Arrangement of the ribosomal RNA genes in *Schizosaccharomyces pombe*

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1. INTRODUCTION

In most eukaryotic cells, the genes for the ribosomal RNAs (rRNAs) occur as tandemly repeated units arranged in head-to-tail arrays within the chromosome. The transcription unit for the 18 S, 5.8 S and 25 S rRNAs alternates with a non-transcribed 'spacer' region within each repeat [1]. The 5 S RNA genes are usually organized in separate, tandemly repeated clusters; however, those of the yeasts *Saccharomyces cerevisiae* [2] and *Torulopsis utilis* [3] are located within the ribosomal DNA (rDNA) repeating unit. This is not a universal characteristic of lower eukaryotes as the 5 S RNA genes of *Neurospora crassa* are not located in the major rDNA unit nor do they appear to be tightly clustered [4,5].

The yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* contain approximately the same amount of DNA per haploid genome [6,7]; however, they differ dramatically in their organization. There are 3 linkage groups for the fission yeast *S. pombe* [8], which is in good agreement with the number of cytologically observed chromosomes [9]. In contrast, the budding yeast *S. cerevisiae* has 17 linkage groups [10]. We have examined the ribosomal DNAs of these yeasts to gain information about the functional organization of the individual repeating units and of repetitive DNA in the chromosomes. Like *S. cerevisiae*, the genes for the large rRNAs of *S. pombe* are organized in clusters of tandem arrays of homogeneous repeating units. In contrast, the 5 S RNA genes of *S. pombe* are not located in this repeating unit but are on multiple fragments surrounded by heterogeneous sequences.

2. MATERIALS AND METHODS

2.1. *Plasmids*

Escherichia coli HBt$^-$ [11] was the bacterial strain used for the preparation of plasmid DNA. The plasmid pJHC11, which contains more than one repeating unit of the *S. cerevisiae* rDNA, has been described [12]. pJHC88, which was created by the same procedure, contains *S. cerevisiae* rDNA *Eco*RI fragment B. pYM21 and pYM124 contain *Hin*dIII fragments of *S. pombe* DNA inserted into pBR322 and were isolated on the basis of hybridization to *S. pombe* 5 S RNA [13]. pYM3.2 contains a 214 basepair (bp) *S. pombe Hpa*I–*Taq*I fragment inserted into pBR322 between the *Bam*HI and *Cla*I sites.

2.2. *DNA preparation*

Plasmid DNA was isolated as described [12]. *S. pombe* whole cell DNA from strain 972h$^-$ [8] was prepared from cells grown in 1% yeast extract–3% glucose described for *S. cerevisiae* [11].

2.3. *RNA preparation*

Yeast cells were grown in low phosphate medium [14] and cellular RNA was labeled in vivo with [^{32}P]orthophosphate. 5 S and 5.8 S rRNAs were isolated from cells permeabilized by phenol at 37°C, and purified by DEAE-cellulose chromatography (Whatman DE-23) [15]. The RNAs were fractionated by polyacrylamide gel electrophoresis [14], identified by autoradiography and eluted as in [16]. 18 S and 25 S rRNAs were isolated from zymolyase-generated (zymolyase 60 000 M_r; Kirin Brewery, Takasuki) spheroplasts which were lysed

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with SDS. The lysates were extracted with phenol and the RNA sequentially precipitated by LiCl and ethanol. The RNAs were fractionated on agarose–urea gels [17], located by autoradiography and eluted at 4°C in 0.6 M NaCl/60 mM Na-citrate. *S. cerevisiae* 5 S and 5.8 S RNAs were isolated from strain S288C obtained from G. R. Fink.

2.4. *Electrophoresis and hybridization*

Restriction enzymes were obtained commercially and used as recommended by the source. Gel electrophoresis, Southern transfer and DNA·DNA hybridization were done as in [11] and RNA·DNA hybridization as in [18]. Autoradiography was at −70°C on Kodak XAR-5 film using intensifying screens.

3. RESULTS AND DISCUSSION

3.1. *Organization of* S. pombe *18 S and 25 S genes*

We have examined the arrangement of the rDNA of *S. pombe* by resolving whole cell DNA restriction digests on agarose gels, transferring the DNA to nitrocellulose filters, and subsequently hybridizing these filters to a mixture of ^{32}P-labelled 18 S and 25 S *S. pombe* RNA. Digestion with the enzymes *Hin*dIII, *Bgl*II or *Sst*I each produces a single fragment of 10.4 kilobasepairs that is visible above the background of other cellular DNA restriction fragments on agarose gels, and which hybridizes to 18 S and 25 S rRNA (fig.1, table 1). The enzymes *Hpa*I, *Kpn*I, *Sal*I and *Xho*I also generate single fragments of the same size that hybridize to *S. pombe* rRNA.

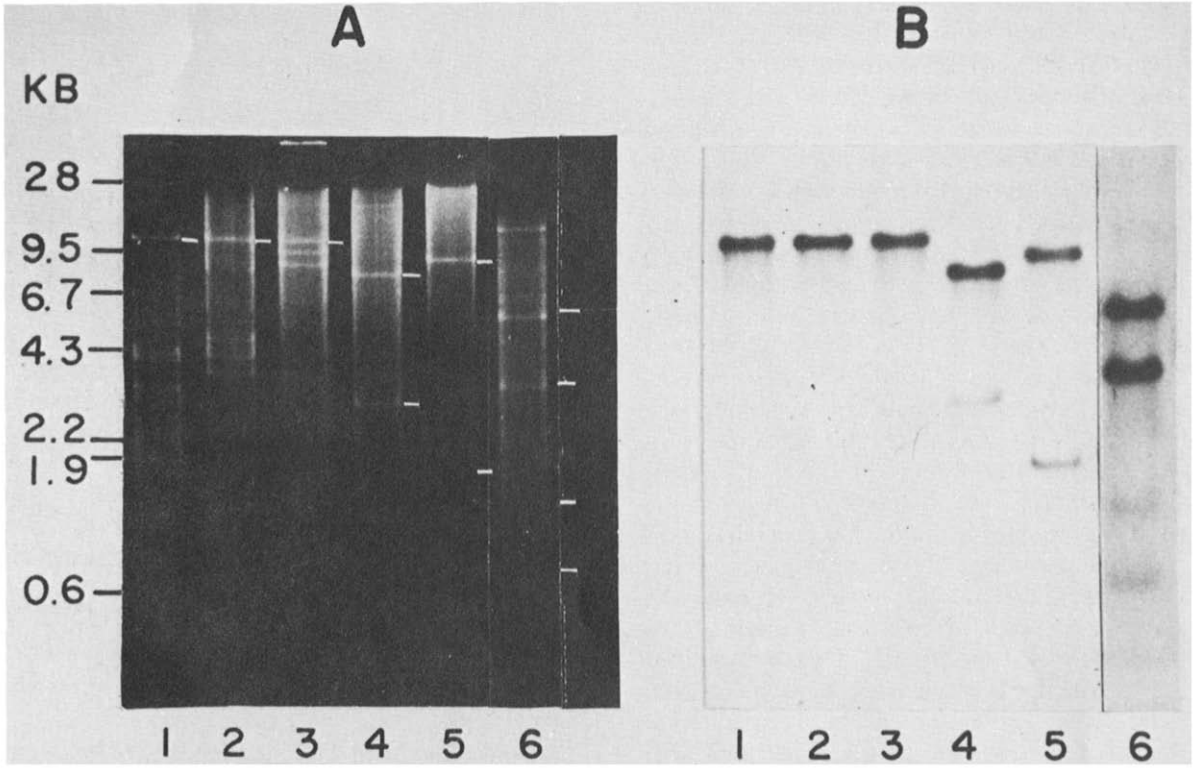

Fig.1. Identification of *S. pombe* genomic rDNA fragments. Restriction endonuclease digests of *S. pombe* whole cell DNA were fractionated on a 1% agarose gel, transferred to a nitrocellulose filter and hybridized to a mixture of ^{32}P-labelled *S. pombe* 18 S and 25 S rRNAs; (1) *Hin*dIII; (2) *Bgl*II; (3) *Sst*I; (4) *Bam*HI; (5) *Bgl*I; (6) *Eco*RI. (A) ethidium bromide-stained gel; (B) autoradiogram following hybridization; white lines, genomic fragments which hybridize to rRNA; black lines, positions of λ *Hin*dIII size standards.

Table 1
Sizes of *S. pombe* rDNA restriction fragments[a]

*Hin*dIII	*Bgl* II	*Sst* I	*Bam*HI	*Bgl* I	*Eco*RI
10.5	10.5	10.4	7.2	8.3	5.2
			2.9	1.9	3.2
					1.3
					0.8
10.5	10.5	10.4	10.1	10.2	10.5

[a] All sizes are given in kilobasepairs
[b] Sizes shown are the average of at least 3 independent determinations. Molecular size markers were *Hin*dIII-digested λ DNA and *Hin*cII-digested pBR322

*Bam*HI and *Bgl*I each yield 2 fragments that hybridize to rRNA, whereas *Eco*RI produces 4 (fig.1, table 1). *Sma*I, *Pst*I and *Pvu*II do not cleave *S. pombe* rDNA. The molecular sizes of the hybridizing restriction fragments in each of these digests totals 10.4 kilobasepairs, indicating that by assessing the homology to the mature 18 S and 25 S rRNA species we are able to detect all or almost all of the rDNA fragments in a genomic digest. The size of the *S. pombe* rDNA repeating unit is somewhat larger than that of *S. cerevisiae* [2,12]. Since the size of the rRNA precursor transcript for both of these yeasts has been reported to be 37–38 S [19,20], the increased length of the *S. pombe* rDNA unit presumably reflects a larger spacer DNA segment separating the rRNA precursor coding regions. The uniformity of the *S. pombe* rDNA repeat size and the absence of heterogeneity in the fragment patterns shows that, like *S. cerevisiae* rDNA [21], most of the *S. pombe* rDNA units are clustered in tandem head-to-tail arrays of homogeneous monomers.

To examine the extent of homology between the rDNAs of *S. cerevisiae* and *S. pombe* we hybridized the ^{32}P-labelled plasmid pJHC11 [12], which contains the entire *S. cerevisiae* rDNA unit, and pJHC88, which contains only non-transcribed spacer and the 5 S RNA gene, to nitrocellulose filters containing *S. pombe* genomic digests. The hybridization pattern for pJHC11 was identical to that in fig.1, whereas no hybridization was observed for pJHC88. In addition, pJHC88 does not hybridize to the *Hin*dIII *S. pombe* rDNA monomer fragment. Purified *S. cerevisiae* and *S. pombe* 5.8 S RNAs each hybridize to the rDNA repeating unit of the other yeast (J.T.B., unpublished).

3.2. *Location of* S. pombe *5 S RNA genes*

The 5 S RNA genes of the yeasts *S. cerevisiae* [2] and *T. utilis* [3] alternate with those for the rRNA precursor in the tandem rDNA repeating units. In *S. pombe*, however, purified ^{32}P-labelled 5 S RNA does not hybridize to the same genomic fragments which are homologous to 18 S and 25 S RNA, nor does 5 S RNA hybridize to a chimeric plasmid which contains the rDNA *Hin*dIII monomer (J.T.B., unpublished). In addition, the 5 S RNA gene contains a *Pst*I site [13,22], an enzyme which does not cleave the rDNA monomer unit. To investigate the genomic organization of the 5 S RNA genes further, we examined the hybridization pattern of the plasmid pYM3.2 to *Hin*dIII, *Bgl*II and *Sst*I digests of whole cell *S. pombe* DNA (fig.2).

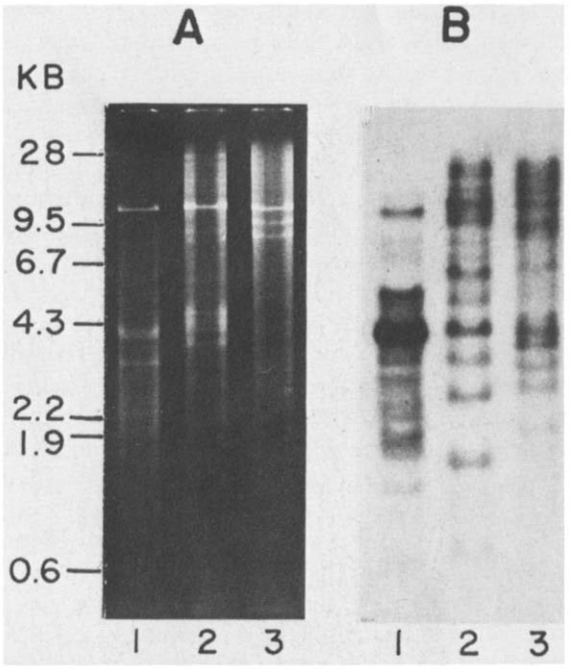

Fig.2. Identification of *S. pombe* genomic 5 S RNA genes. Restriction endonuclease digests of *S. pombe* whole cell DNA were fractionated on a 0.8% agarose gel, transferred to a nitrocellulose filter and hybridized to ^{32}P-labelled pYM3.2: (1) *Hin*dIII; (2) *Bgl*II; (3) *Sst*I; (A) ethidium bromide-stained gel; (B) autoradiogram following hybridization; black lines, positions of λ *Hin*dIII size standards.

pYM3.2 contains the 119 basepair *S. pombe* 5 S RNA gene within a DNA fragment of 214 basepairs inserted into pBR322. In each case, at least 15–20 fragments of different sizes, none of which correspond to the major rDNA repeating unit, contain homology to the plasmid. These results are consistent with those obtained by other investigators who hybridized radioactive 5 S RNA to genomic digests [13,22]. Therefore, the hybridization pattern observed here represents homology between different genomic restriction fragments and the 5 S RNA gene, rather than some other repetitive sequence, on pYM3.2.

The variation in hybridization intensity of the restriction fragments precludes quantitation of the number of different fragments in each digest which hybridize to the 5 S RNA gene. The more intensely hybridizing regions may result from the presence of multiple copies of the same restriction fragment or a number of different restriction fragments on the same molecular size containing 5 S RNA genes. These results demonstrate that the *S. pombe* 5 S RNA genes are not linked to the rRNA precursor gene and that they are not organized as simple repeating units. We cannot discriminate among the possibilities that the 5 S RNA genes are clustered loosely, separated by DNA regions which vary both in size and sequence, that they are dispersed throughout the genome, or some combination of these two arrangements.

When purified ^{32}P-labelled 5 S RNA from *S. pombe* or *S. cerevisiae* is hybridized to plasmids containing the 5 S RNA genes of the other yeast, no hybridization is observed under our standard conditions (J.T.B., unpublished). Six independent *S. pombe* 5 S RNA genes have been sequenced. All of the coding regions are identical and show 32% divergence with respect to the sequence reported for *S. cerevisiae*. The DNA sequences immediately surrounding each of the *S. pombe* 5 S RNA genes were found to be highly heterogeneous [13,22].

Our results indicate that there is considerable homology within the coding region of the rRNA precursor genes of *S. pombe* and *S. cerevisiae* but not within the non-transcribed spacer, the 5 S RNA gene or its flanking sequences. The arrangement of the *S. pombe* rDNA more closely resembles that reported for *N. crassa* [4,5] than that of *S. cerevisiae* [2,12,22].

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