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Nucleotide sequence of 5S ribosomal RNA from Aspergillus nidulans and Neurospora crassa

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ABSTRACT

The nucleotide sequences of 5S rRNA molecules isolated from the cytosol and the mitochondria of the ascomycetes A. nidulans and N. crassa were determined by partial chemical cleavage of 3'-terminally labelled RNA. The sequence identity of the cytosolic and mitochondrial RNA preparations confirms the absence of mitochondrion-specific 5S rRNA in these fungi.

The sequences of the two organisms differ in 35 positions, and each sequence differs from yeast 5S rRNA in 44 positions. Both molecules contain the sequence GCUC in place of GAAC or GAUY found in all other 5S rRNAs, indicating that this region is not universally involved in base-pairing to the invariant GTΨC sequence of tRNAs.

INTRODUCTION

5S rRNA is a component of the large subunit of pro- and eukaryotic ribosomes (1). The relatively conserved primary and secondary structure of this small rRNA (2-4) suggests an important function in protein biosynthesis, and it has indeed been proposed that the invariant GAAC sequence of prokaryotic 5S rRNA interacts with the GTΨC sequence of tRNAs during peptide chain elongation (5).

However, there is increasing evidence that the role of 5S rRNA is not truly universal since mitochondrial ribosomes from N. crassa, yeast and mammalian cells appear to lack a 5S component (6-8). A mammalian mitochondrial 3S RNA believed to be a 5S rRNA equivalent (9) has now been identified as a serine tRNA (10,11). A mitochondrially coded 5S rRNA was claimed to be part of the N. crassa "native" mitochondrial 80S ribosomes (12), although other workers have identified this particle as contaminating cytosolic ribosomes (13).

The present study was undertaken to clear up this matter of discussion by direct sequencing methods. In addition, it was hoped to learn more about the phylogeny of the extensively studied ascomycetes A. nidulans and N. crassa by comparing 5S rRNA sequences.

MATERIALS AND METHODS

Growth of Neurospora crassa (strain Em 5256) and Aspergillus nidulans (strain paba A1, bi A1), and isolation of mitochondria has been described (14,15). Cytosolic and mitochondrial ribosomes were isolated as described (16) but in the presence of 0.5 mg/ml heparin. RNA was extracted from whole mitochondria and from ribosomes according to Edelman et al. (17). 5S rRNA was isolated by preparative polyacrylamide (12 %) gel electrophoresis in the presence of 7 M urea (40 cm x 20 cm x 1 mm slabs) and recovered from gel slices according to Rubin (18).

RNA was labelled at the 5'terminus with adenosine-5'-γ-^{32}P-triphosphate (5 Ci/μM, Amersham) using T4-polynucleotide kinase (New England BioLabs) according to Sugino et al. (19). The 3'-terminal -OH was labelled with cytidine-3',5'[5'-^{32}P]-diphosphate (3 Ci/μM, Amersham), using T4-RNA ligase (PL Biochemicals) according to Peattie (20).

^{32}P-labelled 5'- and 3'-terminal nucleotides were determined by complete digestion with RNase T2 (Sankyo) in 10 mM KH_2PO_4, pH 7, and separation of nucleoside-3'-monophosphates or nucleoside-5', 3'-diphosphates by HPLC chromatography on aminopropylsilica columns (4.6 mm x 250 mm, APS-Hypersil, Shandon Southern, Ltd., packed according to techniques previously described (21)), using 10 mM KH_2PO_4 isocratically for 5 min followed by a linear gradient from 10 mM to 400 mM KH_2PO_4 during 60 min. Digestion mixtures were coinjected with standard nucleotides.

Sequencing of the 3'-terminally labelled 5S rRNA was performed according to Peattie (20).

RESULTS AND DISCUSSION

All experiments described below were performed in parallel with the two ascomycetes A. nidulans and N crassa, and the results were essentially identical, except for nucleotide sequences.

RNA was phenol-extracted from cytosolic and mitochondrial ribosomes, and from whole mitochondria, labelled at the 3' terminal nucleoside with ^{32}pCp using T4 RNA ligase, and resolved by 12 % polyacrylamide gel electrophoresis in the presence of 7 M urea.

The autoradiogram of Fig. 1A shows that mitochondrial ribosomes isolated in the presence of 10 mM $MgCl_2$ contain a strong component comigrating with cytosol 5S rRNA. The same component is present in RNA extracted from whole mitochondria, but considerably reduced after washing mitochondria with EDTA.

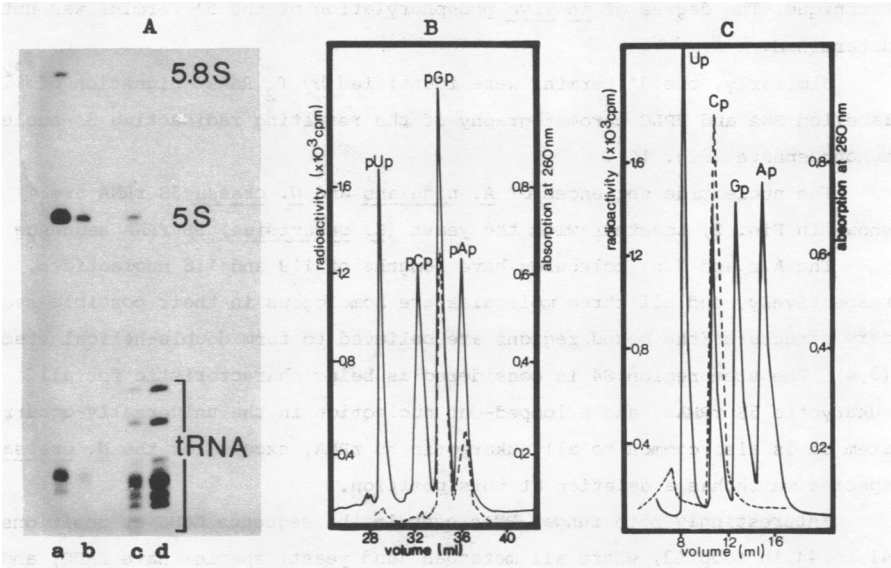

Fig. 1A. Autoradiogram of 3'-terminally labelled N. crassa RNA resolved by 12 % polyacrylamide gel electrophoresis in the presence of 7 M urea. RNA was extracted from cytosolic ribosomes (lane a), from mitochondrial ribosomes isolated in the presence of 10 mM $MgCl_2$ (lane b) and from whole mitochondria isolated in the presene of 10 mM $MgCl_2$ (lane c) or washed with 10 mM EDTA (lane d).
B and C: HPLC chromatography of a RNase T_2 digest of 5'-terminally (B) or 3'-terminally (C) labelled A. nidulans 5S rRNA. For details see Methods.

In order to decide whether this 5S component is mitochondrion-specific or derived from contaminating cytosolic ribosomes, we have determined the nucleotide sequences of 5S rRNA from cytosol and mitochondria, and measured sequence homology to mtDNA by gel transfer hybridization.

The latter experiments (data not shown) clearly excluded a significant homology of both 5S rRNA preparations to mtDNA, in contrast to the 4S (tRNA) fraction of the mitochondrial RNA preparation which strongly hybridized to restriction fragments containing the two mitochondrial tRNA gene clusters (22). The conclusion that the mitochondrial 5S component is derived from contaminating cytosolic ribosomes was further strengthened by the finding, that the nucleotide sequences of 5S rRNAs prepared from cytosol and mitochondria were identical.

Fig. 1B shows the elution profile of the 5' terminal pGp of A. nidulans 5S rRNA. The 5' terminus of N. crassa 5S rRNA was identified as pAp, using the same

technique. The degree of in vivo phosphorylation of the 5' termini was not determined.

Similarly, the 3' termini were identified by T_2 RNAse digestion of ^{32}P-pCp labelled RNA and HPLC chromatography of the resulting radioactive 3'-nucleotide-monophosphate (Fig. 1C).

The nucleotide sequences of A. nidulans and N. crassa 5S rRNA are shown in Fig. 2, together with the yeast (S. cerevisiae) 5S rRNA sequence (23).

The A.n and N.c. molecules have lengths of 119 and 118 nucleotides, respectively, and all three molecules are homologous in their possible secondary structure (the boxed regions are believed to form double-helical stems) (3,4). The stem region S4 is considered as being characteristic for all eukaryotic 5S rRNAs, and a looped-out nucleotide in the universally-occurring stem S5 is also common to all eukaryotic 5S rRNA, except for the N. crassa species which has a deletion at this position.

Interestingly both fungal RNAs contain the sequence GCUC at positions 41 to 44 in loop L3, where all metazoan (and yeast) species have GAUC, and where all plant and bacterial species have GAAC (23). The latter sequence has been suggested to interact with the invariant GTΨC sequence of tRNAs at the A site of prokaryotic ribosomes during peptide chain elongation (5).

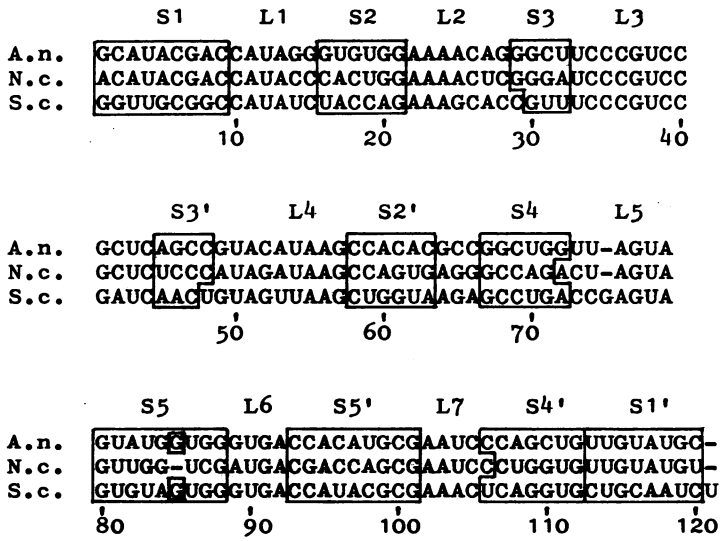

Fig. 2. Nucleotide sequences of 5S rRNA from A. nidulans and N. crassa aligned with that of S. cerevisiae (23). Boxed regions are possibly base-paired (stems S1 to S5).

Whatever the function of eukaryotic 5S rRNA might be, it is obvious that the corresponding sequence GCUC of the two fungal species is not likely to be involved in base-pairing to tRNA sequences.

The two RNA molecules are more related to each other than to all other known 5S rRNA sequences (23). However, they differ in as many as 35 positions (Fig. 2), suggesting an early divergence of euascomycetes into the two groups of plectomycetes and pyrenomycetes, which are represented by A. nidulans and N. crassa, respectively (24).

In contrast, the various petite-positive and petite-negative yeast strains (genera Saccharomyces, Kluyveromyces and Pichia) have diverged much more recently (assuming a similar rate of nucleotide substitution in fungal 5S rRNA genes) because their 5S rRNA sequences are either identical or differ only in few positions (23).

Both eu-ascomycete sequences differ in 44 positions from the S. cerevisiae sequence (and similarly from other proto-ascomycete sequences), and this difference is not much smaller than the average difference between fungal and animal sequences. This suggests that the fungal branch has diverged into proto- and eu-ascomycetes soon after the separation from the animal branch (25).

It might be useful to include data on mitochondrial gene sequences and genome organization patterns in order to study the phylogeny of fungi: the analysis of mitochondrial tRNA genes (22) has already independently established a similar relationship between A. nidulans, N. crassa. and S. cerevisiae, as obtained by the analysis of nuclear-coded 5S rRNAs.

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