PHYSICAL MAPPING OF MITOCHONDRIA rRNA GENES IN *Saccharomyces cerevisiae*

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SUMMARY

We have established previously a physical map of mitochondrial genetic markers for antibiotic-resistance in *Saccharomyces cerevisiae* (1). Using physically and genetically characterised cytoplasmic petite mutants we now physically map the mitochondrial ribosomal RNA (mt-rRNA) genes on the circular grande mitochondrial genome defined to contain 100 units of mtDNA. The gene for 15S-rRNA maps within 13 DNA-units near the *par1* locus and the gene for 21S-rRNA lies within 15 DNA-units between *cap1* and *oli1* close to the *ery1* locus. The minimum distance between the two rRNA genes is at least 27 units.

INTRODUCTION

Molloy et al. (2) have genetically mapped five antibiotic-resistance markers (*cap1, ery1, oli1, mik1, par1*) on a circular mitochondrial genome by scoring the frequency of co-retention of the markers in cytoplasmic petite mutants which are mtDNA deletion mutants. A good correlation between this map and the physical map obtained by DNA-DNA hybridization for the same markers was observed by Sriprakash et al. (1). Recently Sanders et al. (3) concluded by hybridizing the mt-rRNAs to mtDNA fragments obtained after restriction endonuclease treatment of mtDNA that the genes for the two rRNAs are at least a quarter of genome apart in *Saccharomyces carlsbergensis*. In the present communication, we position the genes for the two rRNAs relative to the other markers using a set of well defined petite strains which were characterised for both the antibiotic-resistance markers and the fraction of the grande mtDNA they retain as well as for the

amount of sequence overlaps between the various petite mtDNAs (1). The technique described in this paper can be used as a general one for mapping other genes.

MATERIALS AND METHODS

Strains: All the *Saccharomyces cerevisiae* cytoplasmic petite mutant strains employed in the present study except for strain 987-19 were spontaneous isolates and were described earlier (1). The petite 987-19 was derived after a brief ethidium bromide treatment of a grande strain carrying the resistance markers *cap1 ery1 oli1 mik1* and *par1*. the retention and loss of the mitochondrial markers in the petite strains used are shown in Table 1. The genetic stability of the petites (i.e. the percent of subclones that retained all the mitochondrial markers present in the parent petites) was greater than 80% (cf. Sriprakash et al. (1)) except for Y8 and 987-19 which showed a genetic stability of 15% and 70% respectively. Y8 and other spontaneously arising petites which retain the *ery1, cap1, par1* loci are intrinsically unstable; even on repeated subcloning no highly stable petites containing *ery1, cap1, par1* were obtained.

Extraction and purification of nucleic acids: DNA from whole cells was extracted as described earlier (1). The rRNAs were prepared from the mitochondrial ribosomes isolated from the grande strains (4) and purified according to Forrester et al.(5).

Labelling the rRNA with ^{125}I: The preparation of [^{125}I] rRNA was similar to that described by Getz et al. (6) with several modifications. The reaction mixture (0.2 ml) contained 0.1M sodium acetate (pH 4.8); 25 µM KI; 500 µCi ^{125}I (The Radiochemical Centre Ltd., Amersham); 40-50 µg rRNA and 2 mM thallium chloride (K and K Laboratories Inc., Plainview, N.Y.). Both KI and thallium chloride solutions were made freshly, and used within a minute. The reaction mixture was assembled in the cold; RNA and thallium chloride were added last and the mixture was immediately heated to 60° and held at that temperature for 10 min. The reaction mixture was chilled, Tris-HCl (pH 9.0) was added to a final concentration of 0.4 M and the solution was reheated to 60° for 10 min, recooled and then mixed with 150 µg of whole rat liver RNA. The labelled mt-rRNA was purified by passing the whole mixture through a Sephadex G25 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (8 x 200 mm) supported on a pad of 10 glass filters (GF/C, Whatman). The Sephadex was presaturated by running about 150 µg of whole rat liver through the column. The glass filters remove contaminating materials which are likely to give rise to high background counts in the hybridization experiments (7). Approximately 6% of the radioactivity in the reaction mixture was recovered in the column's excluded volume which contained [^{125}I]rRNA. The [^{125}I]rRNA was further purified by adsorption to a column (1.5 ml) of hydroxylapatite (Bio-Rad. Laboratories, Cal.). The column was eluted with 9 ml of 10 mM potassium phosphate (pH 6.8) followed by 4.5 ml of 250 mM potassium phosphate (pH 6.8).

The labelled rRNA which elutes in the 250 mM potassium phosphate solution was treated with 50 µg/ml of RNase-free Pronase

TABLE 1: Hybridization of 15S- and 21S-rRNA to mtDNA of different genetically marked strains.

Strain	Mitochondrial markers					Relative hybridization	
	cap1	*ery1*	*oli1*	*mik1*	*par1*	15S-rRNA	21S-rRNA
Grande							
770-7B	+	+	+	+	+	100	100
863-2C	+	+	+	+	+	100	100
Petite							
U3	-	-	-	+	-	0	0
U4	+	+	+	+	-	0	100
U5.1	+	+	+	-	-	0	85
U5.2	+	+	-	-	-	6	94
U5.3	-	+	+	-	-	10	170
U6	+	+	-	-	-	0	124
Y1.2	-	-	+	+	-	0	4
Y1.3	-	-	+	+	+	193	30
Y1.4	-	-	-	+	+	220	18
Y2	-	-	+	+	-	13	8
Y6	-	-	-	-	+	243	22
Y9	-	-	-	+	-	0	18
Y8	+	+	-	-	+	346	98
987-19	+	+	-	-	+	430	170

The mitochondrial genetic markers were determined as described by Molloy et al. (2). + denotes retention of the locus; - denotes loss of the locus. The hybridization procedure was as described in the Methods except for Y8 and 987-19 in which the amount of mtDNA was 1 µg and 0.7 µg respectively.

In the controls in which no DNA was added 0.2% of the input counts was retained on the filter, while the controls with 2 µg of DNA from a *rho-o* strain which lacks mtDNA bound 1% of the input counts. The hybridization values are expressed as percentages relative to the counts hybridized to the grande mtDNA less the correction for the counts bound in the *rho-o* controls. The mtDNA from the grandes hybridized about 300 and 500 cpm above the controls for 15S- and 21S-rRNA respectively.

(Pronase was preincubated at 500 µg/ml for 1 hr at 37°) for 30 min at room temperature. The solution was deproteinised by extracting thrice with an equal volume of a mixture of 1 volume of phenol (presaturated in 2 x SSC) and 1 volume of chloroform-isoamylalcohol (99:1, v/v). The rRNA was precipitated from the aqueous phase with absolute ethanol in the presence of 2% potassium acetate as described earlier (6); the precipitated [^{125}I]rRNA was finally dissolved in 2 x SSC.

RNA-DNA hybridization: The hybridization mixture (0.2 ml) contained 0.2 - 0.25 µg, unless otherwise stated, of denatured mtDNA and 0.1 - 0.2 µg of [^{125}I]rRNA in 30% formamide and 2 x SSC. After incubation for 2 hr at 37° and subsequent digestion with RNase for 1 hr at room temperature the RNA-DNA hybrids were collected on nitrocellulose filters as described by Nagley *et al.* (4). The radioactivity was measured in a Philips Automatic Gamma Analyser. The input radioactivity in hybridization mixtures was 10^4 cpm for 21S-[^{125}I]rRNA and was 2 x 10^4 cpm for 15S-[^{125}I]-rRNA.

RESULTS

Table 1 summarises the results of the hybridization of various DNA preparations with 15S-rRNA and 21S-rRNA. The RNA preparations bound only to the extent of 1% of the input radioactivity to 2 µg of nuclear DNA (Table 1 legend) indicating the absence of contamination of the mitochondrial rRNA by cytoplasmic RNA. It was also observed that even in the presence of a ten-fold excess of nuclear DNA the hybridization of mitochondrial RNA to the mtDNA was not affected (data not shown). The whole-cell DNA from two grande strains hybridized to both the rRNA species (Table 1). Moreover, the counts hybridized to the DNA from the grande strains were proportional to the amount of mtDNA up to 0.5 µg (data not shown). These observations established that there was a specific interaction between the mtDNA and the [^{125}I]rRNAs.

A set of genetically and physically characterised petites were selected and the abilities of their mtDNAs to hybridize to the rRNAs were compared to those of grande mtDNAs (Table 1). It is evident from the results that 15S-rRNA hybridized only to those petites that have retained the *par1* locus and thus the 15S-rRNA gene is close to *par1*. The 21S-rRNA hybridized to all the petites that had retained the *ery1* locus confirming previous observations (4, 8, 9). Some limited hybridization was also observed in strains Y1.3, Y1.4, Y6 and Y9 which do not contain the *ery1* locus. However, these observations do not vitiate

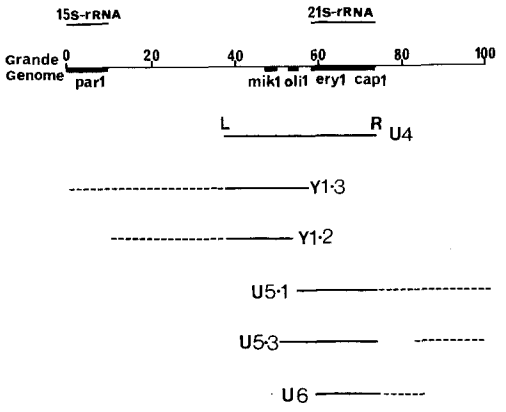

Figure 1: Mapping of 15S and 21S mitochondrial rRNA genes.

A linear representation of the circular mitochondrial genome of *S. cerevisiae* showing the established relative positions of the five antibiotic-resistance markers (1) and the map positions of 15S- and 21S-rRNA genes. The full lines and the dotted lines represent respectively, the homologous and non-homologous sequences to the U4 mitochondrial genome. L and R correspond to left and right ends of U4 genome arbitrarily defined for convenience. The lengths of the genomes are drawn to scale. Grande mtDNA contains 100 units by definition (1).

the results as among several possibilities, the retention of a segment of the 21S-rRNA gene not containing the *eryl* locus could account for the limited hybridization observed in these strains.

As established by Sriprakash et al. (1), Figure 1 depicts the antibiotic markers retained in the petites, the fraction of the grande mtDNA sequences they retain and the DNA sequence overlaps between particular petite genomes. The petite U4 which retains four antibiotic-resistance markers and corresponds to 36 units of the grande mtDNA was chosen as our reference petite. The mtDNA of Y1.3 and Y1.2 extend respectively 40 and 27 units to the left end of the U4 genome (Fig. 1); while 15S-rRNA hybridized to Y1.3, it did not hybridize to Y1.2. Therefore, the gene for 15S-rRNA is within the 13 units near the mapped *par1* locus which is present in the Y1.3 genome but not in the Y1.2 genome. The results obtained in the study of the other petite genomes are consistent with this mapping.

The occurrence of petites which retain both the large and small rRNA genes together in the mitochondrial genomes is rare;

to date there is only one such petite strain reported (10). We have tested the ability of mtDNA from petites which retain the *eryl, capl* and *parl* loci to hybridize to the two rRNA species, and as would now be predicted these petites retained both the genes (Table 1). However, the spontaneous petites which retain *eryl, capl* and *parl* loci are genetically unstable (see Materials and Methods). On the other hand, the strain 987-19, which was derived after mild ethidium bromide treatment, has a moderate stability; perhaps a regions reponsible for the instability of the spontaneous petites retaining the same loci was deleted in the strain 987-19. In order to compensate for the low stability of the strains, we used large amounts of mtDNA from these petites in the hybridization mixtures (Table 1 legend).

DISCUSSION

The genes for large and small rRNAs in the mitochondrial genomes of *Neurospora* and HeLa cells are close to each other (11,12). In contrast, we estimate from this work and the physical map of yeast mitochondrial genome that the minimum distance between the genes for the two rRNAs is 27 units in *S. cerevisiae*. A similar extent of separation of these two genes was observed recently in *S. carlsbergensis* (3). These findings pose some intriguing questions on the mode of regulation of the synthesis of mitochondrial rRNAs.

We further conclude from our studies that the gene for 15S-rRNA is encompassed within 13 units which include the *parl* locus and the gene for 21S-rRNA is encompassed within 15 units which include the *eryl* locus. The mapping of 21S-rRNA gene is consistent with the earlier findings of several laboratories (4, 8, 9).

Whether *eryl* and *parl* loci are part of 21S-rRNA and 15S-rRNA genes respectively is yet an open question. Mutants that carry *eryl-r* locus are also cross resistant to spiramycin, carbomycin and lincomycin (13). The ribosomes isolated from grande strains carrying the *eryl-r* locus have also been shown to be resistant to these three antibiotics (14). Although the resistance to erythromycin is due to a change in the mitochondrial ribosome (15), the altered ribosomal component is yet to be definitively identified. We are pursuing the effect(s) of *parl* mutation on the ribosomes.

REFERENCES

1. Sriprakash, K.S., Molloy, P.L., Nagley, P., Lukins, H.B. and Linnane, A.W. (1976) Manuscript submitted for publication.

2. Molloy, P.L., Linnane, A.W. and Lukins, H.B. (1975) J. Bact. 122, 7-18.

3. Sanders, J.P.M., Heyting, C. and Borst, P. (1975) Biochem. Biophys. Res. Commun. 65, 699-707.

4. Nagley, P., Molloy, P.L., Lukins, H.B. and Linnane, A.W. (1974) Biochem. Biophys. Res. Commun. 57, 232-239.

5. Forrester, I.T., Nagley, P. and Linnane, A.W. (1970) FEBS Letters 11, 59-61.

6. Getz, M.J., Altenberg, L.C. and Saunders, G.F. (1972) Biochem. Biophys. Acta 287, 485-595.

7. Scherberg, N.H. and Refetoff, S. (1974) J. Biol. Chem. 249, 2143-2150.

8. Faye, G., Fukuhara, H., Grandchamp, C., Lazowska, J., Michel, F., Casey, J., Getz, G.S., Locker, J., Rabinowitz, M.,Bolotin-Fukuhara, M., Coen, D., Deutsch, J., Dujon, B., Netter, P. and Slonimski, P.P. (1973) Biochemie 55, 779-792.

9. Faye, G., Kujawa, C. and Fukuhara, H. (1974) J. Mol. Biol. 88, 185-203.

10. Nagley, P., Molloy, P.L., Lukins, H.B. and Linnane, A.W. (1975) In "The Eukaryote Chromosome" (W.J. Peacock and R.D. Brock, eds.) pp 155-167, A.N.U. Press, Canberra.

11. Kuriyama, Y. and Luck, D.J.L. (1973) J.Mol.Biol. 73, 425-437.

12. Robberson, D., Aloni, Y., Attardi, G. and Davidson, N. (1972) J. Mol. Biol. 64, 313-317.

13. Trembath, M.K., Bunn, C.L., Lukins, H.B. and Linnane, A.W. (1973) Mol. Gen. Genet. 121, 35-48.

14. Spithill, T.W. and Maxwell, R.J. (1975) Proc. Aust. Biochem. Soc. 8, 67. Also personal communication.

15. Grivell, L.A., Reijnders, L. and de Vries, H. (1971) FEBS Letters 16, 159-163.