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Cloning and characterisation of the ribosomal RNA genes of the dimorphic yeast, *Yarrowia lipolytica*

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Summary. The ribosomal RNA genes of *Yarrowia lipolytica* have been identified, both in restriction digests of total genomic DNA and in a pBR322 gene bank, by hybridisation with cloned *Saccharomyces cerevisiae* rDNA. The *Y. lipolytica* rDNA repeat unit is 8.9 kb in size and contains the genes for the 25S and 18S, but not the 5S, rRNA species. The number of copies of these repeat units is approx. 50 per haploid genome. Several clones were found which did not conform to the standard restriction map due to differences outside the coding region. It appears that there is either heterogeneity of the spacer sequence within a strain or that the *Y. lipolytica* rDNA genes may be present as a number of separate clusters within this yeast's genome.

Key words: rRNA genes – Yeast – *Yarrowia lipolytica*

Introduction

Most eukaryotic organisms contain multiple copies of the genes encoding the four rRNA species (25S, 18S, 5.8S and 5S rRNAs). The genes for the first 3 species are usually organised into a single transcriptional unit which is transcribed by RNA polymerase I. These units are commonly arranged in an extensive tandem array, usually on a single chromosome. The 5S rRNA genes are transcribed by RNA polymerase III and may be organised into the same repeating unit as the 5.8S–25S–18S cluster, or distributed in separate clusters elsewhere in the genome. Both types of rRNA gene organisation are exhibited by the fungi (Bollen 1982) and their distribution is suggestive of an evolutionary progression. The budding yeasts *Saccharomyces* (Bell et al. 1977; Verbeet et al. 1983) and *Torulopsis* (Tabata 1980), the dimorphic fungus *Mucor racemosus* (Cihlar and Sypherd 1980), the water mould *Achlya ambisexualis* (Rozek and Timberlake 1979) and the cellular slime mould *Dictyostelium discoideum* (Maizels 1976) all incorporate their 5S rRNA genes as part of the major rDNA repeating unit which contains the genes for the other three species. In contrast, the fission yeast *Schizosaccharomyces pombe* (Barnitz et al. 1982) and the filamentous ascomycetes *Aspergillus nidulans* (Borsuk et al. 1982; Lockington et al. 1982) and *Neurospora crassa* (Free et al. 1979) have their 5S rRNA genes located separately to the major rDNA array. In order to study the genome organisation of the dimorphic yeast *Yarrowia (Saccharomycopsis) lipolytica* and to examine its relatedness to other yeasts we have cloned and characterised the rRNA genes from this organism.

Materials and methods

Organisms and culture conditions. Yarrowia lipolytica ATCC-18944 was used as a source of DNA for both direct hybridisation experiments and the construction of a gene bank. It was grown routinely in YEPD medium (1% w/v yeast extract; 2% w/v peptone; 2% w/v glucose) at 30 °C.

Escherichia coli JA221 *recA1 leuB-6 trpES hsdR⁻ hsdM⁺ lacY* was used as the host for both the vector plasmid pBR322 and the recombinant plasmids containing either *Y. lipolytica* or *S. cerevisiae* DNA inserts. It was grown at 37 °C in L-broth containing 40 μg/ml ampicillin or 20 μg/ml tetracycline as required.

DNA preparation. High molecular weight DNA was extracted from *Y. lipolytica* using the method of Specht et al. (1982) except that the lyophilisation and grinding steps used with filamentous fungi proved to be unnecessary with this yeast. Nuclear DNA was separated from mitochondrial DNA using a CsCl-

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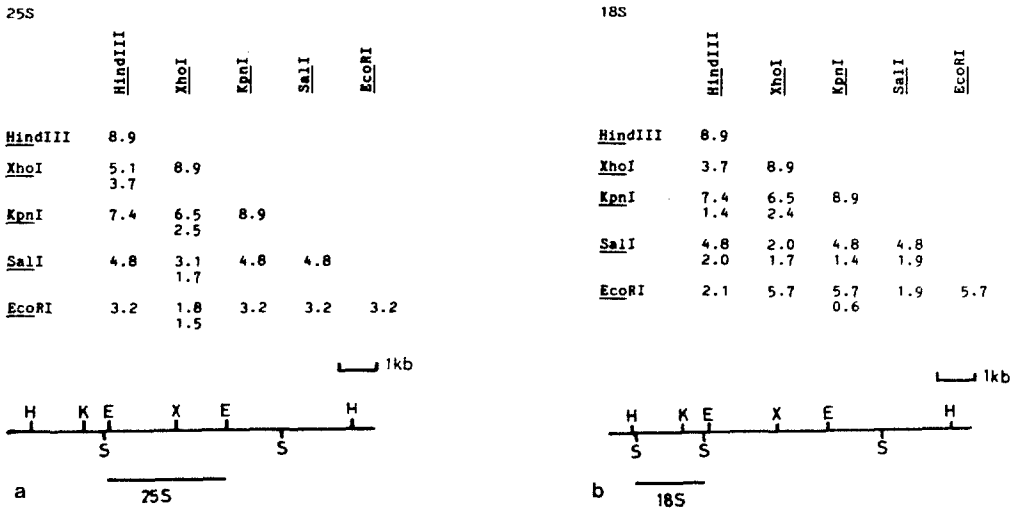

Fig. 1a, b. The sizes (kb) of DNA fragments produced by single and double restriction enzyme digests of total *Y. lipolytica* DNA that hybridised to each probe are given. For each probe the map derived from the data is also shown. In a the relative position of the *Eco*RI sites with respect to the *Xho*I site cannot be deduced and the arrangement shown is that which is compatible with the other data. In b the shortest *SalI-Eco*RI fragment was not measured since it is too small to be detected in these experiments (see Fig. 4 for a size determination). H, HindIII; X, XhoI; S, SalI; E, EcoRI; K, KpnI

Hoechst 33258 gradient (Williamson and Fennell 1975). The DNA was freed of the dye using CsCl-saturated isopropanol.

Preparation of hybridisation probes. DNA fragments containing all or part of the *S. cerevisiae* genes for the 25S, 18S and 5S rRNA were prepared as follows:

25S – Plasmid A12 (Petes et al. 1978) was digested with the enzymes *Kpn*I and *Eco*RI. The 2.0 kb DNA fragment which contains the 25S coding region, except for 131 bp at the 5′ end and 866 bp at the 3′ end (Bayev et al. 1981), was isolated by electrophoresis in 1.0% low gelling temperature agarose followed by phenol extraction and ethanol precipitation of the melted gel fragment containing this band.

18S – The *Eco*RI "C" fragment from *S. cerevisiae* which contains the 18S coding region except for approximately 186 bp at the 3′ end was isolated from plasmid G12 (Petes et al. 1978) in a similar manner to that described above.

5S – The *Eco*RI "B" fragment containing the 5S coding region was isolated from G12 in a similar manner. These probes were labelled to a specific activity of 10^6–10^7 cpm per μg DNA by nick translation (Rigby et al. 1977) using 5 μCi of ^{32}P-α-dATP (Amersham International).

Hybridisation conditions. DNA was transferred from agarose gels to nitrocellulose filters as described by Southern (1975). Filters were pre-hybridised at 37 °C for 3–4 h in 5 x Denhardt's solution (Denhardt 1966) containing 50% formamide, 2 x SSC, 0.1% SDS and 50 μg ml^{-1} of sonicated denatured calf thymus DNA. ^{32}P labelled probe (10^5–10^6 cpm per filter) was then added and hybridisation continued overnight (18–20 h). The following washes were then performed at 37 °C: 2 x 30 min in hybridisation fluid; 2 x 30 min in 2 x SSC, 0.1% SDS; 2 x 30 min in 2 x SSC. Filters for colony hybridisation were prepared using the method of Grunstein and Hogness (1975) and hybridisation was performed exactly as described above.

Materials. Restriction enzymes were purchased from either BRL or Boehringer Mannheim and used in accordance with the maker's instructions. DNA polymerase I, phage T4 ligase and deoxynucleoside triphosphates were all from Boehringer Mannheim. Agarose and low gelling temperature agarose were obtained from Sigma and Seakem respectively.

Results

Hybridisation analysis of total Y. lipolytica DNA

High molecular weight nuclear DNA extracted from *Yarrowia lipolytica* was subjected to single and double digests with a variety of restriction endonucleases. After agarose gel electrophoresis DNA fragments specifying rRNA were identified by Southern analysis using nick translated probes carrying the *Saccharomyces cerevisiae* 25S, 18S and 5S rRNA genes. This proved a quicker and more reliable approach than the use of rRNA probes isolated from *Yarrowia lipolytica*, an organism which is noted for the abundant production of hydrolytic enzymes, including ribonucleases (Ogrydziak and Mortimer 1977). Digestion with the enzymes *Kpn*I and *Xho*I each produced a single fragment of about 8.9 kb that was visible above the background of other restriction fragments and which hybridised to the 25S and 18S probes. Both *Eco*RI and *Sal*I endonucleases produced two fragments (5.9 kb, 3.5 kb and 4.8 kb, 1.9 kb respectively) which hybridised to the 25S and 18S rDNA probes whereas the enzyme HindIII gave a single band of about 8.9 kb.

The restriction maps generated in these experiments are shown in Fig. 1, the maps produced by each probe are completely consistent. None of these bands hybridised to the 5S probe (Fig. 2) and this indicates that *Yar-*