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Mitochondrial protein synthesis is required for maintenance of intact mitochondrial genomes in *Saccharomyces cerevisiae*

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The genes of *Saccharomyces cerevisiae* coding for the mitochondrial threonine and tryptophan tRNA synthetases and for a putative mitochondrial ribosomal protein have been cloned. These, and the previously cloned gene for a mitochondrial elongation factor, were used to disrupt or partially delete the wild-type chromosomal copies of the genes in the respiratory-competent strain W303. In each case, inactivation of a gene whose product is required for mitochondrial protein synthesis causes an instability in mitochondrial DNA. Although intact mitochondrial genomes are rapidly and quantitatively eliminated in the protein synthesis defective strains, specific ϱ^- genomes can be maintained stably over many generations. These results indicate that mitochondrial protein synthesis is required for the propagation of wild-type mitochondrial DNA in yeast.

Key words: Saccharomyces cerevisiae/mitochondria/protein synthesis/ϱ^- genome/mitochondrial DNA

Introduction

The morphogenesis of mitochondria depends on the expression of both mitochondrial and nuclear gene products (Schatz and Mason, 1974; Tzagoloff *et al.*, 1979; Dujon, 1981). To gain insights into the genetic contribution of nuclear DNA towards this process, several laboratories have isolated collections of respiratory-deficient strains of *Saccharomyces cerevisiae* impaired in various functions associated with mitochondrial biogenesis (Dieckmann *et al.*, 1982; Michaelis *et al.*, 1982; Pillar *et al.*, 1983). The mutation collection used in this study is comprised of 1300 independent isolates that have been assigned to 207 separate complementation groups by genetic crosses. Mutations in ~25% of the genes represented by the 207 complementation groups lead to a defect in mitochondrial protein synthesis and result in a pleiotropic phenotype characterized by severe reductions in cytochromes *a*, a_3, cytochrome *b* and oligomycin-sensitive ATPase.

In the course of studying this class of *pet* mutants we observed that they tend to be unstable and readily lose or sustain large deletions in mitochondrial DNA leading to the appearance of a high proportion of secondary ϱ^- mutants in the population. This suggested a requirement of mitochondrial protein synthesis for the maintenance of wild-type but not necessarily of ϱ^- mitochondrial DNA. In the present study we have assessed directly the extent to which maintenance of wild-type mitochondrial DNA depends on the presence of a functional system of mitochondrial protein synthesis. Four nuclear genes which code for either mitochondrial aminoacyl-tRNA synthetases, an elongation factor, or a putative protein of mitochondrial ribosomes have been inactivated by gene disruption or deletions of segments of the genes. The resultant mutant strains, totally blocked in mitochondrial protein synthesis, undergo a rapid quantitative conversion to ϱ^- derivatives. These results show that mitochondrial protein synthesis is needed for the stable propagation of wild-type mitochondrial DNA and further indicate a qualitative difference in the replication of wild-type and of ϱ^- mitochondrial genomes.

Results

Properties of mitochondrial protein synthesis-defective pet mutants and cloning of the genes

The mutants reported here belong to four different complementation groups (Table I). They show a reduced efficiency in mitochondrial protein synthesis as determined by *in vivo* labeling of mitochondrial translation products in the presence of cycloheximide. In addition they lack cytochrome oxidase, coenzyme QH_2- cytochrome *c* reductase, and oligomycin-sensitive ATPase. Since each of the above three enzymes contain subunit polypeptide(s) synthesized in mitochondria (Schatz and Mason, 1974; Tzagoloff *et al.*, 1979), the mutant phenotype is in each case consistent with a lesion in a component required for mitochondrial protein synthesis. Mutants in the four complementation groups studied exhibit variable degrees of leakiness indicative of an incomplete mitochondrial block. In most cases we have observed that protein synthesis-defective strains are unstable and emit ϱ^- mutants at a high frequency. The significance of these properties will be discussed below.

The genes coding for the two aminoacyl synthetases and the putative ribosomal protein were cloned by transformation of the appropriate mutants with a recombinant plasmid library consisting of a partial *Sau*3A digest of wild-type yeast nuclear DNA ligated to the unique *Bam*HI site of the shuttle vector YEp13 (Broach *et al.*, 1979). The genes for the threonine- and the tryptophan-tRNA synthetases were sequenced and the derived protein sequences found to be 35–40% homologous to the known sequences of the *Escherichia coli* enzymes (Mayaux *et al.*, 1983; Hall *et al.*, 1982) (L.Pape, A.Myers, unpublished studies). The putative ribosomal protein gene codes for a 321 amino acid long basic protein which, based on immunological evidence, is associated with yeast mitochondrial ribosomes. A positive identification of this gene as a *bona fide* ribosomal protein gene, however, needs to be further verified.

The gene for the mitochondrial elongation factor was cloned by Nagata *et al.* (1983). This gene was selected from a genomic library of yeast by colony hybridization using a fragment of the homologous *E. coli tufB* gene as the hybridization probe (Nagata *et al.*, 1983). The cloned yeast gene (*tufM*) provided by Dr Nagata was transferred to the shuttle vector YEp13. The resultant plasmid pG130/T1 was capable of transforming *pet* mutant N415 to respiratory competency, indicating that this strain carries a mutation in the mitochondrial elongation factor gene *tufM*. This was also shown by genetic analysis of a strain in which the wild-type gene for the elongation factor was disrupted (see below).

Pertinent information regarding the various mutant strains and their properties are summarized in Table I.

Table I. Designation and properties of mitochondrial protein synthesis-defective strains

Mutant	Gene	Complementation groups	Number of mutants	Gene product	Disruption	Stability[a]	Growth on[b] glycerol
C110	*MST1*	G69	4	$tRNA_1^{Thr}$ synthetase	*MST1::LEU2*	64%	–
E569	*MSW*	G181	2	$tRNA^{Trp}$ synthetase	*MSW::URA3*	40%	–
N415	*tufM*	G130	1	elongation factor	*tufM::URA3*	99%	–
E795	*MRP1*	G195	1	ribosomal protein	*MRP1::HIS3*	24%	+

[a]Stability refers to the percent ϱ^+ clones scored in a stationary phase culture of the mutants (C110, E569, etc.) grown from a freshly isolated ϱ^+ colony.
[b]Observable growth on solid glycerol medium after 3 days at 30°C is indicated by + and lack of growth by –.

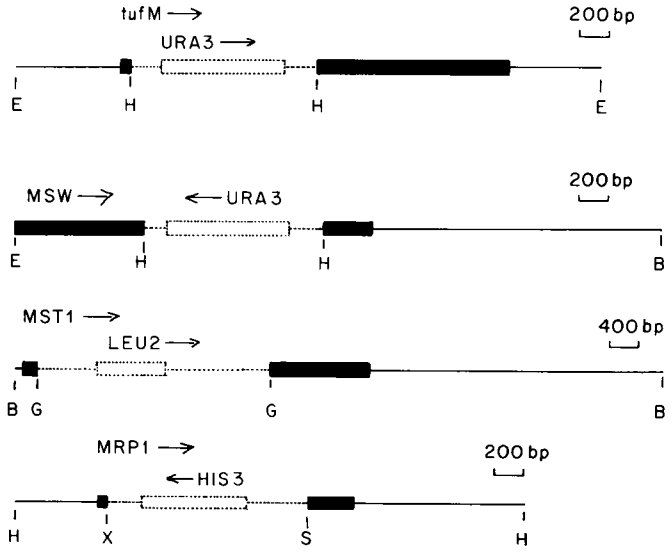

Fig. 1. Linear DNA fragments used to disrupt the wild-type copies of *tufM*, *MST1*, *MSW* and *MRP1*. Solid lines represent coding and flanking regions of the disrupted genes and dotted lines the coding and flanking regions of the inserted DNA. Solid and open bars indicate the coding regions of the disrupted and inserted genes, respectively, with direction of transcription indicated by arrows. Restriction enzyme recognition sites used to construct the disruptions are indicated for *Hind*III (H), *Eco*RI (E), *Sac*I (S), *Bam*HI (B) and *Bgl*II (G). The *tufM* and *MSW* constructions are simple insertions of the disrupting DNA, while the *MST1* and *MRP1* constructions have internal deletions of 30 bp and 600 bp, respectively, in the coding sequences of these two genes.

Table II. Genetic properties of mitochondrial protein synthesis mutants

	B110 (*mst1-1*)	aN415 (*tufm-1*)	aE569 (*msw-1*)	aE795 (*mrp1-1*)	51ϱ°	M7-40 (*cob1*)	M9-3 (*oxi2*)	M10-150 (*oxi3*)
MST1::LEU2	–	+	+	+	–	+	+	+
tufM::URA3	+	–	+	+	–	+	+	+
MSW::URA3	+	+	–	+	–	+	+	+
MRP1::HIS3	+	+	+	–	–	+	+	+
KL14-4B ϱ°	+	+	+	+	–	–	–	–

Haploid cells of opposite mating type were mixed in patches on YPD plates, grown for 8–16 h at 30°C, and replica plated onto WO medium. Diploid colonies selected on WO were replica plated onto EG medium and growth scored after 1–2 days at 30°C.

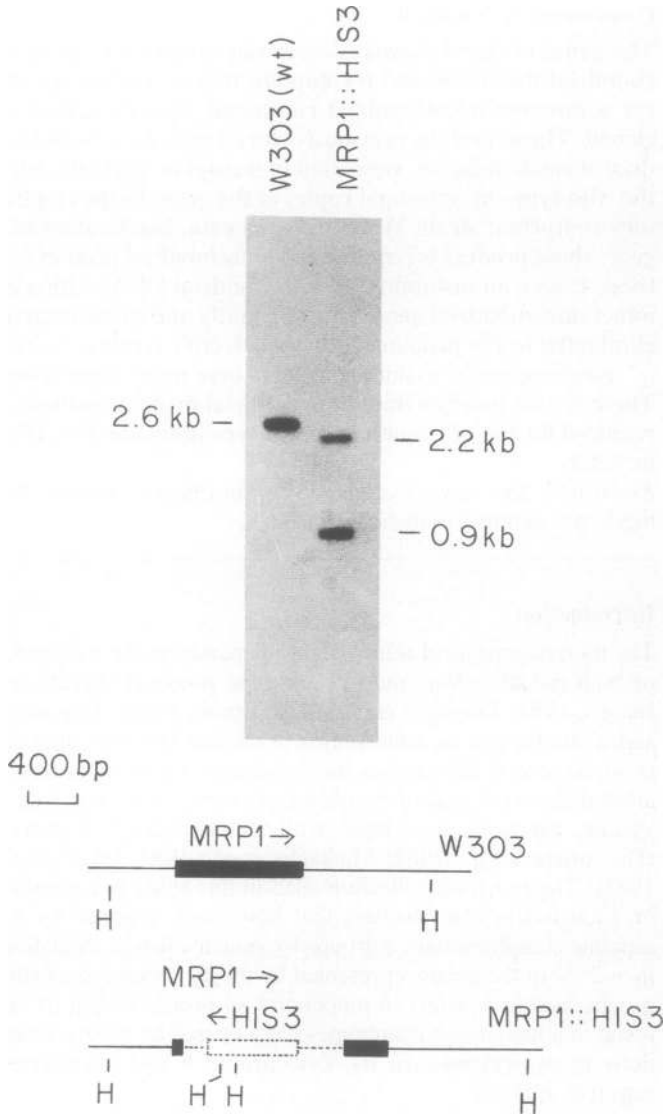

Fig. 2. Chromosomal disruption of *MRP1*. Genomic DNA from wild-type strain W303-1B and the congenic strain containing *MRP1::HIS3* was digested with *Hind*III, separated by agarose gel electrophoresis, transferred to a nitrocellulose sheet and probed with the radioactively labeled 2.6-kb *Hind*III fragment containing the wild-type copy of *MRP1*. This probe is seen to hybridize to a single 2.6-kb fragment of wild-type DNA and to both a 2.2-kb and 0.9-kb fragment from the disrupted strain. The sizes of the two genomic fragments from the disrupted strain that hybridize to this probe correspond to those predicted from the restriction map of the disrupted gene shown in the lower part of the figure.

Construction of protein synthesis-defective mutants by gene replacement

The availability of cloned genes for various constitutents of the mitochondrial protein synthesis machinery made it possible to construct tight mutants by the method of gene replacement (Rothstein, 1983). These constructions entailed an initial transfer of the genes and flanking sequences into pBR322 or a pUC vector. The recombinant plasmids were used to either disrupt the coding sequence by introduction of the yeast *URA3* gene or by substi-

Table III. Mitochondrial genotypes of ϱ^- clones derived from strains with disruptions in *MSW*, *tufM* and *MRP1*

Genotype			Number of clones				
oxi2	*oxi3*	*cob1*	*MSW::URA3*	*tufM::URA3*	*tufM::URA3**	*tufM::URA3***	*MRP1::HIS3*
−	−	−	21	14	73	43	16
−	−	+	20	20	17	26	21
−	+	+	1	3	0	1	5
−	+	−	1	2	3	0	1
+	+	+	20	23	0	0	21
+	+	−	1	3	0	1	3
+	−	−	27	22	6	27	22
+	−	+	9	13	1	2	3
Number of colonies scored			100	100	100	100	92

The primary transformants with deletions in the indicated genes were grown overnight in 1 ml YPD. They were spread for single colonies on YPD plates and ~100 mitotic segregants were crossed to mit$^-$ testers with mutations in *oxi2*, *oxi3* and *cob1*. The diploids were checked for growth on glycerol by replica plating of the diploid cells onto EG medium. Growth of a diploid colony on EG indicates retention of the mitochondrial marker and is scored as a +. The strain designated as *tufM::URA3** is a secondary clone that had retained all three markers. It was subjected to a second round of mitotic segregation. *tufM::URA3*** is a respiratory-deficient meiotic segregant issued from the diploid strain W303 carrying one copy of the disrupted *tufM* gene. This spore was not complemented by a ϱ° but had the three mitochondrial markers prior to the outgrowth.

tution of a deleted region of the gene with the yeast *LEU2* or *HIS3* gene. Linear fragments of DNA encompassing the disrupted/deleted gene and flanking regions (see Figure 1) were used to transform the respiratory-competent strain W303 multiply marked for *leu2*, *ura3* and *his3*. In such transformations complementation of the auxotrophic markers can result by integration of the transforming DNA either at the chromosomal site homologous to the disrupted gene or at some other location, *viz.* the auxotrophic marker. Only the former event is expected to cause loss of respiration.

Respiratory-deficient transformants complemented for the auxotrophic requirement were identified by their inability to grow on glycerol and chromosomal integration of the input DNA was demonstrated by >98% co-segregation of the prototrophic and respiratory-deficient phenotypes during vegetative growth. That the transformants had sustained a replacement of the wild-type genes was demonstrated in several ways. No respiratory-competent diploid cells were issued from crosses of the original mutants to the transformants (Table II). These complementation tests, therefore, showed the transformants to have a genotype consistent with a simple replacement of the wild-type gene copy. This was corroborated by Southern hybridization analysis of genomic DNA from the wild-type and from each transformant. The results of the hybridization analyses indicated that the respiratory-deficient transformants lacked the wild-type restriction fragment with the *PET* gene and instead had a new fragment(s) expected from the particular constructions used for the gene replacements. This is illustrated in Figure 2 for the *in situ* disruption of *MRP1*. A probe spanning the entire length of the *MRP1* gene hybridized to a 2.6-kb *Hind*III fragment of wild-type DNA. The same probe detected two distinct *Hind*III fragments in genomic DNA of the transformant. The two fragments had estimated sizes of 0.9 kb and 2.2 kb. Based on the restriction map of the disrupted gene used in the transformation, the 0.9-kb fragment contains the 5′-flanking sequence, 60 bp of *MRP1* coding sequence and part of the *HIS3* insert. The 2.2-kb fragment contains the carboxy-terminal part of *MRP1* and 3′-flanking sequences plus a 0.8-kb segment of the *HIS3* insert used for the disruption.

Genetic analysis of strains with disruptions in MST1, MSW, MRP1 and tufM

Several genetic tests were used to determine whether transformants with disruptions in genes specifying components of mitochondrial protein synthesis are capable of maintaining a full size mitochondrial genome. Thirty one independent transformants (19 with disruptions in *tufM*, 10 in *MSW*, and one each in *MST1* and *MRP1*) failed to be complemented by a ϱ° tester (Table II). Crosses of the disrupted strains to mit$^-$ testers with mutations in *OXI2*, *OXI3* and *COB1* revealed a differential retention of the mitochondrial markers, suggestive of deletions in, rather than total loss of, mitochondrial DNA.

The types of ϱ^- genomes generated in the protein synthesis-defective strains were examined by testing the mitochondrial genotypes of the transformants following segregation of their mitochondrial genomes during vegetative growth. A randomly picked transformant with a disruption in *MRP1*, *MSW* or *tufM* was grown overnight in liquid YPD and spread for single colonies on YPD plates. Approximately 100 mitotic segregants were tested by crosses to *oxi2*, *oxi3* and *cob1* mutants. The results of the crosses are summarized in Table III. Although the original transformants used for the segregation tests complemented all three mit$^-$ markers, the secondary clones obtained after 20–30 generations of vegetative growth in YPD had an assortment of mitochondrial genotypes. Some 20% of the secondary clones still appeared to have unsegregated mitochondrial genomes based on their ability to restore respiratory competence to all three mit$^-$ testers. One of these with a *tufM* disruption was subcloned a second time and 100 segregants were again checked for their genotypes. The results of the crosses indicated a complete segregation of mitochondrial DNA in the tertiary subclones (Table III).

To exclude the possibility that loss of full-length mitochondrial DNA is an artifact of the transformation process, the effect of the *tufM* disruption on genome stability was tested by two other methods. First, an isogenic diploid strain of W303 was transformed with the linear fragment containing the disrupted copy of the *tufM* gene. Ten independent transformants complemented for the uracil auxotrophy were verified to be respiratory competent indicating that disruption of *tufM* is recessive. Four of the transformants were sporulated for tetrad dissections. Five complete tetrads tested showed a 2:2 segregation of respiratory-competent uracil-dependent and respiratory-deficient uracil-independent phenotypes. None of the respiratory deficient spores were complemented by the ϱ° tester, indicating a loss of wild-type mitochondrial DNA. Furthermore, growth of one spore

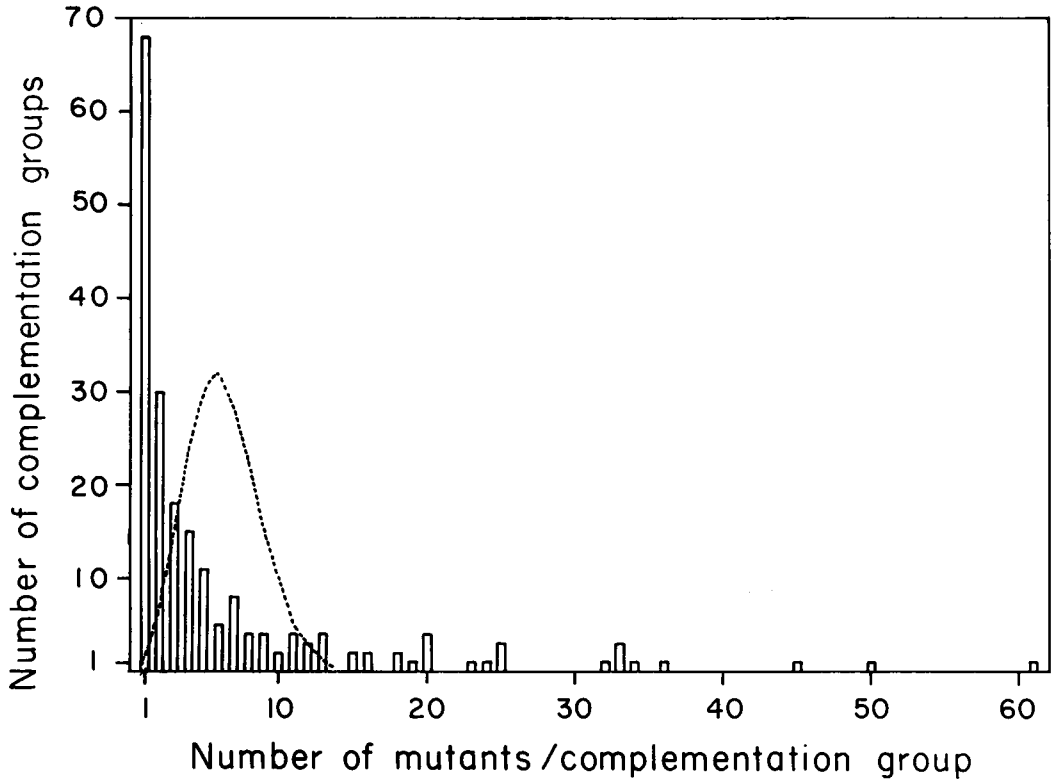

Fig. 3. Distribution of *PET* complementation groups relative to the number of mutants per complementation group. Data are derived from 1300 independent respiratory-deficient mutants grouped into 207 complementation groups. Open bars indicate the observed distribution and the dotted line shows the predicted Poisson distribution based on the mean number of 6.3 mutants per complementation group.

(*tufM*::*URA3***, Table III) under non-selective conditions led to a segregation of different ϱ^- genomes among the mitotic progeny.

The effect of mutations in *tufM* and *MST1* on the stability of mitochondrial DNA was also examined by crossing the disrupted strains to a respiratory-competent haploid yeast with the *kar1* mutation. In such crosses, the heterokaryon is unstable thus allowing for efficient transfer of mitochondria from the *kar1* donor to the recipient cell. Fully segregated haploid strains with the genotypes α,ϱ^-,*MST1*::*LEU2*,*COB1*°,*OXI2*°,*OXI3*° and α,ϱ^-, *tufM*::*URA3*,*COB1*°,*OXI2*°,*OXI3*° were mated to the respiratory-competent *kar1* strain JC3/A1 with the genotype a,ϱ^+, *kar1-1*,*ade2*,*lys2* and lysine-independent haploid cells were selected on the appropriate medium. Out of 50 haploid progeny with the *tufM*::*LEU2* disruption, 21 were not complemented by the ϱ° tested but had acquired different combinations of the mitochondrial markers. The remaining 29 clones displayed the parental mitochondrial genotype and therefore probably did not receive wild-type mitochondrial DNA from the *kar1* donor. Similar results were obtained in the cross with the *MST1* disruption. These data confirm that stringent mutations in genes coding for components of the mitochondrial protein synthesizing machinery cause a rapid conversion of wild-type mitochondrial DNA to ϱ^- genomes.

The specific ϱ^- genomes generated in the mitochondrial protein synthesis-defective strains are stably propagated over many generations. For example, several ϱ^- derivative clones of an *MRP1* disrupted mutant showed 92% and 95%, respectively, retention of the *COB1* and *OXI2* markers among mitotic segregants. Finally, the stable ϱ^- genomes formed in the disrupted strains do not appear to be of the suppressive type (Ephrussi *et al.*, 1966) since crosses of protein synthesis-defective mutants to a respiratory-competent haploid do not lead to a loss of the respiratory-competent phenotype among the diploid progeny.

Isolation of non-conditional protein synthesis-defective pet mutants

In the course of our studies of the *pet* mutant collection, we have noticed that the number of complementation groups with single members is overrepresented based on the average value of 6–7 mutants per complementation group. As shown in Figure 3, the actual distribution in a plot of the number of complementation groups *versus* mutants per complementation group deviates significantly from the calculated Poisson distribution. Although there are a number of factors that might be responsible for the skewing towards groups with fewer members, instability of mitochondrial DNA in certain nuclear backgrounds could be the dominant factor. This is supported by the observation that most of the mutants in complementation groups with one or two members have a leaky phenotype. Moreover, most of the protein synthesis-defective strains we have found belong to such complementation groups. The requirement of mitochondrial protein synthesis for stability of mitochondrial DNA, therefore, has a practical consequence. Stringent mutants in genes specifying components of the mitochondrial protein synthesis apparatus would be scored as mitochondrial mutants due to the secondary loss of the wild-type genome. This problem can be circumvented by isolation of temperature-sensitive protein synthesis mutants.

Discussion

The present study indicates that stringent mutations in yeast nuclear genes required for mitochondrial protein synthesis promote a loss of wild-type mitochondrial DNA and therefore such

Table IV. Strains of *Saccharomyces cerevisiae*

Strain	Genotype	Source
D273-10B/A1	α,ϱ⁺,*met6*	Tzagoloff *et al.* (1976)
CB11	a,ϱ⁺,*ade1*	ten Berge *et al.* (1974)
W303-1B	α,ϱ⁺,*ade2-1,his3-11,15,leu2-3,112,ura3-1,can1-100*	R.Rothstein
W303	α/a,ϱ⁺,*ade2-1,his3-11,15,leu2-3,112,ura3-1,can1-100*	R.Rothstein
KL14-4Bϱ°	α,ϱ°,auxotroph	Wolf *et al.* (1973)
51ϱ°	a,ϱ°,auxotroph	P.P.Slonimski
aM9-3-6C	a,ϱ⁺,*ade1,oxi2*	Slonimski and Tzagoloff (1976)
aM10-150-4D	a,ϱ⁺,*ade1,oxi3*	Slonimski and Tzagoloff (1976)
aM7-40-5B	a,ϱ⁺,*ade1,cob1*	Slonimski and Tzagoloff (1976)
JC3ϱ°	a,ϱ°,*ade2,lys2,kar1-1*	Alexander *et al.* (1980)
JC3/A1	a,ϱ⁺,*ade2,lys2,kar1-1*	JC3ϱ° x D273-10B/A1
C110	α,ϱ⁺,*met6,mst1-1*	This study
B110	a,ϱ⁺,*ade1,mst1-1*	C110 x CB11
N415	α,ϱ⁺,*met6,tufm-1*	This study
aN415	a,ϱ⁺,*ade1,tufm-1*	N415 x CB11
E795	α,ϱ⁺,*met6,mrp1-1*	This study
aE795	a,ϱ⁺,*ade1,mrp1-1*	E795 x CB11
E569	α,ϱ⁺,*met6,msw-1*	This study
aE569	a,ϱ⁺,*ade1,msw-1*	E569 x CB11

mutants behave genotypically as ϱ° or ϱ⁻ clones. These observations provide strong support for the notion that mitochondrial translation is required for the maintenance of mitochondrial DNA, as suggested by earlier findings that growth of yeast in the presence of inhibitors of mitochondrial protein synthesis induces a high frquency of ϱ⁻ (Williamson *et al.*, 1971; Weislogel and Butow, 1970; Carnevali *et al.*, 1971). The fact that strains exist with mutations in either nuclear or mitochondrial genes required for protein synthesis probably means that even a low level of this activity is sufficient to provide whatever function is necessary for maintenance of the mitochondrial genome. In addition to the nuclear genes studied here, mutations have been identified in several mitochondrial tRNA genes (Trembath *et al.*, 1978; Berlani *et al.*, 1980) and in the mitochondrial ribosomal protein *var1* (Zassenhaus and Perlman, 1982). Interestingly, with the exception of aspartyl-tRNA mutants which appear to be completely deficient in respiratory functions and yet have stable wild-type mitochondrial DNA, the isolation of mutants in other mitochondrial tRNAs was made possible only by selection of strains that grow slowly on glycerol (Berlani *et al.*, 1980).

The loss of mitochondrial DNA in protein synthesis-deficient strains may be a primary effect due to a block in the synthesis of DNA replication or repair protein(s) encoded by mitochondrial DNA and translated on mitochondrial ribosomes. All products of mitochondrial protein synthesis detected by *in vivo* labeling of yeast in the presence of cycloheximide or *in vitro* labeling in isolated mitochondria have been identified (Schatz and Mason, 1974; Tzagoloff *et al.*, 1979; Dujon, 1981; McKee and Poyton, 1984). It is conceivable, however, that other mitochondrial translation products exist but are present at levels not detectable by the usual assays. The occurrence of stringent mutations in the mitochondrial aspartyl-tRNA may be explained by the absence of this amino acid in the hypothetical protein.

Alternatively, the observed loss of mitochondrial DNA may be a secondary effect caused by the absence of one or more mitochondrial translation products not directly involved in DNA replication. An electrochemical gradient across the inner membrane has been shown to be needed for transport of certain cytoplasmically synthesized proteins to the mitochondrial matrix (Gasser *et al.*, 1982). Perturbation of inner membrane structure and/or function due to the absence of a mitochondrially synthesized component might prevent transport of nuclearly encoded proteins necessary for replication or repair of wild-type mitochondrial DNA. Mutations in the mitochondrial genes *PHO1* and *PHO2* coding, respectively, for subunits 6 and 9 of the ATPase, have been shown to induce a secondary instability in mitochondrial DNA (Foury and Tzagoloff, 1976; Coruzzi *et al.*, 1978) similar to the protein synthesis mutants reported here. A transport defect, however, would have to apply only to specific proteins, since the great majority of cytoplasmically synthesized constituents of mitochondria are transported in ϱ° and ϱ⁻ mutants that are totally deficient in protein synthesis.

The fact that certain ϱ⁻ genomes are maintained stably in the stringent mutants constructed by gene disruption suggests a qualitative difference in the replication of the petite and wild-type DNAs. The stability of the deleted mitochondrial genomes during vegetative growth cannot be due simply to relatively rapid rates of replication since they do not outcompete wild-type mitochondrial DNA in diploid cells.

Materials and methods

Strains and media

The yeast strains used in this study are listed in Table IV. All *pet* mutants were derived from the wild-type haploid strain D273-10B/A1 by the method of Tzagoloff *et al.* (1975). Plasmid DNA was amplified in *E. coli* RR1. Non-selective medium (YPD) contained 1% yeast extract, 2% peptone and 2% glucose. Minimal medium minus amino acids (WO) was supplemented as needed with histidine, leucine, uracil, tryptophan and adenine at a final concentration of 20 μg/ml. Respiratory competence was scored as growth on medium (EG) containing 1% yeast extract, 2% peptone, and 3% glycerol. Diploid cells were sporulated on medium containing 1% potassium acetate, 0.1% yeast extract and 0.05% glucose. *E. coli* was grown in Luria broth supplemented with 40 μg/ml ampicillin for selection of resistant clones.

Genetic crosses

Procedures for complementation tests (Slonimski and Tzagoloff, 1976), dissection of the tetrads (Mortimer and Hawthorne, 1969) and transfer of mitochondria between yeast strains in crosses using the *kar1-1* mutation (Conde and Fink, 1975; Perlman and Mahler, 1983) have been described previously.

Preparation and analysis of plasmid DNA

Standard techniques were used for preparation of recombinant plasmids from *E. coli*, restriction digestions, agarose gel electrophoresis, isolation of restriction fragments from agarose gels, ligation of specific fragments, transformation of

E. coli, and screening of transforming DNAs (Maniatis *et al.*, 1982). Wild-type copies of the *LEU2*, *HIS3* and *URA3* genes of yeast were isolated from YEp13 (Broach *et al.*, 1979), YIp1 (Orr-Weaver *et al.*, 1981), and YEp24 (Shortle *et al.*, 1982), respectively. They were inserted into the multiple cloning sites of pUC8 (Vieira and Messing, 1982) and pUC18 (Norrander *et al.*, 1983) to provide additional restriction sites for use in constructing deletions and disruptions.

Transformation of yeast

Isogenic haploid and diploid strains of *S. cerevisiae* W303 were transformed with 1 – 3 μg of purified linear DNA by the method of Hinnen *et al.* (1978).

Preparation of yeast genomic DNA and Southern hybridizations

Yeast grown to stationary phase in liquid YPD were converted to spheroplasts (Bonitz *et al.*, 1980). The spheroplasts were lysed in 1.5% SDS, 20 mM Tris-Cl, pH 7.5, 0.2 M NaCl, 1 mM EDTA, extracted twice with an equal volume of phenol:chloroform:isoamyl alcohol (25:25:1) and once with an equal volume of chloroform. Three volumes of ethanol were layered over the aqueous phase and large DNA was collected by spooling with a glass rod. The DNA was dissolved in 10 mM Tris-Cl, pH 7.5, 0.1 mM EDTA, dialyzed against an excess volume of the same buffer to remove ethanol, and purified by density gradient centrifugation in cesium chloride. Genomic DNA digested with restriction enzymes and separated by electrophoresis in 1% agarose gels was transferred to nitrocellulose sheets by the method of Southern (1975). Linear fragments of DNA extracted from agarose gels were radioactively labeled by nick-translation for use as hybridization probes. Hybridization overnight at 65°C in 6 x SSC, 1% Sarkosyl, 50 μg sonicated sperm DNA, was followed by two washes at 65°C in 2 x SSC and four washes at room temperature in 5 mM Tris-Cl, pH 8.

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References

Alexander,N.J., Perlman,P.S., Hanson,D. and Mahler,H.R. (1980) *Cell*, **20**, 199-203.

Berlani,R.E., Pentella,C., Macino,G. and Tzagoloff,A. (1980) *J. Bacteriol.*, **141**, 1086-1097.

Bonitz,S.G., Coruzzi,G., Thalenfeld,B.E., Tzagoloff,A. and Macino,G. (1980) *J. Biol. Chem.*, **255**, 11927-11941.

Broach,J.R., Strathern,J.N. and Hicks,J.B. (1979) *Gene*, **8**, 121-133.

Carnevali,F., Leoni,L., Morpurgo,G. and Conti,G. (1971) *Mutat. Res.*, **12**, 357-363.

Conde,J. and Fink,G.R. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 3651-3655.

Coruzzi,G., Trembath,M.K. and Tzagoloff,A. (1978) *Eur. J. Biochem.*, **92**, 279-287.

Dieckmann,C.L., Bonitz,S.G., Hill,J., Homison,G., McGraw,P., Pape,L., Thalenfeld,B.E. and Tzagoloff,A. (1982) in Slonimski,P., Borst,P. and Attardi,G. (eds.), *Mitochondrial Genes*, Cold Spring Harbor Laboratory Press, NY, pp. 213-223.

Dujon,B. (1981) in Strathern,J.N., Jones,E.W. and Broach,J.R. (eds.), *The Molecular Biology of the Yeast Saccharomyces. Life Cycle and Inheritance*, Cold Spring Harbor Laboratory Press, NY, pp. 391-396.

Ephrussi,B., Jakob,H. and Grandchamp,S. (1966) *Genetics*, **54**, 1-29.

Foury,F. and Tzagoloff,A. (1976) *Eur. J. Biochem.*, **68**, 113-119.

Gasser,S.M., Daum,G. and Schatz,G. (1982) *J. Biol. Chem.*, **257**, 13034-13041.

Hall,C.V., van Cleemput,M., Muench,K.H. and Yanofsky,C. (1982) *J. Biol. Chem.*, **257**, 6132-6136.

Hinnen,A., Hicks,J.B. and Fink,G.R. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 1929-1934.

Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.

Mayaux,J.-F., Fayat,G., Fromant,M., Springer,M., Grunberg-Manago,M. and Blanquet,S. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 6154-6156.

McKee,E.E. and Poynton,R.O. (1984) *J. Biol. Chem.*, **259**, 9320-9331.

Michaelis,G., Mannhaupt,G., Pratje,E., Fischer,E., Naggert,J. and Schweizer,E. (1982) in Slonimski,P., Borst,P. and Attardi,G. (eds.), *Mitochondrial Genes*, Cold Spring Harbor Laboratory Press, NY, pp. 311-321.

Mortimer,R.K. and Hawthorne,D.C. (1969) in Rose,A.H. and Harrison,J.S. (eds.), *The Yeasts*, Vol. **1**, Academic Press, NY, pp. 385-460.

Nagata,S., Tsunetsugo-Yokota,Y., Naito,A. and Kaziro,Y. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 6192-6196.

Norrander,J., Kempe,T. and Messing,J. (1983) *Gene*, **26**, 101-106.

Orr-Weaver,T.K., Szostak,J.W. and Rothstein,R.J. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 6354-6358.

Perlman,P.S. and Mahler,H.R. (1983) *Methods Enzymol.*, **97**, 374-395.

Pillar,T., Lang,B.F., Steinberger,I., Vogt,B. and Kaudewitz,F. (1983) *J. Biol. Chem.*, **258**, 7954-7959.

Rothstein,R.J. (1983) *Methods Enzymol.*, **101**, 202-210.

Schatz,G. and Mason,T.L. (1973) *Annu. Rev. Biochem.*, **43**, 51-87.

Shortle,D., Haber,J.E. and Botstein,D. (1982) *Science (Wash.)*, **217**, 371-373.

Slonimski,P.P. and Tzagoloff,A. (1976) *Eur. J. Biochem.*, **61**, 27-41.

Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503-517.

ten Berge,A.M.A., Zoutwelle,G. and Needleman,R.B. (1974) *Mol. Gen. Genet.*, **131**, 113-121.

Trembath,M.K., Macino,G. and Tzagoloff,A. (1978) *Mol. Gen. Genet.*, **158**, 35-45.

Tzagoloff,A., Akai,A. and Needleman,R.B. (1975) *J. Biol. Chem.*, **250**, 8228-8235.

Tzagoloff,A., Akai,A. and Foury,F. (1976) *FEBS Lett.*, **65**, 391-396.

Tzagoloff,A., Macino,G. and Sebald,W. (1979) *Annu. Rev. Biochem.*, **48**, 419-441.

Vieira,J. and Messing,J. (1982) *Gene*, **19**, 259-268.

Weislogel,P.O., and Butow,R.A. (1970) *Proc. Natl. Acad. Sci. USA*, **67**, 52-58.

Williamson,D.H., Maroukas,N.G. and Wilkie,D. (1971) *Mol. Gen. Genet.*, **111**, 209-223.

Wolf,K., Dujon,B. and Slonimski,P.P. (1973) *Mol. Gen. Genet.*, **125**, 53-90.

Zassenhaus,H.P. and Perlman,P.S. (1982) *Curr. Genet.*, **6**, 179-188.

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