MAPPING OF THE MITOCHONDRIAL 16S RIBOSOMAL RNA GENE AND ITS EXPRESSION IN THE CYTOPLASMIC PETITE MUTANTS OF *SACCHAROMYCES CEREVISIAE*.

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Abstract. The 16S ribosomal RNA gene of yeast mitochondria was titrated in various cytoplasmic petite mutants by DNA-RNA hybridization. The gene was located close to the prolyl transfer RNA gene. The properties of the rho⁻ strains suggest that the gene order would be : - P_I - 16S - prolyl tRNA - valyl tRNA - (tRNAs) - R_I - R_{III} - ; the 23S ribosomal gene is far from the 16S one. Several petite mutants were found which have retained, in addition to many transfer RNA genes, both of the 23S and 16S ribosomal RNA genes. The two genes seem to be transcribed in these mutants.

The mitochondrial DNA (mtDNA) of *Saccharomyces cerevisiae* contains one copy each of 23S and 16S ribosomal RNA (rRNA) genes (see ref. 1). The genome codes also for twenty to twenty five 4S RNA genes (2,3) among which 19 individual transfer RNA (tRNA) genes have been identified (4, N. Hinckley and M. Rabinowitz, unpublished observations).

Five mitochondrial genetic loci, R_I, R_{III}, O_I, O_{II} and P_I, conferring resistance to chloramphenicol, erythromycin, oligomycin and paromomycin, have been extensively studied (see 5,6,7). These loci can be lost or retained in various combinations in cytoplasmic petite mutants (rho⁻) as a result of large deletions of mtDNA (see 15). Many of the mutant genomes appear to arise from the loss of a single continuous segment of the wild type DNA, though some of them result from several non-contiguous deletions (9,10,11). Using these deletion mutants, it has been shown that the 23S rRNA gene is very closely associated to the R_I-R_{III} region (8), while the 16S rRNA gene lies in the vicinity of the P_I locus (9). By a similar approach, we have localized (10) twelve mitochondrial tRNA genes, among which prolyl and valyl tRNA genes have been mapped in the region of P_I. In the present report, we examined the order of 16S rRNA, prolyl tRNA and valyl tRNA genes with respect to the P_I locus.

MATERIALS AND METHODS

Petite deletion mutants of various configurations are listed in Table 1. They were obtained by mutagenesis of the wild type (rho⁺) strains

Table 1

Strains	Short names	Mitochondrial markers* rho	R_I	R_{III}	P_I	O_I	O_{II}	tRNA gene* prolyl	valyl	Hybridization of 16S rRNA (α)**
MH41-7B/R12	CEP 1	-	R	R	R	0	0	+	+	102
MH41-7B/AB83	CEP 2	-	R	R	R	0	0	+	+	133
TR3-15A/F857	CEP 6	-	R	R	R	0	0	+	+	54***
TR3-15A/F5262	CEP 7	-	R	R	R	0	0	+	+	98
MH32-12D/J4762	CEP 4	-	R	R	R	0	0	0	0	2
MH41-7B/AC219	CEP 3	-	R	R	R	0	0	+	0	100
TR3-15A/F218	CEP5	-	R	R	R	0	0	+	0	51***
MH41-7B/P31	P 3	-	0	0	R	0	S	+	+	79***
MH41-7B/P11	P 1	-	0	0	R	0	0	+	+	121
TR3-15A/F5262/P27	P 27	-	0	0	R	0	0	+	+	111***
MH41-7B/R12/P22	P 22	-	0	0	R	0	0	+	0	84***
MH41-7B/P21	P 2	-	0	0	R	0	0	0	0	72***
MH32-12D/H241	P 4	-	0	0	R	S	0	0	0	0.5
MH32-12D/L434	P 5	-	0	0	R	0	0	0	0	3
MH32-12D/C25	P 7	-	0	0	R	0	0	0	0	2
MH41-7B/AB83/P25	P 25	-	0	0	R	0	0	0	0	0.1
MH41-7B/L661	0_IP 1	-	0	0	R	R	0	+	+	110
MH41-7B/L721	0_IP 2	-	0	0	R	R	S	+	+	91
MH41-7B		+	R	R	R	R	S	+	+	100
IL8-8C		+	R	R	S	S	S	+	+	100

*Data from Fukuhara et al. (10) ; **The hybridization per cent (α) is determined as described in text ; ***Average of two experiments.

labelled with several drug resistance markers : C^R (locus R_I), E^R (locus R_{III}), 0^R (locus O_I or O_{II}) and P^R (locus P_I). Detailed description of the genotypes is to be found in Fukuhara et al. (10).

Isolation of mtDNA, ^{3}H-labelled 16S rRNA has been described previously (8). DNA-RNA filter hybridization was performed in 8M urea, 0.3 M NaCl and 0.03 M trisodium citrate at 37°C for 48 hours (8). Purity and molecular size of mitochondrial RNA were analyzed by polyacrylamide gel electrophoresis (8).

RESULTS AND DISCUSSION

1) Mapping of the 16S rRNA gene

Eighteen rho$^-$ clones were examined (Table 1). All of the clones contain the P_I locus. The clones CEP-1, 2, 6 and 7 are known to contain eleven

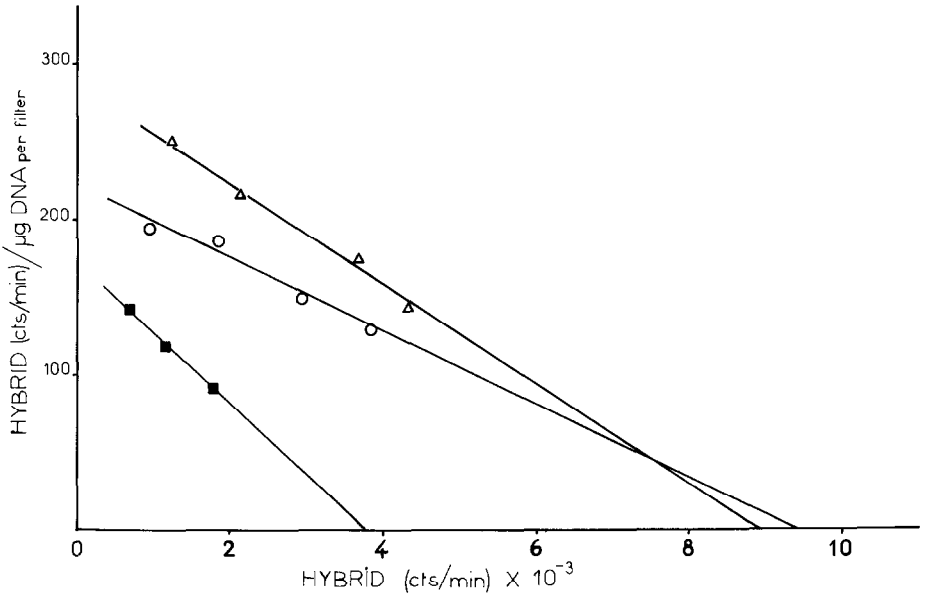

Figure 1. Hybridization of ^{3}H-16S rRNA to the mtDNA of the petite clone CEP7 (△——△), P 27 (○——○) and CEP6(■——■). Data were expressed in Scatchard plot. See text.

identified tRNA genes, while CEP-3, 4 and 5 have lost, probably by a secondary deletion, several of the tRNA genes (10). Other clones contain the loci O_I and O_{II} in different combinations.

A constant amount (about 0.3 µg/ml) of mitochondrial ^{3}H-16S rRNA from the rho^{+} strain IL8-8C was incubated with increasing amounts of filter-bound mtDNA of each rho^{-} clone (5, 10, 20 or 30 µg per filter). The hybridization data were analyzed by using a Scatchard plot (13), i.e., the hybrid counts on the abscissa and the hybrid counts per µg DNA on the ordinate. The straight lines obtained were extrapolated onto the abscissa to evaluate "α" (see ref. 8), that is, the survival fraction of the sequence of the 16S rRNA gene (for rho^{+}, α= 100 % ; for rho^{-}, $0 \leq \alpha \leq 100$ %). Fig. 1 illustrates some examples.

The results of hybridization are shown in Table 1, last column. The hybridization levels, α, of various rho^{-} mtDNA can be grouped into three classes : (a) 133 - 72 %, (b) 54 - 51 % and (c) 3 - 0.1 %. The high levels indicate that the mtDNA contains a large part, or more likely all, of the 16S rRNA gene sequence. The two intermediate values, 54 and 51 %, may mean that the clones have kept only a part of the 16S rRNA gene, and the low levels indicate that the sequence has been deleted.

The results summarized in Table 2 show the frequency of association of

Table 2

P_I	16S rRNA	prolyl tRNA	valyl tRNA	Number of observed clones
I	I	I	I	8
I	I*	I	I	1
I	I	I	0	2
I	I*	I	0	1
I	I	0	0	1
I	0	0	0	5

I : the sequence is present.
I*: the sequence is present in part (for 16S rRNA).
0 : the sequence is absent.

different genes. We may try to order the four markers : P_I, 16S rRNA, prolyl and valyl tRNA genes by using the disjunction coefficients (18) for the six pairwise associations between these four markers. (The more distant two markers are, the greater the disjunction coefficient).

It is apparent from Table 3 that the three genes of 16S rRNA, prolyl tRNA and valyl tRNA are associated to each other and the P_I marker is outside the mtDNA segment defined by the three preceding genes.

The choice between the six possible arrangements :

P_I, 16S, Pro, Val P_I, Pro, Val, 16S P_I, Pro, 16S, Val
P_I, Val, 16S, Pro P_I, 16S, Val, Pro P_I, Val, Pro, 16S

may be made by the following arguments : (a) first we will suppose that single continuous deletions are more frequent than multiple deletions, (b) when the P_I marker and the valyl tRNA gene are retained, the prolyl tRNA gene and 16S rRNA gene are always present, (c) when the P_I marker and the prolyl tRNA gene are present, the 16S rRNA gene is always detected while the valyl tRNA gene is not always found, in particular in the strain P22 that we may suppose to result from a single deletion , (d) a petite clone (D21) is known in which the R_I-R_{III} region is present together with the prolyl and valyl tRNA genes without retaining the P_I marker and the 16S rRNA gene (17, 8, 10). These considerations lead to the conclusion that the arrangement P_I, 16S, Pro, Val is the most likely order.

It should be noted however that this arrangement does not readily explain why the clones CEP 5 and 6 show a partial hybridization level with 16S rRNA. Several interpretations are possible. For instance, if the 16S RNA

Table 3

Disjunction coefficients (DC)

16S rRNA	prolyl tRNA	valyl tRNA	
1.00	1.00	1.00	P_I
	0.72	0.72	16S rRNA
		0.66	prolyl tRNA

The disjunction coefficients (18) were calculated from the data of Table 2 with the formula :

$$DC = \frac{X}{N} \cdot \frac{Y}{N} \Big/ \frac{XY}{N}$$

X : number of rho$^-$ carrying the X marker
Y : number of rho$^-$ carrying the Y marker
XY : number of rho$^-$ carrying X and Y
N : total number of clones (N = 18)

gene sequence is engaged in a local palindromic structure (cf. 14), the titration may not reach completion. Alternatively, a small secondary deletion may have occurred between P_I and prolyl tRNA gene.

The four markers of the P_I region may be oriented with respect to the R_I-R_{III} segment (23S rRNA gene region) by using the data of Fukuhara et al. (10) (and the above argument (d)) who assigned the positions of most tRNA genes between the P_I and R_I loci : --- P_I - 16S rRNA - prolyl tRNA - valyl tRNA ----- (tRNAs) ---------- R_I - (23S rRNA, R_{III}) ----. The two rRNA genes appear to be separated by a series of tRNA genes.

2) Transcription of the 16S rRNA gene in petite mutants.

We have previously shown that the petite mtDNA retaining the R_I-R_{III} region (the two linked loci) contain the 23S rRNA gene and transcribe it into 23S rRNA. This was confirmed with the petites presently studied (see later). On the other hand, some of the petites carrying the P_I locus have been found to contain the 16S rRNA gene (9). This was also confirmed by the above hybridization experiments. The petite mutants which retain both of the R_I-R_{III}

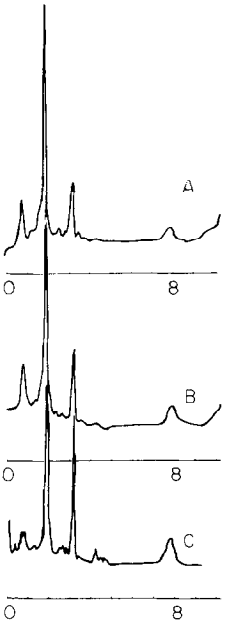

Figure 2. Electrophoretic patterns of mitochondrial RNAs of the clone CEP 1 (A), CEP 2 (B) and the wild type (C). The flow was left to right. The peaks were detected by ultraviolet scanning.

region and P_I region are thus expected to possess, with high frequency, both of the two rRNA genes. The petites CEP's in Table 1 are such multimarker mutants. We studied the transcription *in vivo* of the rRNA genes in some of these clones.

When RNA was extracted from the purified mitochondria of the CEP petites, we found two major components migrating in electrophoresis at the exact positions of 23S and 16S rRNA (Fig. 2). Their molar ratio was however 1 : 0.57 , that is, different from the 1 : 1 ratio usually found in the wild type mitochondrial RNA. The deficit of the 16S component may mean that the efficiency of transcription is different for the two genes in these modified mitochondrial genomes. Alternatively, the two genes may be differently amplified in these mutants (cf. 15).

A close inspection of the 16S peak in the electrophoregrams suggests the presence of two components of very similar molecular weights, a fact that we had noticed previously in the petites carrying the 16S rRNA gene (9). The suggested second component may be either a product of abnormal maturation of 16S rRNA or an RNA of non-ribosomal origin.

Figure 2 also reveals the presence of 4S RNA in these mitochondria.

This is consistent with the fact that these mutants have retained many tRNA genes (10) and some have been shown to transcribe functional tRNAs (16).

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References

1. Borst, P. and Grivell, L.A. (1971) FEBS Letters 13, 73-88.
2. Schneller,J.M., Faye, G., Kujawa, C. and Stahl, A.J.C. (1975) Nucleic Acids Res. 2, 831-838.
3. Reijnders, L. and Borst, P. (1972) Biochem. Biophys. Res. Commun. 47, 126-133.
4. Casey, J.W., Hsu, H.J., Getz, G.S., Rabinowitz, M. and Fukuhara, H. (1974) J. Mol. Biol. 88, 735-747.
5. Avner, P.R., Coen, D., Dujon, B. and Slonimski, P. (1973) Mol. Gen. Genetics 125, 9-52.
6. Wolf, K., Dujon, B. and Slonimski, P. (1973) Mol. Gen. Genetics, 125, 53-90.
7. Dujon, B., Slonimski, P.P. and Weill, L. (1974) Genetics, 78, 415-437.
8. Faye, G., Kujawa, C., and Fukuhara, H. (1974) J. Mol. Biol. 88, 185-203.
9. Faye, G., Kujawa, C., Dujon, B., Bolotin-Fukuhara, M., Wolf, K., Fukuhara,H. and Slonimski, P.P. (1975). J. Mol. Biol. (in press).
10. Fukuhara, H., Hsu, H.J., and Rabinowitz, M. (1975) Submitted for publication.
11. Morimoto, R., Lewin, A., Hsu, H.J., Rabinowitz, M. and Fukuhara, H. (1975) Proc. Natl Acad. Sci., USA (in press).
12. Nagley, P., Molloy, P.L., Lukins, H.B. and Linnane, A.W. (1974). Biochem. Biophys. Res. Commun. 57, 232-239.
13. Marsh, J.L., and McCarthy, B.J. (1973) Biochem. Biophys. Res. Commun. 55, 805-811.
14. Locker, J., Rabinowitz, M. and Getz, G.S. (1974). Proc. Natl Acad. Sci. USA, 71, 1366-1370.
15. 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). Biochimie, 55, 779-792.
16. Hinckley, N., Rabinowitz, M., and Fukuhara, H. (1975). J. Mol. Biol., submitted for publication.
17. Casey, J.W., Hsu, H.J., Rabinowitz, M., Getz, G.S. and Fukuhara, H. (1974) J. Mol. Biol. 88, 717-733.
18. Slonimski, P.P. and Tzagoloff, A. (1975) Europ. J. Biochemistry (in press)