Received: 10 March 1995

Abstract Fission yeasts form a small but heterogeneous group of ascomycetes and it is still unclear whether they should be subdivided into three genera (Schizosaccharomyces, Octosporomyces, Hasegawaea) or remain a single genus (Schizosaccharomyces). In order to decide whether a new genus Hasegawaea should be established for the species Schizosaccharomyces japonicus and Schizosaccharomyces versatilis, we have characterized the entire rDNA cluster in Schizosaccharomyces japonicus var. versatilis and compared it with the homologous region from Schizosaccharomyces pombe and with complete rRNA gene sequences from other yeast genera. From a phage genomic library a recombinant lambda phage containing the entire rDNA repeat unit was isolated. In this paper we report the primary sequence of the 18s, 5.8s and 25s rRNA coding regions. The S. japonicus var. versatilis rRNA genes are 1823 (18s), 158 (5.8s) and 3422 (25s) nucleotides long. The two sequences of the larger rRNA genes exhibit 95.7% (18s) and 93% (25s) similarity with the homologous genes from S. pombe. The differences between the rRNA genes of S. japonicus and S. pombe, however, are much smaller than the intragenic differences within the rDNA sequences of other yeast genera. Therefore, subdivision of fission yeasts into the genera Schizosaccharomyces and Hasegawaea does not seem to be justified. The sequence has been deposited in the EMBL data bank under the accession number Z 32848.

Key words Schizosaccharomyces • Hasegawaea • Ribosomal RNA • Yeast phylogeny

Introduction
The fission yeasts are phylogenetically distant from the “budding” yeast clade and from the euascomycetes, which has resulted in the reassignment of the fission yeasts to a separate order, the Schizosaccharomycetales (Eriksson et al. 1993; Kurtzman 1993). When the genus Schizosaccharomyces Lindner was established (Lindner 1893; Wickerham and Duprat 1945; Kudriavzev 1960), it was divided into four species (Sloof 1970): Schizosaccharomyces pombe Lindner (1893), S. octosporus Beijerinck (1894), S. japonicus Yukawa et Maki (1931), and S. malidevorans Rankine et Fornachon (1964). Yamada et al. (1973) divided the fission yeasts into three groups according to the type of coenzyme Q: S. pombe and S. malidevorans with CoQ-10, S. octosporus with CoQ-9, and S. japonicus without CoQ. The species S. japonicus was furthermore divided into the two varieties “japonicus” and “versatilis”. On the basis of the presence or absence of linoleic acid, Kock and Van der Walt (1986) divided the fission yeasts into a group with linoleic acid (S. japonicus with its two varieties) and a group lacking linoleic acid (S. pombe, S. malidevorans and S. octosporus). Based on the scanning electron microscopy of ascospores, Yamada and Banno (1987) classified the fission yeasts into three groups: (1) S. pombe and S. malidevorans, (2) S. octosporus, and (3) S. japonicus. Taking into account the morphological and chemotaxonomical characteristics (Bridge and May 1984), together with the finding that S. japonicus strains are respiratory deficient, Yamada and Banno (1987, 1989) and Yamada et al. (1987) divided the fission yeasts at the generic level into Schizosaccharomyces Lindner, with the species “pombe” and “malidevorans”, Octosporomyces Kudriavzev, with the single species “octosporus”, and the new genus Hasegawaea Yamada et Banno gen. nov., with the species “japonica” subdivided into the two varieties “japonica” and “versatilis”. Kurtzman and Robnett (1991) compared partial nuclear ribosomal RNA sequences and determined the
greatest intrageneric nucleotide differences as referenced from the type species as follows: *Saccharomyces* 10.2%, *Debaryomyces/Schwanniomyces* 8.5% and *Schizosaccharomyces* 13.2%. *S. octosporus* and *S. japonicus* are separated by a distance not much greater than that for the extremes in the genus *Saccharomyces*. Therefore, the above mentioned authors suggest that all three presently defined species should remain in the genus *Schizosaccharomyces*. Using nuclear DNA/DNA reassociation techniques, Martini (1991) came to the conclusion that there is a larger difference between *S. japonicus* and the other two species *S. octosporus* and *S. pombe*, but in her opinion it seems more appropriate to maintain the separation of three species within the genus *Schizosaccharomyces*. The universal core of the two major rRNAs is likely to represent functionally equivalent structures in all species (Eckenrode et al. 1984; Cedergren et al. 1988) and therefore appears to be more suited than physiological criteria to establish phylogenetic relationships (Campbell 1974; Berbee and Taylor 1992; Hendriks et al. 1992; Olsen and Woese 1993).

In the present paper we report the sequence of the entire ribosomal RNA cluster of the yeast *S. japonicus* var. *versatilis* and compare it with the sequence established for *S. pombe* and other yeasts for which complete rRNA gene cluster sequences exist.

**Materials and methods**


**DNA isolation.** Was done according to Cryer et al. (1975).

**Establishment of a lambda genomic bank from S. japonicus.** Was done with the Lambda Packaging Kit (Promega, Heidelberg) according to Enquist and Sternberg (1979) and Benson and Taylor (1984).

**Synthesis of oligonucleotides and PCR amplification of DNA probes.** The oligonucleotides used in this work were as follows: 18s nor (5'-CGAGACCTTAACCTG-3') and 25s rev (5'-TTGGA-AGCCCTGCTGGCGG-3'). The amplified product is 2.9 kb and contains 18s rRNA, 5.8s rRNA and parts of the 25s rRNA.

**Identification of positive plaques.** Was done using a DIG-labelled fragment (Mifflin 1987).

**Subcloning.** *Lambda* DNA from a positive clone was subcloned in pUC18 (Ausubel et al. 1992).

**DNA sequencing.** DNA fragments were obtained by the nested deletion method (Henikoff 1984, 1987) using a Nested Deletion Kit (Pharmacia). Sequencing was done with the Sanger method and DIG-labeling with a MWG-Biotech direct blotter using Taq-polymerase and cycle sequencing. Sequence analysis was done with DNASIS 7.0 (Hitachi) and Enhance 1.01 (Scientific and educational software).

**Results and discussion**

The architecture of the rRNA cluster

In eukaryotic cells four different rRNA molecules are found. The 8s rRNA is located in the 40s subunit of the ribosome; 5s, 5.8s and 25s rRNA are components of the 60s ribosomal subunit. The genes for 18s, 5.8s and 25s are organized in tandem repeats. In yeast they are transcribed in a 45s precursor, which is subsequently processed (Barnitz et al. 1982). The repeat units, which contain two internal transcribed spacers, are interrupted by non-transcribed spacers. In *S. pombe* there are 100-150 copies of units of 10.4 kb on both ends of chromosome III. The amount of ribosomal DNA is about 1000 kb, thus covering about some 8.5% of the genome. The amount of rDNA varies from strain to strain (Barnitz et al. 1982; Schaak et al. 1982). By hybridization with a specific gene probe, the amount of rDNA in *S. japonicus* var. *versatilis* was estimated to be about 8% in a genome of some 12000 kb. This indicates that *S. pombe* and *S. japonicus* have about the same composition of rDNA clusters.

Cloning and sequencing of the rRNA cluster

A lambda gene bank was established from *S. japonicus* var. *versatilis* and screened with a PCR-amplified probe. One lambda clone, containing an insert of 19 kb, was subcloned in pUC18. A detailed restriction map of the pUC18 inserts was established (data not shown). A region comprising 6492 bp was sequenced, containing the entire rRNA cluster plus internal transcribed spacers (ITS) and upstream and downstream regions. The 5' and 3' ends of the genes were deduced from the yeast sequences known for *S. cerevisiae* (5.8s rRNA: Rubin 1973; 18s rRNA: Rubtsow et al. 1980; 25s rRNA: Georgiev et al. 1981) and *S. pombe* (5.8s rRNA: Schaak et al. 1982; 18s rRNA: Barnitz et al. 1990; 25s rRNA: Lapeyre et al. 1993).

**Promoter region, internal transcribed spacers and 3' non-transcribed spacer**

Ribosomal RNA genes are transcribed by the RNA-polymerase I. Promoters used by this polymerase are heterogenous and there are no data from yeasts that can be used for a comparative analysis.

Two internal transcribed spacers (ITS) (Musters et al. 1990) are located between the 18s rRNA gene and the 5.8s rRNA gene and also between the 5.8s rRNA gene and the 25s rRNA gene. ITSII contains 186 nucleotides, ITSII 258 nucleotides (Fig. 1C, E). The ITS sequences

**Homology plots and tree analysis.** These were obtained according to the distant matrix method using a Higgins algorithm by analysis of the "nearest neighbour" (Van de Peer et al. 1990).