Mutations in the genes for mitochondrial RNA polymerase and a second mitochondrial transcription factor of *Saccharomyces cerevisiae*

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**Summary.** In our previous work (Lisowsky et al. 1987; Lisowsky and Michaelis 1988) we have identified two nuclear *pet* genes of yeast that are required for mitochondrial transcription. In this report we show that one of these *pet* mutations, *pet-ts798*, maps in the *RP041* gene encoding mitochondrial RNA polymerase. The temperature-sensitive lesion of mutant *pet-ts798* can be suppressed by a second nuclear gene *RF1023* (*mfs1*) when inserted into a high copy number plasmid. Our assumption that *mfs1* codes for a 40 kDa mitochondrial transcription factor is supported by the fact that the cloned gene acts as an intergenic suppressor of a temperature-sensitive RNA polymerase mutant. A third nuclear gene (*mfs2*) for mitochondrial transcription was identified by analysing mutant *pet-ts3504*. The in vitro transcriptional activity of isolated mutant mitochondria is temperature sensitive suggesting the presence of an altered component of transcription inside mitochondria. The defect was confirmed by studies with a transcriptionally active DNA-protein complex and by testing the DNA-binding ability of mitochondrial proteins.

**Key words:** Yeast *Saccharomyces cerevisiae* – Nuclear *pet* genes – Mitochondrial RNA polymerase – Mitochondrial transcription – Two transcription factors

**Introduction**

Mitochondria possess their own genetic system whose expression depends on a specific RNA polymerase activity. In yeast the core enzyme of mitochondrial RNA polymerase has been characterized as a 145 kDa protein (Kelly and Lehman 1986; Kelly et al. 1986) which is homologous to the DNA-dependent RNA polymerase of bacteriophages T3 and T7 (Masters et al. 1987). Like most mitochondrial proteins yeast mitochondrial RNA polymerase is encoded by a nuclear gene, translated in the cytosol and imported into mitochondria. Disruption of the structural gene *RP041* results in a loss of respiratory function (Greenleaf et al. 1986).

Biochemical evidence for additional factors of 43 and 70 kDa interacting with mitochondrial RNA polymerase has been reported (Schinkel et al. 1987; Ticho and Getz 1988; Wilcoxen et al. 1988). The 43 kDa factor especially has been intensively studied for interactions with the core enzyme and the promoter regions of mitochondrial DNA (Schinkel et al. 1988a, b).

Using temperature-sensitive yeast mutants as a tool we have identified the first nuclear gene for a mitochondrial transcription factor. Molecular cloning and sequencing of this gene revealed an open reading frame with the coding potential for a polypeptide of 341 amino acids (Lisowsky and Michaelis 1988). This gene, *RF1023*, acts as an intergenic suppressor of the mutant *pet-ts798* which has previously been characterized as having an altered mitochondrial transcription apparatus (Lisowsky et al. 1987). Here we report that *pet-ts798* is a mutation of the *RP041* gene for yeast mitochondrial RNA polymerase. Further insight into the mitochondrial transcription process is given by a new mutant *pet-ts3504*. The mutated gene of this strain is shown to be non-allelic with the aforementioned two genes and causes a severe defect in mitochondrial transcription.

**Materials and methods**

**Yeast strains.** The strains used were: M12 (a ilv5, trp2, ura3; Wolf et al. 1973); Hs-d8 = HS 3324 (a his1, leu2, trp2, ura3) and Hsrp1 = HS 3224 :: Tn10/URA3+ (a his1, leu2, trp2, ura3, rpo41 :: Tn10/URA3+; W.L. Fangman, personal communication); pet-ts798 (a ade1, pet798*); pet-ts3504 (a ade1, pet3504*), GM88-1B (a adel, leu2-1, pet798*); GM88-6B (a ade1, leu2-1, trp, ura, pet798*); GM89-3D (a trp, ura, pet3504*).

**Plasmid.** The yeast and *Escherichia coli* shuttle vector YEp13 (Broach et al. 1979) with the gene *RF1023* on a 4.2 kb *HindIII* fragment of genomic yeast DNA (Lisowsky and Michaelis 1988) was used.

**Hybridisation probes for mitochondrial genes.** The following gene probes were isolated from the mitochondria of petite strains (o−): 21 S rDNA (o− E41), *olig DNA* (o− DS300/A3), *cob DNA* (o− P26 and L30), *oxil DNA* (o− J26), *oxil2 DNA* (o− B37), and *oxil3 DNA* (o− G2). The probe for the 15 S rDNA was an *MboI* fragment cloned in pBR322 (Osinga et al. 1981). The DNA probes, their origin and function are described in detail in a previous paper (Lisowsky et al. 1987).

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Isolation of mitochondria. Mitochondria were prepared from early logarithmic cultures grown in YPGaLa (2% galactose, 1% Bacto yeast extract, 1% Bacto peptone, 0.002% adenine sulphate) as described earlier (Faye et al. 1974). The preparation of protoplasts from yeast cells was done using zymolyase-20T (Seikagaku Koyo) according to the instructions of the producers.

In vitro experiments. In vitro experiments with isolated mitochondria, dot blot hybridisation, and preparation of transcriptionally active DNA-protein complexes from mitochondria were done exactly as described in Lisowsky et al. (1987).

Results

Pet-ts798 and RP041 are allelic

The intergenic suppressor RF1023 was isolated from a gene bank of yeast DNA inserted into the YEpl3 vector in an attempt to clone the nuclear gene pet-ts798 by functional complementation. Although detailed analysis of the complementing clone made clear that the suppressor defines the first gene (mtf1) for a mitochondrial transcription factor, the pet mutant still required characterization. We wanted to know whether pet-ts798 and the recently identified RP041 gene for mitochondrial RNA polymerase are allelic or not. Crosses were performed between pet-ts798 and a mutant (Hsrpl) with a disrupted RP041 gene. The result is shown in Table 1a. The heterozygous diploids failed to grow at 36 °C. The temperature-sensitive phenotype of pet-ts798 can be complemented by the wild-type but not by the disrupted RP041 gene. Thus, the two mutations pet-ts798 and RP041 are allelic and define one single complementation group.

Pet-ts3504, a third nuclear gene (mtf2) for mitochondrial transcription

Mutant pet-ts3504 belongs to a large collection of temperature-sensitive pet strains (Michaelis et al. 1982). In previous studies this mutant had shown a strong reduction in mitochondrial transcripts in vivo at the non-permissive temperature (Lisowsky 1987). Does mutant pet-ts3504 represent a new complementation group for mitochondrial transcription? Crosses of this mutant were performed with pet-ts798 and rpo41. Complementation was found: the heterozygous diploids grew normally at 36 °C (Table 1b). In a second experiment mutant pet-ts3504 was transformed with the gene RF1023 (mtf1) inserted into a high copy number plasmid. No complementation and no suppression could be observed (Table 1c). Thus, mutation pet-ts3504 defines a third gene essential for mitochondrial transcription.

Temperature-sensitive transcription in isolated mitochondria of pet-ts3504 (mtf2)

The identification of a novel gene for mitochondrial transcription initiated a detailed biochemical characterization of the new mutant. In initial experiments transcription was studied in isolated mitochondria that had been prepared from mutant and wild-type strains grown at 23 °C. Radioactive [β-32P]UTP was added to an in vitro system to label the transcripts synthesized under these conditions. Transcriptional activity was compared at the permissive (23 °C) and non-permissive (36 °C) temperature by hybridizing the newly formed RNA against dot blots of six mitochondrial genes. The in vitro RNA synthesis of isolated mutant mitochondria was found to be temperature sensitive for all the genes tested (Fig. 1).

Table 1. Complementation analysis of different pet genes

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<th>Strains</th>
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The yeast strains are listed in Materials and methods. The crosses in a and b were performed on glucose medium and growth of the resulting diploids was tested on glycerol medium at 23 °C and 36 °C. c. Transformation of the two pet-ts mutants was with the RF1023 gene cloned in YEpl3, a high copy number vector. Growth of the transformants was tested on glycerol medium at the indicated temperatures. +, growth; −, no growth and no complementation. The three complementation groups are represented by RP041 (Hsrpl and pet-ts798), mtf1 (RF1023), and mtf2 (pet-ts3504).

Fig. 1. Transcriptional activity of isolated mitochondria. Mitochondria of the wild type and mutant pet-ts3504 were isolated from 300 ml cultures grown in YPGalA and incubated at 23 °C. Aliquots of 1 mg mitochondrial protein from the same preparations were incubated at 23 °C or 36 °C in an in vitro RNA synthesis system containing [β-32P]UTP. The labelled transcripts were hybridized against dot blots of six mitochondrial gene probes, each consisting of 200 ng DNA. Salmon sperm DNA served as an internal control for stringent hybridization conditions. The autoradiograph illustrates the differences between the wild type and mutant at 36 °C.