The Mitochondrial Genome of the Fission Yeast *Schizosaccharomyces pombe*

I. Isolation and Physical Mapping of Mitochondrial DNA

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Summary. 1) We have identified by electron microscopy and isolated a circular DNA species of approximately 6 µm contour length from DNase treated mitochondrial fractions of the petite negative yeast *Schizosaccharomyces pombe* (S. pombe).

2) Another molecular species of about 3 µm length is also present in mitochondrial fractions. These molecules, however, disappear after DNase treatment or extensive washing, indicating their extramitochondrial location. There is evidence (Fournier et al. 1981) that these molecules represent a multicopy plasmid coding for the genes of cytoplasmic ribosomal RNAs.

3) A restriction enzyme cleavage map of the 6 µm species was constructed using twelve enzymes. Physical mapping revealed a genome length of approximately 18.9 kilobase pairs, thus confirming the electron microscopic data.

4) Northern hybridization of mitochondrial RNA with restriction fragments of mitochondrial DNA revealed two major signals which are attributed to the small and large ribosomal RNA. Apparently both rRNA genes map close together.

5) Spontaneous mit⁻ deletion mutants were characterized phenotypically and the location of their deletions was determined.

6) In strains carrying a cytoplasmic mutator (Seitz-Mayr and Wolf 1982) or derived from mutator strains four restriction sites have been mapped, which are not present in the mitochondrial DNA of the parental strain. These extra sites are very likely consequences of the action of the mutator.

7) In conclusion we have presented evidence that the 6 µm circular DNA species is the mitochondrial genome of fission yeast. Special features of this genome are discussed in comparison with other mitochondrial genomes.

Key words: *Schizosaccharomyces* – Physical map – Mitochondria – Genome organization

Introduction

One of the landmarks in molecular biology was the publication of the complete sequence of the human mitochondrial genome (Anderson et al. 1981). The analysis of mitochondrial transcripts (Montoya et al. 1981; Ojala et al. 1981) has shown there to be an economy of genome organization never before encountered, with genes tightly packed that there are few or no non-coding bases between them. In addition, punctuation between genes appears to be provided by tRNA genes. This compact organization is highly conserved among mammals (Bibb et al. 1981) and is shared with other lower eukaryotes such as sea urchins, flatworms and insects (Borst and Flavell 1976) even including the relative sizes and positions of the mitochondrial genes.

An entirely different situation has been found in the group of ascomycetes, and especially in the yeast family. At present the best characterized fungal mitochondrial genome is that of *Saccharomyces cerevisiae* (Borst and Grivell 1981). In it, the largely equivalent set of genes found in human mitochondrial DNA is spread luxuriously over approximately 78 kilobase pairs. The gene order is different from that of human mitochondrial DNA and there are present in the yeast genome long intergene spacers at least some of which are transcribed (Perlman et al. 1980) and at least three mosaic genes.

Among the yeasts, the largest mitochondrial genome reported to date is that of *Brettanomyces custersii* CBS
The mitochondrial genome of *S. pombe* has a very short mitochondrial genome, which is, according to analysis of the organization and expression of that short genome by mutation. As a first step in the analysis of mitochondria, identification and physical map of that short mitochondrial genome may deepen our insight into these evolutionary pressures and may also aid in defining the minimal requirements for a functional genetic system.

O'Connor et al. (1975) have reported that *Schizosaccharomyces pombe*, the petite negative fission yeast, has a very short mitochondrial genome, which is, according to electron microscopic data, approximately 6 µm long. Since we have been active in the genetic analysis of genes on the mitochondrial DNA of *S. pombe* we set out to confirm their result and to begin the detailed analysis of the organization and expression of that short mitochondrial genome. It should be stressed that, unlike other yeasts with small mitochondrial genomes, *S. pombe* is as genetically maleable as is baker's yeast and can tolerate the inactivation of the mitochondrial genome by mutation. As a first step in the analysis of the mitochondrial DNA of *S. pombe* we report the isolation, identification and physical map of that genome.

**Materials and Methods**

**Yeast Strains.** The *S. pombe* strain ade7-50h- and ura1-171h+ were kindly provided by U. Leupold, Berne. The antimony resistant mitochondrial mutant ana8-8 (Lang et al. 1975) was derived from strain ade7-50h+ by nitrosoguanidine mutagenesis. This mutant carries a cytoplasmic mutator (mut-); the corresponding wild type allele is designated mut+. This point mutations and deletions exclusively in the mitochondrial genome (Seitz-Mayr and Wolf 1982). From the cross ana8-8 mut- ade7-50h- x ana8 mut+ ura1-171h+ ascospores with various combinations of mating types and nuclear markers were isolated. Concerning the extrachromosomal phenotypes a series of spores were isolated having the genotypes ana8 mut- (parental), ana8 mut+ (parental), and ana8 mut+ (recombinant), and ana8 mut- (recombinant).

The mitochondrial deletion mutants DPA1, DPA3, and DPA4 are spontaneous respiratory deficient mutants derived from mut- strains. Their characteristics are summarized in Table 4.

**Media and growth conditions** are described by Wolf et al. (1976)

**Preparation of mitochondria** is described by Del Giudice et al. (1978)

**Assay of cytochrome c oxidase** (EC 1.10.3.2) and NADH – cytochrome c reductase (EC 1.9.3.1.) is described by Needleman and Tzagoloff (1975).

**DNase treatment of mitochondrial fractions** is described by Tabak and Weijers (1976).

**Preparation of mitochondrial DNA for restriction enzyme digests** is according to Tabak and Weijers (1976). For CsCl gradient centrifugation the refractive index was adjusted to 1.3950 and 7.5 µg/ml bisbenzimid was added (Müller and Gauthier 1975). Gradients were centrifuged at 35,000 rpm for 48 h at 20 °C. The fraction containing mitochondrial DNA was identified under UV light and the gradient fractionated. Bisbenzimid was first removed by isopropanol extraction, and then fractions were dialyzed against 5 l of buffer containing 5 mM Tris, 5 mM NaCl, pH 8.0 for 36 h, with 3 changes.

**Restriction enzyme digestion and gel electrophoresis.** Restriction enzymes were purchased from BRL (Bethesda Research Labs) and Boehringer, Mannheim, digestions were conducted as indicated by them. Electrophoresis was done with vertical gels containing 0.7 or 1.0% agarose. HindIII digested DNA from phage λ (Szybalski 1976) was used as marker.

**Electron microscopy.** Isolation of mitochondrial DNA and 3 µm DNA from osmotically shocked mitochondria (Del Giudice et al. 1978) was performed by the protein monolayer method of Kleinschmidt (1968). DNA of phage φ X174 was used as standard (Sanger et al. 1978).

**Preparation of mitochondrial RNA** was performed according to Morimoto et al. (1979).

**Electrophoresis of RNA** was done according to McMaster and Carmichael (1977).

**Isolation of DNA fragments.** Bands were cut out from agarose gels and transferred into dialysis tubes containing buffer (10 mM EDTA, 10 mM Tris HCl, pH 7.4 plus 1 µg/ml ethidium bromide). Electrophoresis was carried out at 100 V for 30 min. DNA was removed from dialysis tube, mixed with 5 M NaCl (final concentration 1 M), and extracted with isoamyl alcohol. DNA was precipitated with ethanol at −20 °C.

**Nick translation of mitochondrial DNA fragments** was carried out according to Rigby et al. (1977)

**Northern blotting** was performed according to Alwine et al. (1977).

**Results**

**Isolation of Circular Molecules from Mitochondrial Fractions**

Mitochondrial fractions were isolated from lysates of spheroplasts prepared by digestion of cell walls. After osmotic shock of mitochondria, two types of circular molecules were observed with approximately 6 µm and 3 µm circumference. An electron micrograph of these molecules is shown in Fig. 1, were both types of molecules are marked by arrows. When mitochondria were treated with DNase, only the 6 µm molecular species was recovered. These results indicate that while the 3 µm