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 to 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
has a very short mitochondrial genome, which is, accord-

S. pombe analysis of the organization and expression of that short

saccharomyces pombe, some viral genomes or into an arrangement more typical

between different yeasts raises the question of what

long. Since we have been active in the genetic analysis

point mutations and deletions exclusively in the mitochondrial

resistant mitochondrial mutant

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Materials and Methods

Yeast Strains. The S. pombe strain ade7-50h− and ura1-171h+

were kindly provided by U. Leupold, Berne. The antimiycin

resistant mitochondrial mutant ana2-8 (Lang et al. 1975) was
derived from strain ade7-50h− by nitrosoguanidine mutagenesis.
This mutant carries a cytoplasmic mutator (mut−; the corre-
sponding wild type allele is designated mut+)
which induces point mutations and deletions exclusively in the mitochondrial

geno(Lange-Mayr and Wolf 1982). From the cross ana2-8 mut− ade7-50h− × ana2 mut+ ura1-171h+
ascospores with various combinations of mating types and nuclear markers were

isolation, identification and physical map of that genome.

Materials and Methods

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

Assay of cytochrome c oxidase (EC 1.10.2.2.) 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 bisbenzimide 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. Bis-
benzimide 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. Restrict-
ton 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 φX174 was used as standard

(Sanger et al. 1978).

Preparation of mitochondrial RNA was performed according

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Electrophoresis of RNA was done according to McMaster and

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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 concentra-

tion 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.


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 mole-
cules 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

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