only a single stem-loop structure and is not conserved in other mammalian mitochondrial L-rRNAs [29–32]. It is of course possible that mammalian mitochondria contain a free counterpart of 5 S rRNA that has thus far escaped detection. Since both small- and large-subunit rRNAs from these mitochondria are substantially reduced in size, the 5 S rRNA analog might also be a truncated RNA molecule which comigrates with tRNAs or with breakdown products of the larger rRNAs. A candidate 5 S rRNA equivalent of approx. 3 S was recently identified in hamster mitochondria [33], but the presence of -C-C-A-OH at the 3′-terminus of this RNA and the fact that a similar RNA molecule from bovine mitochondria can be aminoacylated with serine argue that it is a functional, although most unusual, tRNA [34]. Furthermore, we cannot exclude the possibility that ribosomal protein(s) perform the role of 5 S rRNA in mammalian mitochondria, particularly as proteins comprise an exceptionally large proportion of the ribosomal mass in these organelles [2].

In summary, segments inserted into domain V of the L-rRNAs from two fungal mitochondria exhibit a number of secondary-structure features which resemble those in free 5 S rRNA. Whether or not these inserts fulfil a role analogous to free 5 S rRNA in protein synthesis cannot at present be determined. A definitive answer to this question will require a clearer understanding of exactly how the small rRNA molecules participate in the translational process. Nonetheless, if our hypothesis is correct, it leads to an important inference. Because the similarities between free 5 S rRNA and the inserts in fungal mitochondrial L-rRNAs occur at the level of secondary structure,

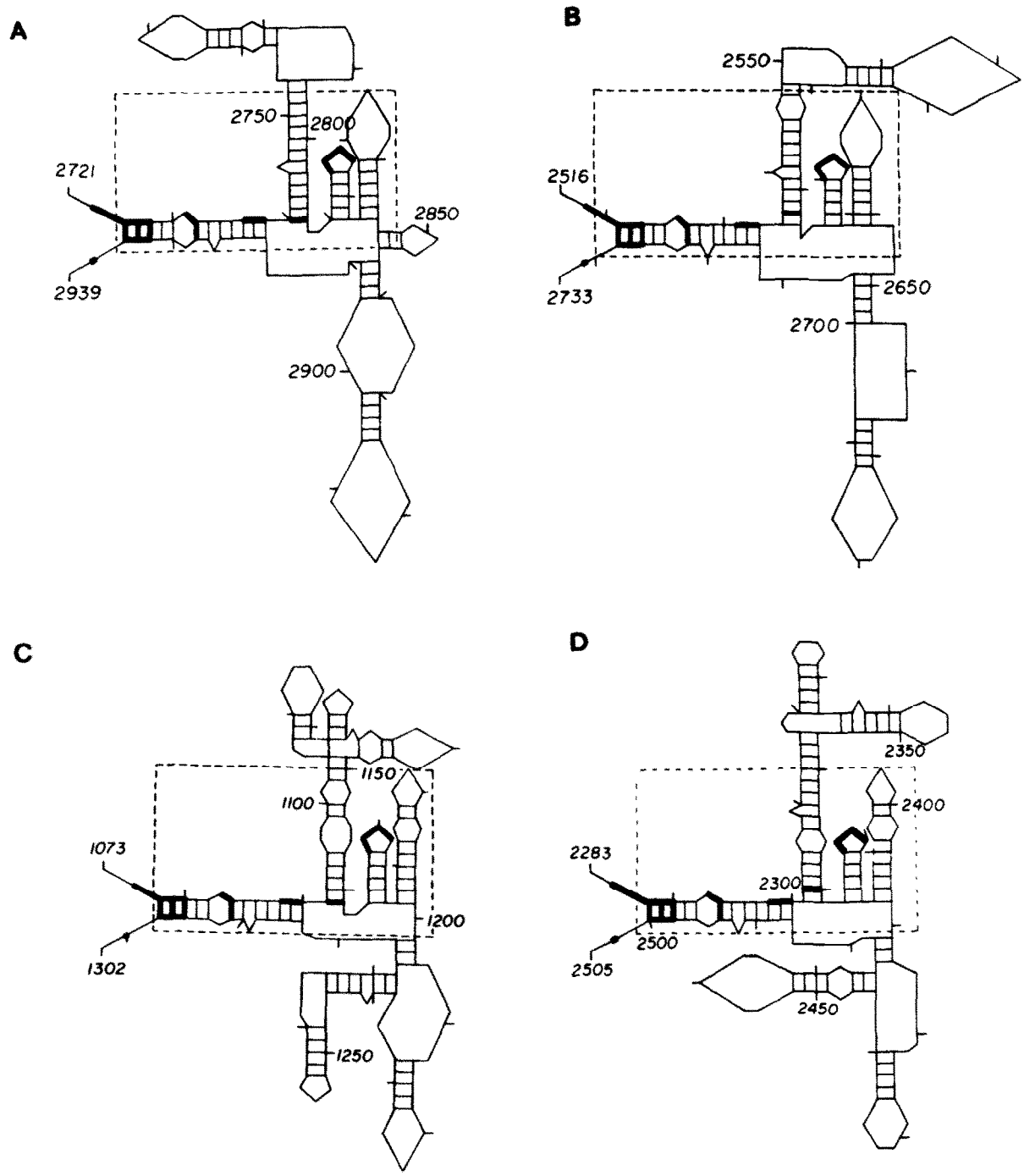

Fig.4. Secondary-structure diagrams for sequences within domain V of mammalian mitochondrial L-rRNAs. (A) Human, (B) bovine, (C) rat and (D) mouse mitochondrial 16 S rRNAs; nucleotides are numbered according to [28], [29], [30] and [31], respectively. Sequences common to L-rRNAs of *E. coli*, fungal mitochondria and mammalian mitochondria are indicated by thick lines. The models contain several conserved helices (boxed) as discussed by Noller et al. [5], but exhibit no inserted sequences with structures similar to free 5 S rRNA. Two G-A base pairs are included in the proposed models.

and do not entail sequence homologies, we suggest that the higher-order structure of 5 S rRNA or its equivalent, and not the primary structure, determines the function of 5 S rRNA in protein biosynthesis.

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