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Mitochondrial L-rRNA from *Aspergillus nidulans*: potential secondary structure and evolution

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ABSTRACT

The alignment of gene sequences coding for A. nidulans mitochondrial L-rRNA and E. coli 23S rRNA indicates a strong conservation of primary and potential secondary structure of both rRNA molecules, except that homologies to the 5'-terminal 5.8S-like region and the 3'-terminal 4.5S-like region of bacterial rRNA are not detectable on mtDNA. The structural organization of the A. nidulans mt L-rRNA gene corresponds to that of yeast ω^+ strains: both genes are interrupted by a large intron sequence (1678 and 1143 bp, respectively) and by another smaller insert (91 and 66 bp) at homologous positions within domain V. An evolutionary tree derived from conserved L-rRNA gene sequences of yeast nuclei, E. coli, maize chloroplasts and six mitochondrial species exhibits a common root of organelle and bacterial sequences separating early from the nuclear branch.

INTRODUCTION

The comparative analysis of L-rRNA (large ribosomal subunit RNA) gene sequences from E. coli (1), maize chloroplast (2) and yeast nuclei (3,4) has led to a general secondary structure model of prokaryotic and eukaryotic L-rRNA containing six more or less conserved domains (5,6). Only few regions of this model are retained in the highly deleted L-rRNA gene sequences of mammalian mitochondria (5,6), and a complete secondary structure model of a mitochondrial L-rRNA has not yet been presented.

In this study we demonstrate that the A. nidulans mitochondria L-rRNA gene sequence (7) is homologous to the E. coli 23S rRNA sequence, and that both L-rRNA molecules can be folded in a very similar way. A phylogenetic tree analysis of a region common to all sequenced L-rRNA genes supports the previous notion (8,9) that fungal and mammalian mitochondrial rRNAs are more related to eubacterial than to nuclear rRNAs.

MATERIALS AND METHODS

The EcoRI fragment E3 containing the split L-rRNA gene (10) was isolated from recombinant plasmid panE3 (11) and sequenced by chemical cleavage methods (7). The alignment of L-rRNA sequences and the phylogenetic tree analysis of

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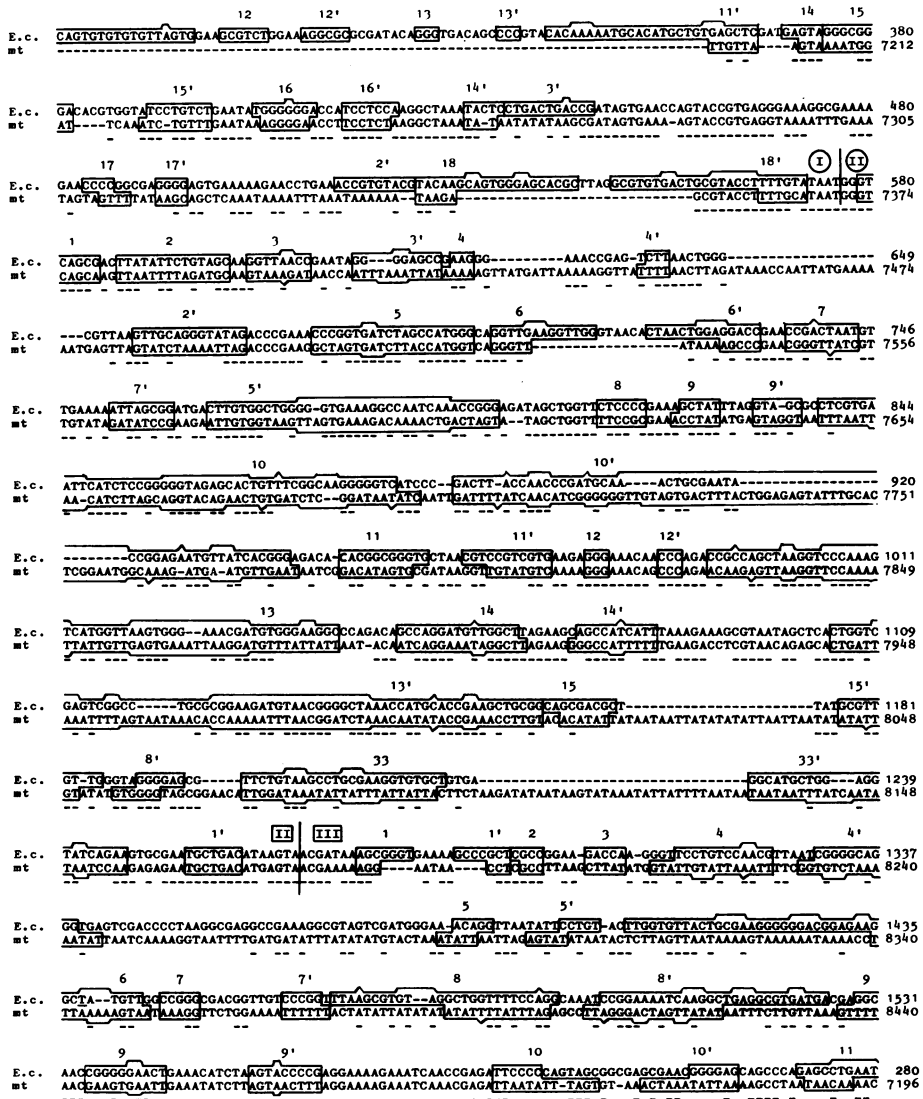
conserved sequences was performed as previously described (12,9).

RESULTS AND DISCUSSION

Fig. 1 shows an alignment of the two exon sequences of the A. nidulans mitochondrial L-rRNA gene (7) with the E. coli 23S rRNA sequence (1). The termini of the mitochondrial sequence are operationally defined by the start and end of sequence homology to the bacterial molecule.



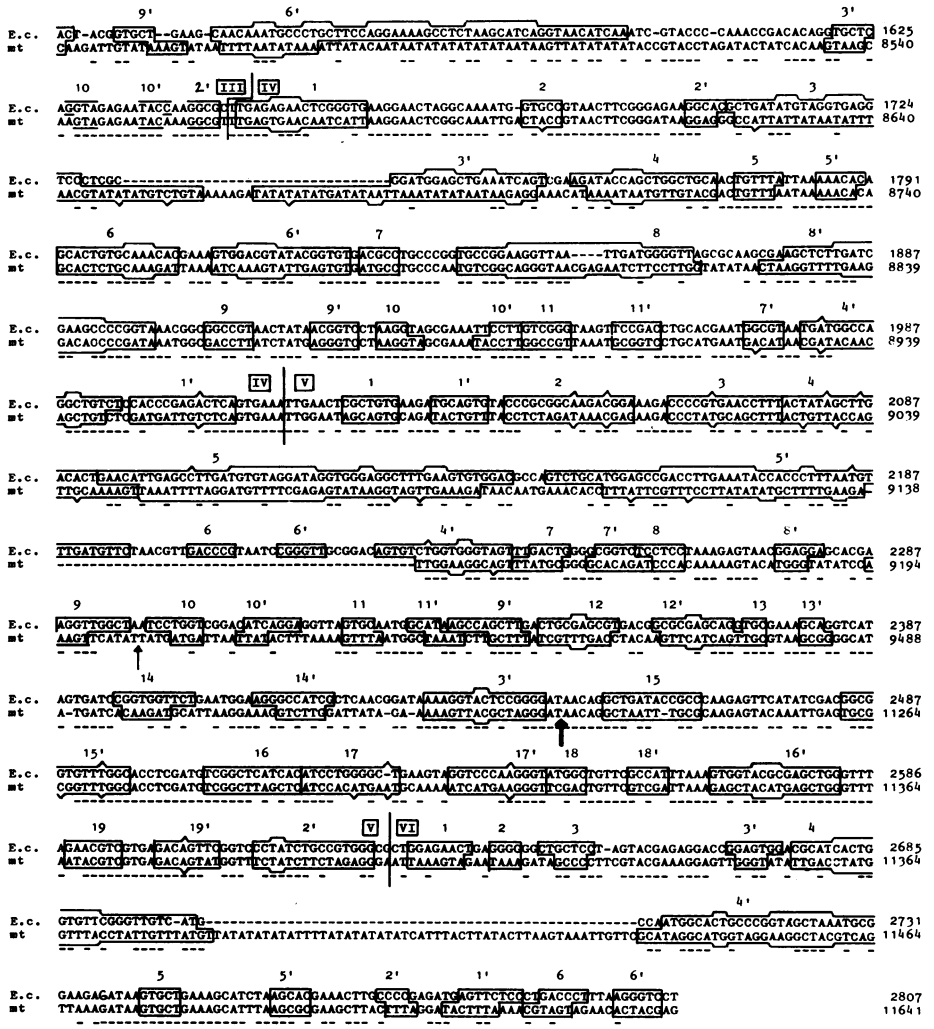


Figure 1. Alignment of the A. nidulans mitochondrial L-rRNA gene sequence (nucleotides 7097 to 9547 of exon I and nucleotides 11226 to 11641 of exon II) (7) with the E. coli 23S rRNA gene sequence (nucleotides 180 to 2807) (1). The boxed regions form base-pairs according to the secondary structure of Noller et al. (6). The six domains of this model are indicated in roman numbers. The thick arrow gives the position of the 1678 b intron (splice point), the thin arrow the position of a 194 b "mini-insert" (14).

No significant homology could be found between the two flanking sequences of the mitochondrial gene (166 bp downstream the proline tRNA gene and 120 bp upstream the threonine tRNA gene) (7) and the two terminal regions of the E.

coli sequences corresponding to the 5.8S and 4.5S rRNA sequences of eukaryotic L-rRNAs (6). It remains to be shown by RNA sequencing whether these two regions are deleted from the mature mt L-rRNA or are replaced by rapidly diverging sequences of reduced functional importance.

The position of the 1678 b intron sequence within domain V (splice point) could accurately be localized by the strong homology between the two flanking exon sequences and nucleotides 2430 to 2520 of E. coli 23S rRNA. A comparison with a corresponding sequence around the splice point of yeast (ω^+) mitochondrial L-rRNA (14) indicates a strong conservation of the splice point in both fungal mitochondria.

The A. nidulans mt L-rRNA sequence exhibits several inserts (21 to 53 nucleotides) relative to the bacterial molecule, most of them in domain II. A longer insert of 194 nucleotides is found at the same position in domain V

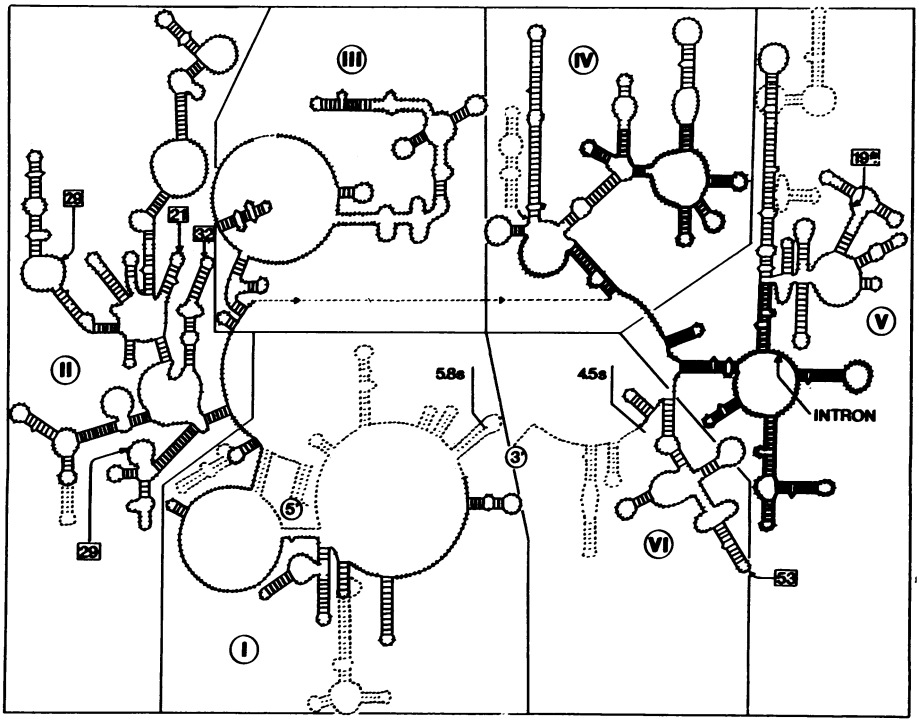


Figure 2. Secondary structure model of A. nidulans mt L-rRNA, based on the alignment of Fig. 1 and the E. coli 23S rRNA model of Noller et al. (6). The dotted parts of the molecule are found only in the bacterial species, the boxed numbers are nucleotides inserted in the mitochondrial species. Heavy lines indicate those parts of domains IV and V which are conserved in all sequenced L-rRNA species, including mammalian mt L-rRNAs (15-17).

(thin arrow of Fig. 1) as the "mini-insert" (66 nucleotides) of mt L-rRNA in ω^+ strains of yeast (14). Possibly this insert represents another intron not detected by electron microscopy of RNA/DNA hybrids (10), since the corresponding yeast mini-insert is absent in ω^- strains, like the large intron (14).

The alignment of Fig. 1 demonstrates a high coincidence of primary and secondary structure homology. The A. nidulans mt L-rRNA molecule deduced from the gene sequence can be folded almost exactly like E. coli 23S rRNA, as shown in Fig. 2.

Only few of the bacterial hairpin structures are deleted or shortened in the mitochondrial species, and one of the bacterial hairpin stems (stem 3 of domain IV) is extended by a mitochondrial insert. The other inserts are of low potential secondary structure and appear to extend hairpin loops or side loops.

The parts of domains IV and V drawn in heavy lines are conserved in all mitochondrial L-rRNAs, and all splice points of L-rRNAs coded by nuclear and mt genes are found in the conserved regions (6).

Fig. 3 shows a compilation of domain V sequences (around the fungal mitochondrial splice point) from nine species, including six mitochondrial sequences. All sequences have the potential to form the stem structures 15 to 19 (see Figs. 1 and 2).

Fig. 4 shows a phylogenetic tree based on the sequence compilation of Fig. 3. The difference matrix indicates that all organelle sequences are more related to the bacterial than to the nuclear sequence. As previously reported for conserved S-rRNA gene regions (9) the eubacterial affinity decreases with the order: chloroplasts (84 % homology to the E. coli sequence), fungal mt

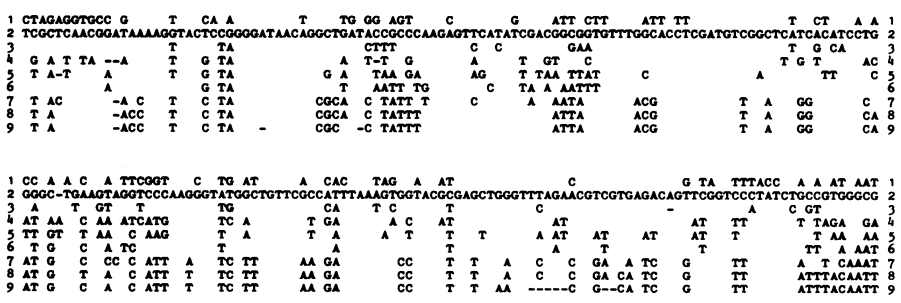


Figure 3. Alignment of nine L-rRNA sequences (3'-terminal part of domains V) from yeast cytosol (nucleotides 2784-2992, ref. 3), (2) E. coli (2418-2627, ref. 1), (3) maize chloroplast (2515-2722, ref. 2), (4) A. nidulans mt (9518-11405, ref. 7), (5) S. cerevisiae mt (-90-118, ref. 14), (6) Paramecium primaurelia mt (1888-2097, ref. 18), (7) human mt (2907-3113, ref. 15), (8) mouse mt (2472-2680, ref. 16) and rat mt (1269-1469, ref. 17). Nucleotides homologous to the E. coli sequence are omitted.

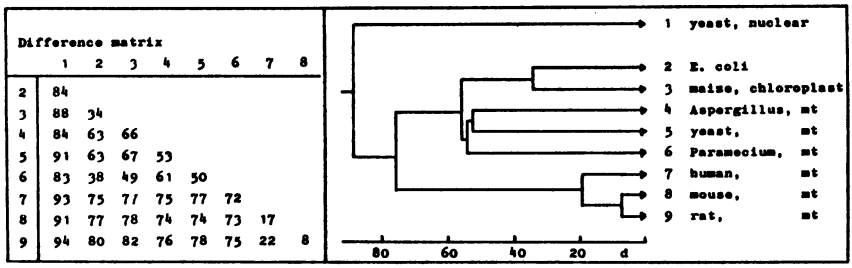


Figure 4. Difference matrix and evolutionary tree derived from L-rRNA gene sequences of Fig. 3. The tree was constructed as previously described (12,9).

(68-70 %) and animal mt (62-64 %). The ratio: bacterial affinity versus nuclear affinity of all organelle sequences is higher for the L-rRNA domain V than for the six conserved S-rRNA domains previously tested (9), indicating a higher functional constraint and a stronger preservation of the ancestral eubacterial L-rRNA domain V in mitochondria.

The compilation also includes a protozoan mt sequence (from Paramecium primaurelia) (18) having a surprisingly high affinity (82 %) to the bacterial sequence (but only 60 % homology to the nuclear one).

Many different tree topologies were tested, but only the topology of Fig. 4 produced increasing difference values for successively constructed nodal points (12). This topology is identical to that of the S-rRNA trees (9): the nuclear sequence diverges first from a common ancestor of organelle and eubacterial sequences. The two groups of fungal and mammalian mt sequences do not exhibit a common root. Instead, the fungal mt protosequence is more related to the bacterial/chloroplastic protosequence than to the mammalian mt protosequence. The protozoan mt sequence is more related to the fungal than to the mammalian mt protosequence.

In contrast to recent claims (19) the eubacterial ancestry of both fungal and animal mitochondria is quite evident from all available sequence data, and any alternative to the endosymbiont hypothesis will be increasingly difficult to reconcile with experimental data.

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