THE ORGANIZATION OF GENES IN YEAST MITOCHONDRIAL DNA. I. THE GENES FOR LARGE AND SMALL RIBOSOMAL RNA ARE FAR APART

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SUMMARY

We have determined the position of the two rRNA cistrons on the physical map of the mtDNA of Saccharomyces carlsbergensis obtained with restriction endonucleases. Hybridization of ^{125}I-labelled rRNA with DNA fragments of known location on the map shows that the two rRNA cistrons are at least 25 000 base pairs apart on this DNA of 70 000 base pairs.

INTRODUCTION

Earlier work in this laboratory has shown that yeast mitochondria contain 73S ribosomes, consisting of 50S and 37S subunits, both of which are required for protein synthesis in a sub-mitochondrial system [1,2]. The 21S (mol. wt. 1.3×10^6, see [3]) and 14S (mol. wt 0.7×10^6, see [3]) rRNAs of these ribosomes are coded for by the mtDNA, each 25-μm mtDNA circle (cf. [4]) containing only one cistron for each rRNA [5].

Earlier attempts to detect in yeast mitochondria a precursor RNA containing both rRNAs like the 32S RNA found in Neurospora mitochondria [6], have been unsuccessful [7]. A possible explanation for this negative result was suggested by recent experiments of Faye et al. [8,9]. They studied the hybridization of yeast mitochondrial rRNAs with the deleted mtDNAs of yeast petite mutants and found no linkage at all between the genes for large and small subunit rRNAs.

Although the interpretation of these experiments is not unambiguous, they did raise the possibility that a conventional rRNA precursor is lacking in yeast mitochondria, because the genes for the two rRNAs are far apart on the DNA.

The recent construction of a physical map of yeast mtDNA [10] has allowed us to unambiguously test this possibility. The results of our experiments, which were summarized in part by Dr. L.A.Grivell at the VIIth International Conference on Yeast Genetics and Molecular Biology at Brighton, are presented in this note.

METHODS

Agarose gel electrophoresis of DNA

DNA fragments were electrophoresed through 0.5% agarose slab gels with a buffer containing 0.5 μg ethidium bromide per ml (cf. [11]), as described in detail by Sanders et al. [10]. To detect the bands the gels were placed on an UV lamp (Ultra Violet Products, Inc., San Gabriel, Calif.) and photographed through a Kodak Wratten filter Nr. 16, with a Nikon F camera, equipped with Agfa copex pan rapid film. Exposure times were adjusted to avoid saturation of the film. Suitable negatives were scanned with the aid of a Gilford 2400 spectrophotometer scanner, equipped with a linear transport accessory and a home-made adaptor.

DNA-RNA filter hybridization

Fragments of mtDNA digested by the endonucleases HindII+III or EcoRI were obtained from a 0.5% agarose gel by the freeze-squeeze method, described by Thuring et al. [12]. The DNA fragments were dialysed overnight against 10 mM Tris-HCl, 0.5 mM sodium EDTA (pH 8.0) and an amount equivalent to 1 μg (or 0.3 μg) undigested mtDNA, determined from the fluorescence of the ethidium-DNA complex, was

loaded on nitrocellulose filters (diameter 11 mm) by the procedure of Gillespie and Spiegelman [13], as previously described [14]. These filters, and filters loaded with different amounts of undigested mtDNA and blank filters were incubated together for 16 h at 58°C in 2 ml 0.45 M NaCl, 0.045 M sodium citrate (pH 7.0), 0.1% sodium dodecylsulphate, containing about 70 ng ^{125}I-labelled 14S rRNA (42 000 cpm) or 21S rRNA (54 000 cpm), and 2 µg unlabelled Escherichia coli RNA, added as carrier. After the hybridization the filters were incubated in 10 ml 0.3 M NaCl, 0.03 M sodium citrate (pH 7.0) with 100 µg pancreatic ribonuclease A for 1 h at 25°C, as described by Tabak et al. [15] with 10 µg E. coli RNA added as carrier. Further conditions are mentioned in the legend to Fig. 3.

rRNA isolation and labelling

Yeast mitochondrial ribosomal subunits were prepared as described by Reijnders et al. [5]. RNA was extracted from these subunits by the hot phenol-sodium dodecylsulphate method, as described by Penman [16]. The RNA was labelled in vitro with ^{125}I according to Getz et al. [17]. It was further purified by a methoxy-ethanol extraction and a cetyltrimethylammoniumbromide precipitation, according to Ralph and Bellamy [18].

RESULTS

Fig. 1 shows the separation in agarose gels of the fragments of yeast mtDNA obtained after complete digestion with restriction endonucleases EcoRI (Fig. 1A) and HindII+III (Fig. 1B). Since we (and others) have not succeeded in isolating this DNA intact on a large scale, the endonuclease digestion was done with fragmented DNA, varying in size between about 1/3rd and full-length wild-type DNA. This accounts for the relatively low yield of larger fragments and the

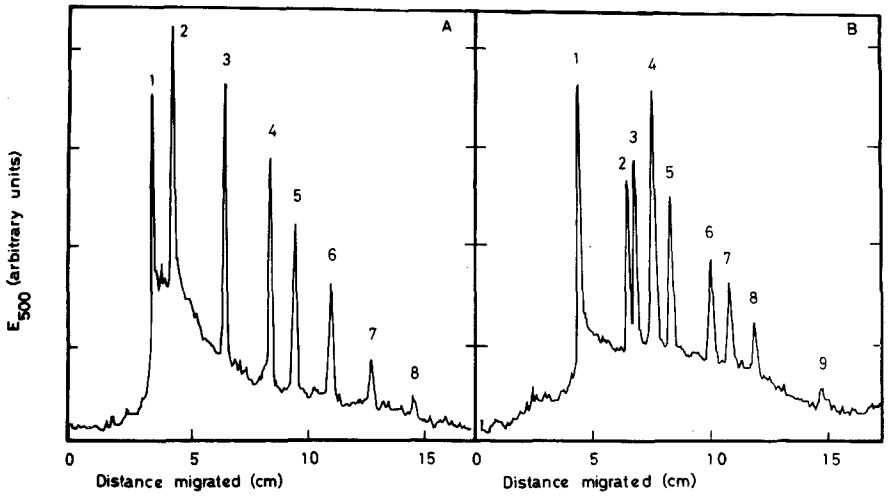

Fig. 1. EcoRI and HindII+III cleavage products of the mtDNA of S. carlsbergensis. Fragments of mtDNA digested by the endonucleases EcoRI or HindII+III were separated on a 0.5% agarose slab gel and the photographs of gels stained with ethidium bromide were scanned as described in Methods. A) EcoRI fragments, R1-R8, the smallest fragment has run off; B) HindII+III fragments, H1-H9.

DNA background migrating between the bands. The physical map constructed with these fragments, shown in Fig. 2, is circular and 23 μm long. The position of the EcoRI fragments R1, R2, R3, R5 and R8 has been determined by two independent methods, the sequence of the other EcoRI and the HindII+III fragments by one method only.

The DNA fragments obtained either with endonuclease EcoRI or HindII+III were isolated from agarose slab gels and hybridized with mitochondrial rRNA as described in Methods. The results of these experiments are shown in Fig. 3 and some additional results comparing hybridization of fragments obtained with restriction enzymes and total mtDNA are given in Table 1.

The RNA from the small subunit predominantly hybridizes with fragments H2 and R2, the RNA from the large subunit RNA with fragments H6 and R1. The significant hybridization of the small subunit RNA with H6 and R1 is clearly due to a minor contamination of this

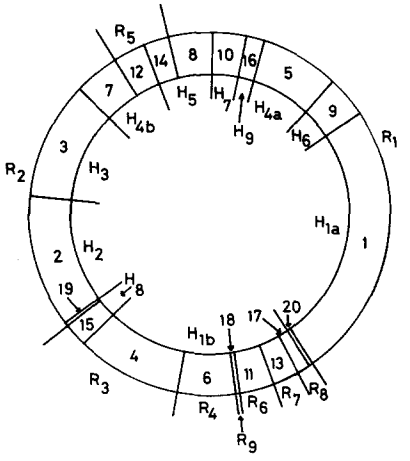

Fig. 2. The physical map of mtDNA of S. carlsbergensis. Outer ring, EcoRI fragments: inner ring, HindII+III fragments: middle ring, fragments obtained with a combination of EcoRI and HindII+III. The construction of the map, using partial digests and double digests, is described in detail by Sanders et al. [10].

RNA with large subunit RNA, because it is competed out with excess cold large subunit RNA (Fig. 3C). We attribute the low hybridization with other fragments either to incomplete separation of DNA fragments that run closely together in the gel (e.g. H2 and H3; R1 and R2) or to the unavoidable contamination of small fragments with pieces of the larger fragments (e.g. R1 and R2 in R3; H2 in H3).

The small subunit RNA hybridizes only with fragments which far exceed its length. A further splitting of fragment H2 with additional restriction enzymes will be required to obtain a more precise location of this RNA on the map. The large subunit RNA hybridizes with a fragment (H6) which is smaller than this RNA: our estimate of the RNA is 3900 bases [3] and of the DNA 2500 base pairs [10]. Nevertheless, no significant hybridization ($>$ 150 base pairs) is observed with any of the neighbouring fragments H4a or H1a. This suggests that either the size of the RNA has been over-estimated, or that of the DNA under-estimated. Further experiments are required to settle this point.

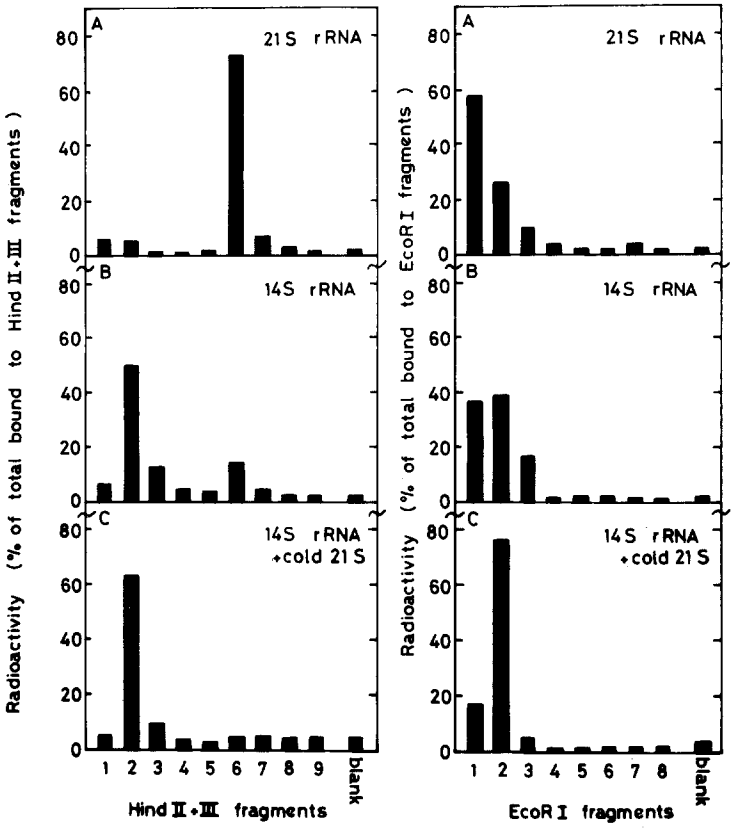

Fig. 3. Hybridization of 21S and 14S rRNA with the HindII+III and EcoRI fragments of yeast mtDNA. DNA-RNA hybridization was carried out as described in Methods. Panels A represent the average values of two hybridizations with 21S rRNA (Expts 1 and 2 in Table 1). Panels B represent the average values of two hybridizations with 14S rRNA (Expts 3 and 4 in Table 1). Panels C represent the hybridization of labelled 14S rRNA in the presence of 1 μg unlabelled 21S rRNA (values between brackets in Expt. 4 in Table 1). The hybridization data are corrected for the binding of ^{125}I-labelled rRNA to blank filters. In all experiments the amount of fragment DNA was equivalent to 1 μg except for one of the hybridizations with 14S rRNA (Expt. 4 in Table I) in which about 0.3 μg was present. Expts 1-3 were done with one batch of HindII+III and EcoRI fragments, Expt. 4 with another batch. The decrease in hybridization of 14S rRNA to R3 in panel C is only apparent, because R3 was contaminated with R2 in the DNA batch used for Expt. 3 and not in the batch used for Expt. 4.

DISCUSSION

Our results establish that the two rRNA cistrons of yeast mtDNA are far apart on the physical map and separated by at least 25 000

TABLE 1: Hybridization of 21S and 14S rRNA with the HindII+III and EcoRI fragments of yeast mtDNA

DNA-RNA filter hybridization was carried out as described in Methods and in the legend to Fig. 3. Summation of the total radioactivity bound to filters containing individual fragments from the results presented in Fig. 3, are given in this table and compared to filters loaded with unfractionated mtDNA. Further details are given in Fig. 3.

DNA on filter	Radioactivity bound (cpm)			
	21S rRNA		14S rRNA	
	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Hind II+III fragments	1250	1425	1120	310 (630)*
EcoRI fragments	2900	2080	1610	230 (730)
Unfractionated mtDNA:				
0.5 µg	780	-	625	116 (244)
1 µg	1465	2540	1229	197 (-)
3 µg	-	4512	-	- (-)
6 µg	-	4912	-	- (-)
Blank filter	19	17	14	17 (18)

* Values between brackets were obtained when 2 µg of unlabelled 21S rRNA was added to the hybridization mixture as a competitor.

base pairs. This is the first example in nature of rRNA cistrons that are not more or less adjacent and it shows that the transcription of both RNAs into a conventional precursor is not essential for the proper processing of rRNA and ribosome assembly. The production of both RNAs might, nevertheless, be coordinated by transcribing all of the mtDNA into one single RNA, which is then processed to yield rRNAs, tRNAs and presumably mRNAs. Evidence that such a mechanism operates in HeLa cell mitochondria, which contain a 5-fold smaller DNA than yeast, has been presented by Aloni and Attardi [19,20]. Experiments to identify the primary transcription products in yeast mitochondria are in progress.

A further fine-structure analysis of the cistron for the large rRNA should allow a critical test of our previous suggestion [21]

that the resistance to antibiotics that block mitochondrial protein synthesis, is due to a change in the primary structure of the mitochondrial rRNA. This suggestion is now supported by strong, indirect evidence: the resistance to antibiotics is also found in isolated ribosomes [2,22,23]; there is no evidence for a change in ribosomal protein [22]; the rRNA is minimally modified and contains no base methyl groups [23-25], making changes in methylation, an unlikely explanation for the many antibiotic-resistant phenotypes observed [22]; and in petite mutants there is a strong link between the erythromycin resistance marker and the presence of part or all of the large subunit rRNA cistron [9]. By fine-structure mapping of mtDNAs from petite mutants, containing the E and C markers, it may be possible to obtain a small fragment of the rRNA gene containing one of these markers. By fingerprinting the mutant and wild-type rRNA piece, protected by hybridization with this DNA fragment, even a point mutation in the rRNA should be detectable. Experiments along this line are in progress.

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