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Gene Organization of the Mitochondrial DNA of Yeasts: *Kluyveromyces lactis* and *Saccharomycopsis lipolytica*

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Summary. Restriction fragment maps have been constructed for the mitochondrial DNA from two petite-negative yeasts, *Kluyveromyces lactis* and *Saccharomycopsis lipolytica (Candida lipolytica)*. On these circular genomes, we localized the sequences homologous to the *S. cerevisiae* mtDNA fragments carrying known genes. The arrangement of genes for ATPase subunit proteins, ribosomal RNA and 4S RNA shows a common feature with respect to *S. cerevisiae* mitochondrial genome.

Key words: Physical mapping – Hybridization – Sequence Homology

Introduction

Among various forms of lower eukaryotes, the mitochondrial DNA is very diverse in molecular size. Yeast species seem to be particularly rich in variety. The mitochondrial DNA (mtDNA) of *Saccharomyces cerevisiae* is one of the largest. This mtDNA has been extensively studied by many laboratories (see Bandlow et al. 1977). Most of its genes have been identified and localized on detailed restriction maps, and defined DNA fragments that carry known genes can be isolated by means of rho$^-$ deletion mutations (Faye et al. 1973). Our preliminary survey has indicated that individual genes in various yeasts, even quite distant species, seem to have high degree of sequence homology, with respect to *S. cerevisiae* mtDNA. Therefore, the specific gene sequences isolated from *S. cerevisiae* mtDNA may be used as hybridization probes for the detection and localization of equivalent genes in other related mitochondrial genomes for which genetic analysis is not feasible.

Using these gene probes, we have examined the gene organization of the mtDNA from two aerobic, petite-negative yeasts, *Kluyveromyces lactis* and *Saccharomycopsis lipolytica* whose mtDNA are 37,000 and 44,000 base pairs long respectively, as compared to the 75,000 base pairs of *Saccharomyces cerevisiae* mtDNA.

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Abbreviations: bp: base pairs; mtDNA: mitochondrial DNA; tRNA: transfer RNA; rRNA: ribosomal RNA

Materials and Methods

Yeast Strains. Saccharomyces cerevisiae MH41-7B (a *ade2 his1*, [*rho*$^+$ w^+ $C^RE^RO_I^RO_{II}^R$], Bolotin-Fukuhara and Fukuhara 1976) was used for the preparation of rRNA and 4S RNA. Mitochondrial deletion mutants (rho$^-$) derived from this strain have been described previously (Wesolowski and Fukuhara 1979), and were used for the preparation of gene probes. *Kluyveromyces lactis* CBS 2360-7 (arg$^-$) is a stock from the Genetics Institute, Parma. *Saccharomycopsis lipolytica* (formerly *Candida lipolytica*) W29 was kindly provided by Dr. C. Gaillardin (Institut Agronomique, Paris). All yeast strains were grown aerobically in YGG medium (galactose 2%, Difco Yeast Extract 1%, Difco Bactopeptone 1%, glucose 0.1%) to stationary phase.

Preparation of Mitochondrial DNA. Total DNA was extracted from mechanically broken cells by a conventional phenol method. DNA was further purified by hydroxyapatite chromatography, then banded in a CsCl gradient (initial density 1.56 g · cm^{-3}) containing DAPI (Boehringer), 50 μg/ml. The upper of two fluorescent bands (pure mtDNA) was collected, passed through a Dowex-50 column to remove DAPI, concentrated and dialyzed against 15 mM NaCl, 1.5 mM trisodium citrate. The properties of rho$^-$ mtDNA used as probes are shown in Table 1.

Preparation of RNA. RNA was extracted from the purified mitochondria of MH41-7B (Faye et al. 1974), electrophoresed on a 1% agarose slab gel containing 6M urea. The RNA bands corresponding to 21S and 15S rRNA and 4S RNA were excised from the gel, purified by phenol extraction and precipitated with ethanol.

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Table 1. Rho⁻ mtDNA from *S. cerevisiae*, used as gene probes

Gene	Origin	Unit sequence length of the mtDNA, bp[a]
C^R	rho⁻ C-5	2,000
E^R	rho⁻ E-1	720
O_I^R	rho⁻ O_I-2	2,600
O_{II}^R	rho⁻ O_{II}-3	920
P^R	rho⁻ P-5	800

[a] These rho⁻ mtDNAs are made of tandemly repeated unique sequences. The indicated value is the length of the repeat unit (Faye et al. 1973)

Labeling of DNA and RNA. DNA was labeled with [^{32}P]-α-dCTP and DNA-polymerase I according to Maniatis et al. (1975). RNA was 5'-labelled with [^{32}P]-γ-ATP and T_4 polynucleotide kinase, after alkali-shearing (pH 10, 45°C, 60 min).

Restriction Enzyme Analysis and Hybridization. Restriction enzyme digestion of DNA and electrophoresis were performed as described earlier (Wesolowski et al. 1980). After electrophoresis of the digests on agarose gels, the DNA fragments were transferred onto a nitrocellulose paper (Southern 1975). The DNA-paper was used for the hybridization with labeled nucleic acids. Hybridization conditions were 0.6 M NaCl, 45°C, 24–48 h. The hybridized paper was washed in 0.3 M NaCl at room temperature, rinced once in 10 mM Tris, pH 8.

Melting Curves of the Hybrids formed on the nitrocellulose paper were obtained by incubating the paper in 50% formamide 0.3 M NaCl at increasing temperature, and measuring the radioactivity released (Casey et al. 1974).

Autoradiography. After hybridization, the radioactive DNA paper was dried and autoradiographed for various lenghts of time using Kodak X-ray film (Kodirex) with a tungsten intensifying screen.

Results

Restriction Fragment Maps of K. lactis and S. lipolytica mtDNAs

The mtDNA from many yeasts can be conveniently separated from the nuclear DNA in a DAPI-CsCl density gradient. The isolated DNA showed restriction enzyme digests identical to those from the DNA isolated from purified mitochondria.

K. lactis and *S. lipolytica* mtDNA were digested by a set of restriction enzymes and their combinations. The molecular sizes of the products were determined by gel electrophoresis. The sum of the molecular weights of the fragments from each digest gave the total molecular weight of 37,000 bp for *K. lactis* mtDNA and 44,400 bp for *S. lipolytica* mtDNA, with respect to the reference molecular weight of phage lambda DNA 49,000 bp.

The restriction fragments have been ordered by double and triple digestion by several restriction enzymes. In a few cases, specific fragments were extracted from the electrophoresis gels, labeled by nick translation, and hybridized to southern transfers of various restriction digests to identify contiguous fragments which should show an overlapping hybridization. Both for *K. lactis* and *S. lipolytica* mtDNA, the ordered fragments showed a circular arrangement as shown in Figs. 1 and 2. These maps have been confirmed by the fact that various gene probes (see later) did hybridize to contiguous fragments.

Localization of the Sequences Homologous to the Known Genes of Saccharomyces mtDNA

a) Sequences Homologous to the Antibiotic Resistance Genes of S. cerevisiae. Previously, we constructed a series of rho⁻ deletion mutants of *S. cerevisiae*. Many of them contained only a very short segment of the wild-type mtDNA. Some carried a single known gene. For example, the mtDNA of the mutant, called O_I-2, contained the ATPase subunit 9 gene as well as its genetic marker O_R^I (resistance to oligomycin), and no other known gene. This DNA is made of a 2,600 bp long unique sequence in which the ATPase gene is flanked by almost pure adenine-thymine stretches. This DNA was labeled by [^{32}P]-dCTP and hybridized to the restriction fragments of *K. lactis* (Fig. 3a) and *S. lipolytica* mtDNA that had been transferred from the electrophoresis gels onto nitrocellulose papers. Autoradiography of the hybridized papers showed that specific fragments formed radioactive hybrids, indicating the presence of a sequence homologous to the ATPase gene from *Saccharomyces*. The degree of homology, or the specifity of hybridization, appeared to be quite high as judged from the melting profile of the hybrids. Figure 4 shows such an example. The *Tm* of homologous hybrids ([^{32}P]-O_I-2 DNA x wild-type *S. cerevisiae* mtDNA) was 40.5 °C while the Tm of the heterologous hybrids (^{32}P-O_I-2 DNA x *K. lactis* mtDNA) was 38.5 °C. The mismatch was estimated to be approximately 3%, based on the reported value of 0.7 °C decrease of *Tm* per 1% mismatch (McCarthy and Church 1970). Thus on the restriction maps, the sequence homologous to the ATPase subunit 9 gene of *S. cerevisiae* has been localized between map units 69 and 72 of *K. lactis* mtDNA and between map units 14,5 and 19,5 of *S. lipolytica* mtDNA.

In the same manner, other *S. cerevisiae* gene probes have been used to detect their homologous regions in *K. lactis* and *S. lipolytica* genomes.

The probe O_{II}-3, 920 bp long, contains the genetic marker O_{II}^R (Fig. 3b), another oligomycin resistance locus which is believed to correspond the ATPase subunit 6 gene (Macino and Tzagoloff 1980).