INFORMATIONAL CONTENT OF MITOCHONDRIAL DNA FROM A "LOW DENSITY" PETITE MUTANT OF YEAST

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Received February 5,1973

Summary: Mitochondrial DNA from wild-type yeast Saccharomyces cerevisiae contains the genetic information for some mitochondrial tRNAs including $tRNA_{ser}$ and $tRNA_{phe}$. In a "low density" petite mutant, mitochondrial DNA still retains the information for $tRNA_{ser}$, while the information for $tRNA_{phe}$ is lost.

The permanence of genetic information in this DNA containing only 3.6% G+C supports previous results concerning its intramolecular heterogeneity. An irregular distribution of G+C content along the molecule was further demonstrated by annealing experiments performed with DNA fragmented by sonication and fractionated on CsCl density gradient. These experiments show that the heavy fractions of the gradient preferentially anneal with mitochondrial seryl-tRNA.

Mit-DNA contains part of the information for the specifically mitochondrial protein synthesizing machinery. The existence of genetic information for tRNAs on mitochondrial DNA has been shown for HeLa cells (1), rat liver (2) and yeast (3,4). In Saccharomyces cerevisiae at least a part of mitochondrial genetic information is lost with the petite mutation, which is accompanied by a major alteration of mit-DNA. It is, therefore, interesting to investigate the amount of residual mitochondrial genetic information in petite mutants. As far as information for tRNAs is concerned, Cohen et al. (5,6) have shown that it is retained in some petite strains and lost in others. We investigated the possibility of the permanence of information for tRNA in a mutant, previously

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Abbreviations: mit-DNA, mitochondrial DNA; w.t., wild type; A, adenine; T, thymine; G, guanine; C, cytosine; SSC, standard saline-citrate; EDTA, ethylenediaminetetracetate.

studied (7) in this laboratory, having very low density mit-DNA (ρ = 1.671 g/cm^3; G+C = 3.6%) which, therefore, could have been considered devoid of genetic information. This possibility would in any case be dependent on an intramolecular heterogeneity of mit-DNA, leading to a concentration of G and C in a limited region of the molecule.

This heterogeneity has been actually observed by Carnevali and Leoni (8) and by Bernardi et al. (9) in w.t. yeast mit-DNA and by Carnevali and Leoni in this petite mutant (8).

In this work the localization of the information for mitochondrial tRNA was studied using the Weiss hybridization technique (10) with whole as well as sonicated and fractionated mit-DNA.

MATERIALS AND METHODS

Two diploid strains of *Saccharomyces cerevisiae* were used : strain 42(DM)ρ^+ and strain 41(DM$_1$)ρ^- kindly supplied by prof. G. Morpurgo. These strains were grown as previously reported (8). Wild-type mit-DNA was prepared by the method of Marmur (11) from DNase treated mitochondrial preparations obtained by the method of Duell et al. (12). For the preparation of mit-DNA from petite mutant, DNase treatment was omitted and mit-DNA was isolated by preparative CsCl gradient. For the hybridization experiments performed with fractionated DNA, DNA was prepared, sonicated and fractionated as previously reported (8). The fractions were dialyzed overnight against $\frac{1}{100}$ SSC and denatured by heat treatment followed by quick cooling. The hybridization procedure was essentially that reported by Cohen et al. (5).

Mitochondrial tRNA was prepared by the method reported by Holley (13). The acylation procedure was the same as reported by Cohen et al. (5). For the preparation of mitochondrial aminoacyl-tRNA synthetases, mitochondria were lysed with 0.2 - 0.3% Triton X 100 in 10^{-2}M Tris buffer containing 10^{-3}M EDTA and $2 \cdot 10^{-3}$M mercaptoethanol. Synthetases were stored for one week at -10°C in the presence of 20% glycerol with little loss of activity.

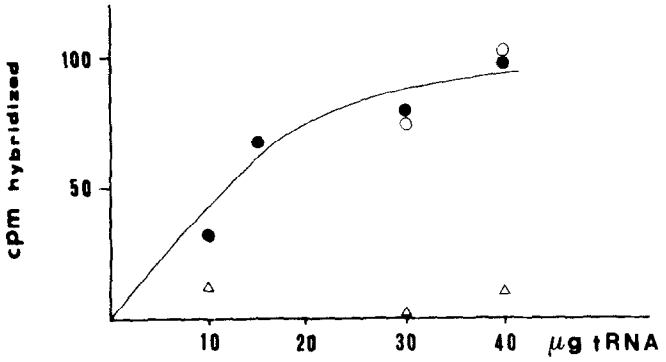

Fig. 1 Hybridization of [^{3}H]-labelled mitochondrial seryl-tRNA with w.t. and petite mitochondrial and nuclear DNA. Mitochondrial seryl-tRNA (239 cpm/μg) hybridized to filters containing 20 μg w.t. mit-DNA (●) or 20 μg petite DNA (○) or 20 μg nuclear DNA (△).

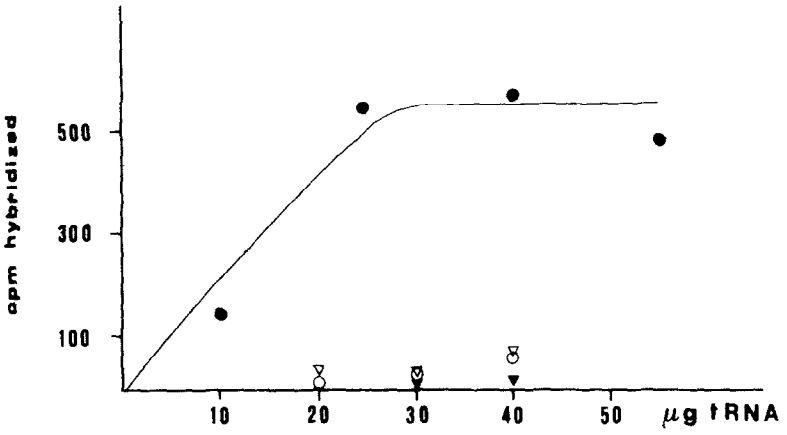

Fig. 2 Hybridization of [^{3}H]-labelled mitochondrial phenylalanyl-tRNA with w.t. and petite mit-DNA, yeast nuclear DNA and E. coli DNA. Mitochondrial phenylalanyl-tRNA (660 cpm/μg) hybridized to filters containing 20 μg of w.t. (●) or 20 μg of petite mit-DNA (○) or 20 μg of yeast nuclear DNA (▽) or 20 μg of E. coli (▼) DNA.

RESULTS

Hybridization of seryl-tRNA with nuclear, w.t. mitochondrial and petite mit-DNA is reported in Fig. 1. As can be seen, the same saturation plateau is attained with w.t. and petite mit-DNA, while no hybridization is observed with nuclear DNA.

As far as phenylalanyl-tRNA is concerned, Fig. 2 shows that

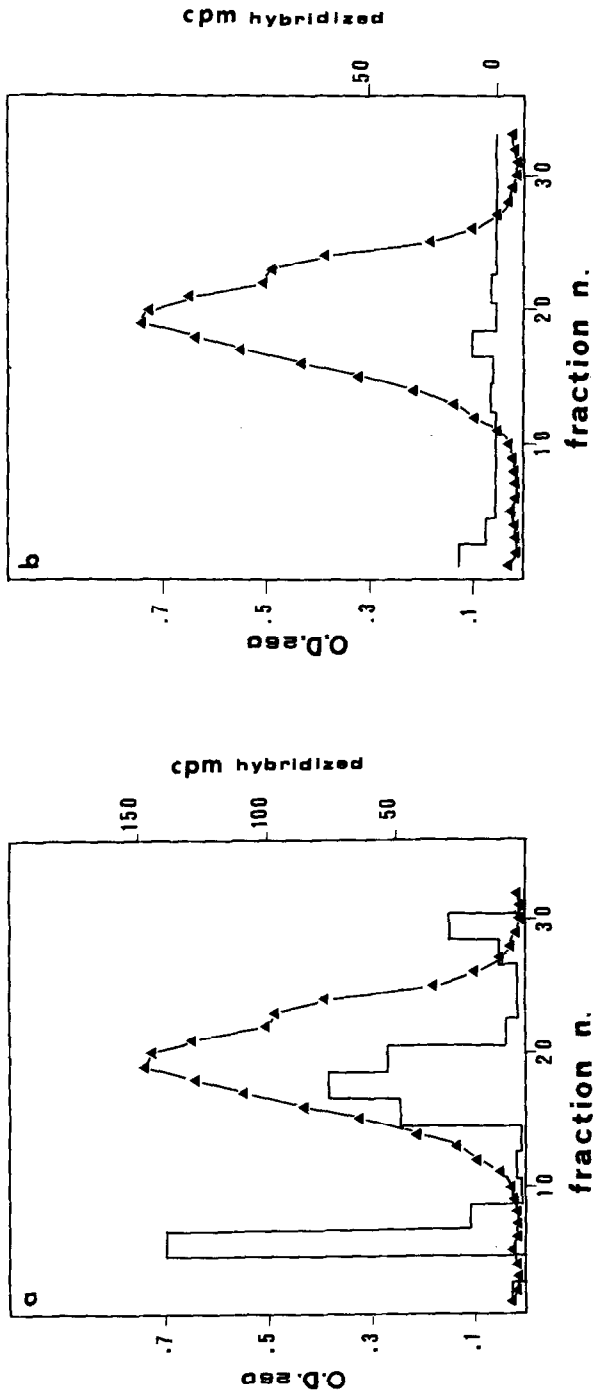
cpm hybridized
150
100
50
a
O.D. 260
.7
.5
.3
.1
10
20
30
fraction n.
cpm hybridized
50
0
b
O.D. 260
.7
.5
.3
.1
10
20
30
fraction n.

Fig. 3.

hybridization is obtained only with w.t. mit-DNA and not with petite mitochondrial, nuclear or Escherichia coli DNA.

The presence of genetic information for $tRNA_{ser}$ in this petite mit-DNA and the heterogeneity of the latter (8) suggested to test the hybridization between seryl-tRNA or phenylalanyl-tRNA and the various fractions obtained by CsCl gradient from sonicated petite mitochondrial DNA. The results reported in Fig. 3a show that seryl-tRNA anneals with the fractions from the DNA peak and also with a heavy zone of the gradient in which the quantity of DNA is very low: consequently the hybridization level in this zone is at least 100 times higher than that obtained in the central zone. On the contrary no hybridization is obtained with phenylalanyl-tRNA (Fig. 3b). As a control, the same experiment was performed with w.t. mit-DNA and the results are reported in Fig. 4. In this case hybridization is much more scattered on the gradient but is higher with heavy fractions for seryl-tRNA (a) and with light fractions for phenylalanyl-tRNA (b). Each experiment was repeated three times and the results were perfectly reproducible.

Fig. 3 Fractionation on a CsCl density gradient of sonicated mit-DNA from DM_1 (petite) strain and annealing of the fractions with [^{3}H]-seryl (a) and [^{3}H]-phenylalanyl-tRNA (b). The DNA sample was run in a Spinco preparative ultracentrifuge model L-2-65B, rotor n.65, for 62 h at 35,000 rpm and 15°C. Fractions of 14 drops were collected from the tubes punctured at the bottom. Optical density was measured at 260 mμ after appropriate dilution with SSC. Fractions were pooled two by two, dialyzed overnight against 1/100 SSC, DNA was denatured by heating for 10 min at 100°C and quick cooling and fixed on two sets of filters which were used for annealing with [^{3}H]-seryl- and [^{3}H]-phenylalanyl-tRNA respectively. The annealing mixture contained in 2xSSC, 33% formamide and 60 μg/ml of [^{3}H]-seryl-tRNA (800 cpm/μg) or [^{3}H]-phenylalanyl-tRNA (700 cpm/μg) final pH was 5.2. This mixture was incubated for 5 h at 32°C. Filters were washed with 2xSSC (pH 6), treated with T_1RNase (5 μg/ml for 30 min at 37°C) washed again and counted in a Beckman scintillation counter. An average value of five blanks with nuclear DNA (20 μg/filter) was subtracted from the data (70 cpm in the case of seryl-tRNA and 145 cpm in the case of phenylalanyl-tRNA). (▲—▲) O.D. at 260 mμ. The radioactivity hybridized on the filters is indicated by the height of the bars.

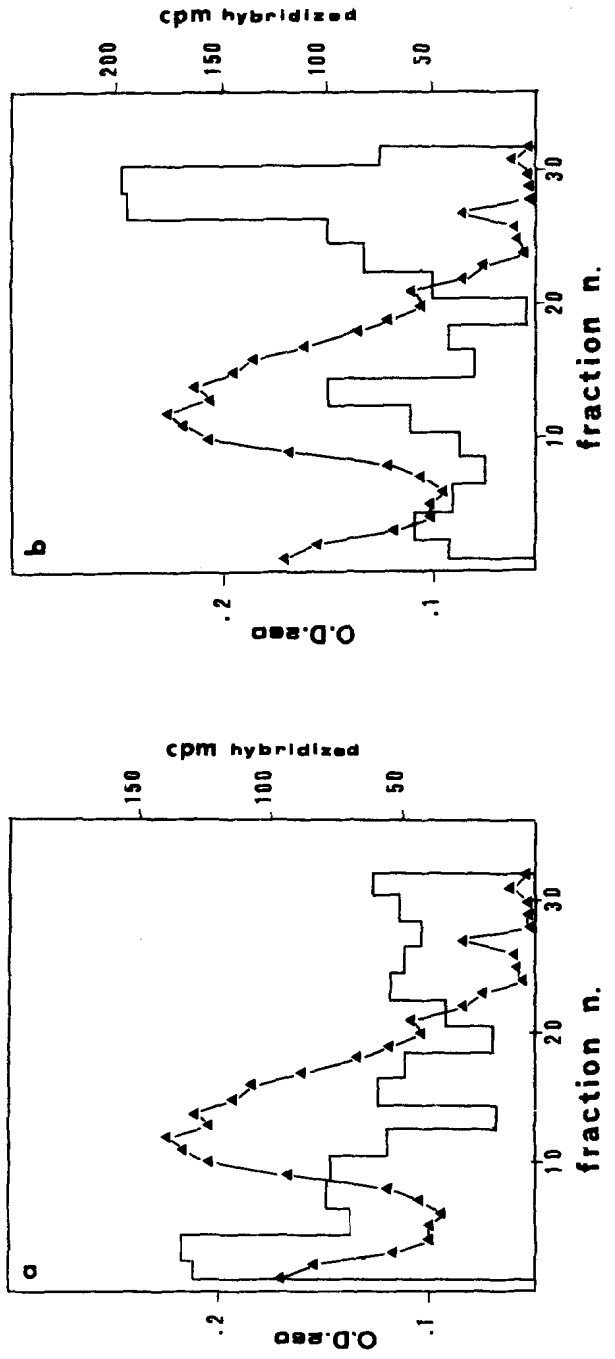

Fig. 4 Fractionation on a CsCl density gradient of sonicated mit-DNA from DM (w.t.) strain and annealing of the fractions with (a) [^{3}H]-seryl-tRNA (2000 cpm/μg) and (b) [^{3}H]-phenylalanyl-tRNA (3000 cpm/μg). The experimental conditions are the same of the experiment reported in Fig. 3. Average values of the blanks subtracted from the data are 130 cpm in (a) and 700 cpm in (b). (▲—▲) O.D. at 260 mμ. The radioactivity hybridized on the filters is indicated by the height of the bars.

DISCUSSION

The results reported above show that w.t. mit-DNA contains the genetic information for $tRNA_{ser}$ and $tRNA_{phe}$. The existence of specifically mitochondrial-tRNA species for serine and phenylalanine different from cytoplasmic counterparts had been previously demonstrated (4) on the basis of preferential acylation by mitochondrial synthetases and of the absence of competition between cytoplasmic and mitochondrial tRNA in annealing experiments with mit-DNA. The annealing experiments performed with petite mit-DNA show that the information for $tRNA_{ser}$ is retained in the mutant while that for $tRNA_{phe}$ is lost.

On the contrary, the experiments performed with nuclear DNA only supply control values but do not rule out the possibility that the information for these tRNAs may be repeated on the nuclear genome. The labelling of the aminoacyl tRNA would actually be too low in any case to show the existence of sites for tRNAs in yeast nuclear genome.

The fact that the information for $tRNA_{ser}$ is retained in this petite mutant, whose DNA only contains 3.6% G+C, supports the possibility of an irregular distribution of G and C along the molecule of the mutated DNA, and indirectly that of a possible irregular distribution of G and C in w.t. mit-DNA.

An intramolecular heterogeneity in density of mit-DNA from w.t. and petite yeast had already been shown (8) and could be interpreted either with an irregular distribution of the G and C content along the molecule or by an unequal distribution of alternated A-T sequences and of adenine and thymine clusters.

The annealing of seryl-tRNA with heavy fractions of petite mit-DNA further supports the intramolecular heterogeneity of this DNA and, on the other hand, demonstrates the first of the two above hypotheses indicating that at least a part of G+C must be concentrated in a limited region of the molecule, which thus retains an informational content sufficient for $tRNA_{ser}$ and, perhaps, for some other tRNAs.

On the other side, the possibility of an irregular distribution of G and C in w.t. DNA too is supported by the results obtained with sonicated and fractionated w.t. mit-DNA which show higher hybridization levels with heavy fractions in the case of seryl-tRNA and with light fractions in the case of phenylalanyl-tRNA. This difference and the hybridization level obtained with light fractions in the case of seryl-tRNA are still under investigation.

The authors wish to thank A. Di Francesco for the help in the CsCl preparative DNA isolation and G. Scotti for the technical assistance.

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