Volume 9 Number 23 1981 Nucleic Acids Research

Nucleotide sequences of the 5S ribosomal RNA genes and their adjacent regions in Schizosaccharomyces pombe

Satoshi Tabata

Institute for Chemical Research, Kyoto University, Uji, Kyoto-fu 611, Japan

Received 15 September 1981

ABSTRACT

The organization of 5S ribosomal RNA (rRNA) genes in the genome of Schizosaccharomyces pombe has been investigated by restriction and hybridization analyses. The 5S rRNA genes were not linked to the other three species of rRNA genes which formed a repeating unit of 6.9 megadaltons, but located in other regions surrounded by heterogeneous sequences. The 5S rRNA gene organization in S. pombe is therefore different from those in other yeasts; Saccharomyces cerevisiae and Torulopsis utilis.

Four restriction segments of different sizes each containing a single 5S rRNA gene were cloned on a bacterial plasmid, and the sequences in and around the RNA coding regions were determined. In the RNA coding regions, the sequences in four clones were identical with an exception that one residue has been substituted in one clone. In the flanking regions, the sequences were extremely rich in the AT-content and highly heterogeneous. The sequences were also markedly different from those in the corresponding regions of the other two yeasts. The presence of T-clusters in the regions immediately after the RNA coding sequences was only notable homology among the four clones and the other two yeasts.

INTRODUCTION

It has generally been accepted that the 5S rRNA gene and the other three species of rRNA genes in eucaryotes are independently organized in tandem arrays of repeating unit (1), but exceptions are those of lower eucaryotes. In Dictyostelium discoideum (2) and two yeast strains; Saccharomyces cerevisiae (3) and Torulopsis utilis (4), the 5S rRNA genes compose the same repeating unit with the other species of rRNA genes.

We have recently cloned one repeating unit of rRNA genes (6.9 megadaltons) from another yeast strain; Schizosaccharomyces pombe. This segment however hybridized with 5.8S, 18S and 25S rRNA but not with 5S rRNA. This implies that the organization of 5S rRNA genes in S. pombe differs from those of S. cerevisiae and T. utilis. It has also been shown that the S. pombe 5S rRNA has a markedly different primary structure from those of the other two yeast strains(5,6,7).

In this study, the structural organization of the 5S rRNA genes in S. pombe was investigated by restriction and hybridization analyses of nuclear DNA. In addition, four different DNA segments each containing a single 5S rRNA gene were cloned on a bacterial plasmid, and the regions encompassing the 5S rRNA genes were sequenced to compare with those in the corresponding regions of the other two yeast strains.

MATERIALS AND METHODS

Strains and plasmids

Schizosaccharomyces pombe (IFO No. 0345) was obtained from Institute for Fermentation, Osaka. Plasmid pKAT1 (Ap^r, Km^r) containing a single HindIII site in the kanamycin resistance gene was constructed from pBR322 (8) and Tn903 (9). Escherichia coli C600 (r_K^-, m_K^-) and LC248 (Hfr, thi, recA) were used as recipients.

Hybridization

DNA segments resolved on agarose gel electrophoresis were denatured and transferred to a nitrocellulose filter (S&S BA85, 0.45μm) as described by Southern (10). For colony hybridization, colonies formed on a agar plate were transferred onto a nitrocellulose filter and lysed on the filter by the procedure of Grunstein and Hogness (11). The filter was dipped into a hybridization mixture containing 50% formamide/5 x SSC (SSC: 0.15 M NaCl/0.015 M Na-citrate) and approximately 1 x 10^6 cpm of ^{32}P-labeled rRNA. After incubation for 10 to 20 hrs at 42°C, the filter was washed with 5 x SSC and dried for autoradiography.

Cloning of the 5S rRNA gene

S. pombe nuclear DNA was digested by HindIII and enriched in the 5S rRNA genes by 1% agarose gel electrophoresis. Restriction segments extracted from agarose gel were ligated with the HindIII digest of pKAT1, and transformation was carried out by using E. coli C600 (r_K^-, m_K^-) as a recipient. Ampicillin-resistant and kanamycin-sensitive colonies were selected and colony hybridization tests were carried out by using ^{32}P-labeled S. pombe 5S rRNA as a probe. Plasmid DNA was first isolated from hybridization-positive clones in a small scale (100 ml) and then propagated in E. coli LC248 after re-transformation.

Other procedures

The procedures for preparation of S. pombe nuclear DNA, plasmid DNA and S. pombe rRNAs, bacterial transformation, restriction analysis, end labeling by polynucleotide kinase reaction and sequence determination have pre-

viously been described (4). The labeling of 5S rRNA was performed by using T4 RNA ligase and [5'-^{32}P]$_p$C$_p$ (12).

RESULTS

Organization of rRNA genes in the S. pombe genome

It has been reported that in yeasts, like many other eucaryotes, rRNA genes tandemly repeat and generate discrete bands when digested with several restriction endonucleases (3,4). Digestion of S. pombe nuclear DNA by BamHI generated two discrete bands of 4.9 Md and 2.0 Md (Fig. 1A and Fig. 2). Southern hybridization analysis with 5.8S, 18S and 25S rRNA respectively showed that 5.8S and 18S rRNA hybridized to the 4.9 Md DNA segment and 25S rRNA to the 2.0 Md segment and slightly to the 4.9 Md segment (Fig. 1A). Digestion of nuclear DNA by HindIII and EcoRI, on the other hand, produced a 6.9 Md and 3.6 Md, 2.1 Md, 0.86 Md and 0.42 Md band, respectively, with strong densities (Fig. 1B and Fig. 2). Hybridization experiments using a

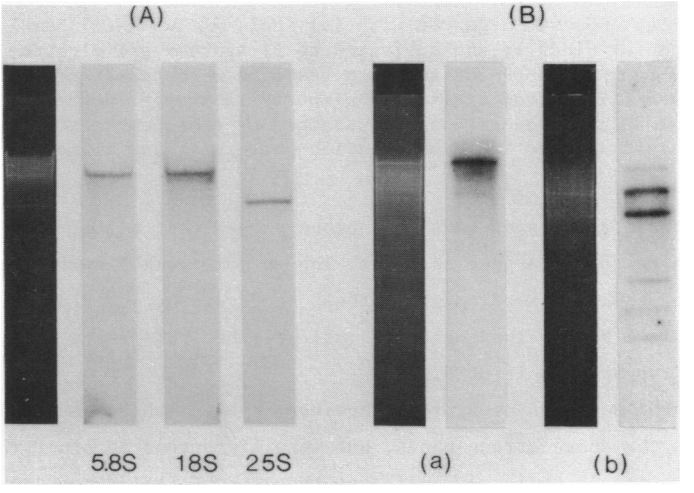

Fig. 1 Agarose gel electrophoresis of the restriction endonuclease-digested S. pombe DNA and hybridization tests with rRNAs. Restriction endonuclease digests of S. pombe DNA were subjected to 1% agarose gel electrophoresis for about 2 hrs at 100 V. DNA segments resolved were transferred to nitrocellulose filters and hybridized with ^{32}P-labeled rRNAs. For each digest, the gel pattern visualized by ethidium bromide staining is shown in the left lane and its hybridization pattern in the right lane(s). (A) BamHI digest of S.pombe nuclear DNA was hybridized with the indicated species of rRNA. (B) (a) HindIII and (b) EcoRI digest of S. pombe DNA were hybridized with a mixture of 5.8S, 18S and 25S rRNA.

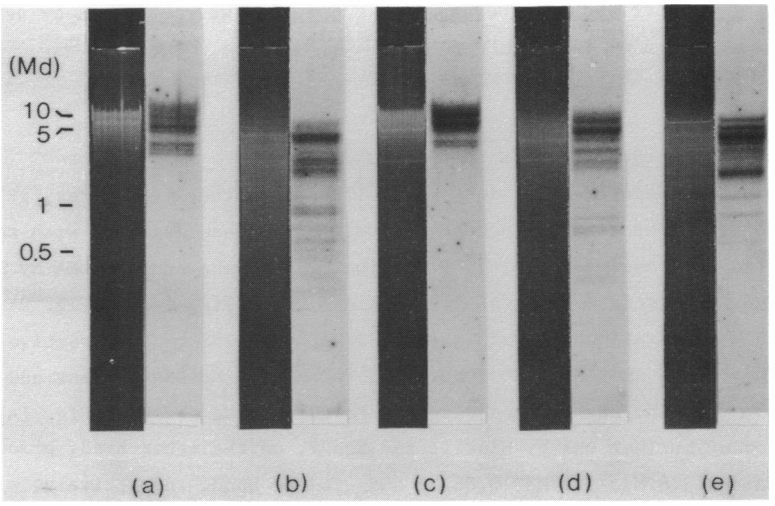

Fig. 2 Agarose gel electrophoresis of S. pombe nuclear DNA digested by restriction endonucleases and hybridization tests with 5S rRNA.
S. pombe nuclear DNA was digested with (a) KpnI, (b) AccI, (c) BamHI, (d) EcoRI and (e) HindIII; and subjected to 1% agarose gel electrophoresis for 2 hrs at 100V. DNA segments resolved were transferred to nitrocellulose filters and hybridized with ^{32}P-labeled S. pombe 5S rRNA. For each digest, the gel pattern stained by ethidium bromide is shown in the left lane and its hybridization pattern in the right lane.

mixture of three species of rRNA as a probe showed that all of these bands corresponded to the rRNA gene segments. Though some weakly hybridizable bands were observed in EcoRI digest, these bands did not overlap with the strongly stained bands. These results indicate that rRNA genes in S. pombe construct a repeating unit of 6.9 Md.

When 5S rRNA was used as a probe, however, hybridizable bands did not overlap with the above strong bands, but were widespread in other regions (Fig. 2). The result obviously indicates that the 5S rRNA genes are not linked to the repeating unit of the other three species of rRNA genes. The same conclusion was drawn from the fact that the cloned HindIII segment corresponding to the one repeating unit of the rRNA genes hybridized with 5.8S, 18S and 25S rRNA but not with 5S rRNA (data not shown).

Cloning of DNA segments containing 5S rRNA genes

The 5S rRNA gene regions in the HindIII digest of the genome were detected by hybridization tests and two heavy band regions indicated in Fig. 3A were cut out from the gel. DNA was extracted from each band and cloning

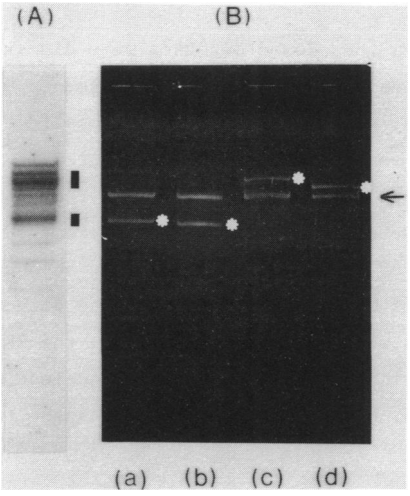

Fig. 3 Agarose gel electrophoresis of HindIII digests of plasmids containing 5S rRNA genes.
(A) Hybridization patterns of the HindIII digest of S. pombe DNA with 5S rRNA. The 5S rRNA gene regions used for cloning are indicated by bars (see text). (B) HindIII digests of pSPr1 (lane a), pSPr11 (lane b), pSPr36 (lane c) and pSPr41 (lane d) were electrophoresed for 2 hrs at 100V on 1% agarose gel, and stained by ethidium bromide. The vector segment is indicated by arrow and the bands hybridized with 5S rRNA by asterisks.

with E. coli plasmid pKAT1 was performed. As a result, four clones carrying 5S rRNA genes were independently isolated. In Fig. 3B, HindIII digests of these plasmids are shown. Three plasmids, pSPr1, pSPr11 and pSPr41, produced two segments and the other plasmid, pSPr36, produced three segments, smaller two of which were very near to each other, respectively. As indicated in Fig. 3B, one of the segments produced from each plasmid corresponds to the vector molecule. The result therefore indicates that a single segment has been cloned in pSPr1, pSPr11 and pSPr41, and two segments in pSPr36, respectively. The hybridization tests with 5S rRNA demonstrated that the cloned segment in each plasmid indeed contained the 5S rRNA genes. Though two segments have been cloned in pSPr36, only one of the segments showed affinity to 5S rRNA. The hybridizable bands are indicated by asterisks on the gel patterns in Fig. 3B. The size of the hybridizable segment in each clone was approximately identical to those of the original segment which was used for ligation.

Localization of 5S rRNA genes within the cloned segments

According to the sequence data of S. pombe 5S rRNA (7), the recognition

sites for AccI and PstI are located near the 5' end and in the middle of the gene, respectively. When the cloned segments were digested with these two enzymes, each of them was cleaved into three fragments by AccI and two fragments by PstI. Thus, restriction maps were constructed by combined digestion of these two enzymes and either EcoRI or HhaI. The 5S rRNA gene was localized on the maps based on the fact that the PstI and AccI sites in the RNA coding region were separated by approximately 50 base pairs (Fig. 4).

Sequence determination of 5S rRNA genes and their adjacent regions

The sequence analysis was performed in both the directions from each of the AccI and PstI sites which were located within the RNA coding regions. Sequenced regions were indicated in Fig. 4. The nucleotide sequences deduced are indicated in Fig. 5, by aligning them with respect to the RNA coding regions.

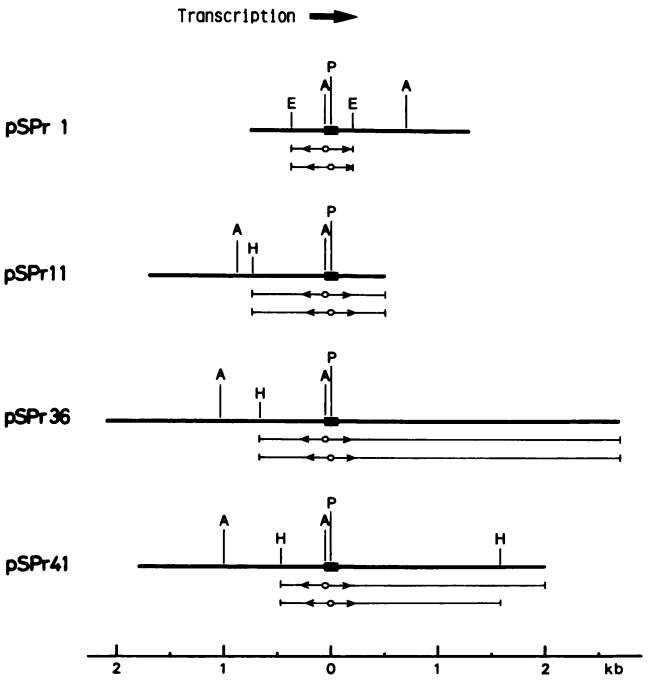

Fig. 4 Physical maps of the four cloned segments and the strategy for sequencing.
The 5S rRNA genes are indicated by thick lines. Thin horizontal lines represent the fragments used for sequence analysis. The AccI and PstI ends labeled by ^{32}P are indicated by open circles and the direction of sequencing by arrow heads. Restriction sites used for sequencing are abbreviated as follows; AccI: A, PstI: P, EcoRI: E, HhaI: H.

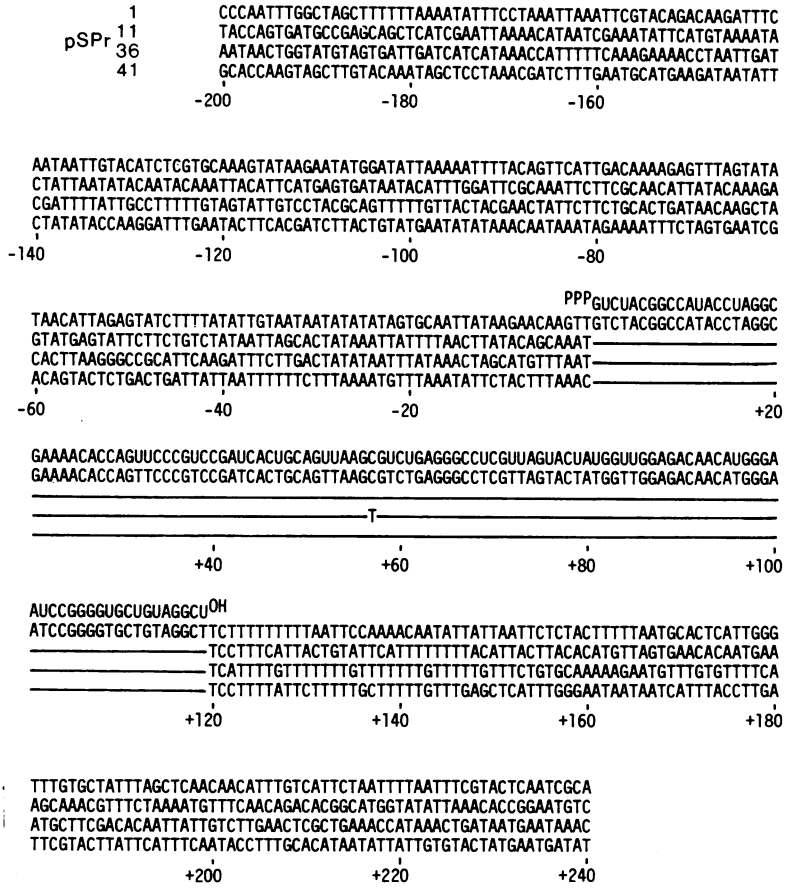

Fig. 5 Nucleotide sequences of S. pombe 5S rRNA genes and their flanking regions.
The sequences in the non-coding strand are shown. In the RNA coding region, the entire sequence is shown in the lane of pSPr1 and in the other lanes, only the substituted residue is indicated. The 5S rRNA sequence determined by Komiya et al. (7) is given in the corresponding region. The sequence is numbered from the first residue of the RNA coding region and the upstream region is denoted by "minus" sign.

DISCUSSION

It has been shown that in two yeast strains, S. cerevisiae and T. utilis, the 5S rRNA gene and the other three rRNA genes are linked on the repeating units of 5.8 Md (3) and 8.0 Md (4), respectively. In S. pombe, however, only the genes for 5.8S, 18S and 25S rRNA constitute a repeating unit of 6.9 Md and the 5S rRNA genes were detected in heterogeneous segments ranging from

0.3 Md to 10 Md when digested with restriction endonucleases. It is therefore clear that the 5S rRNA genes in S. pombe are not linked to the other three species of rRNA genes, but located in other regions surrounded by heterogeneous sequences. This type of rRNA gene organization has been reported in Neurospora crassa (13).

We did not clone a DNA segment large enough to contain two or more 5S rRNA genes, so that it is not known whether the 5S rRNA genes are clustering or not. As seen in Fig. 1, hybridizable bands detected in KpnI and BamHI digests were larger in size and fewer in number than those in AccI, EcoRI and HindIII digests. This may suggest that the 5S rRNA genes in S. pombe are linked in one or more clusters. If this is the case, the spacer regions among the 5S rRNA genes should be highly heterogeneous.

We have cloned four segments each containing a single 5S rRNA gene and the nucleotide sequences in and around the 5S rRNA genes were compared. The sequences in the RNA coding regions were identical with the four clones except that C at position 57 has been replaced by T in one clone. In contrast, the sequences in the flanking regions were highly heterogeneous. Another feature in the flanking regions was their high AT contents. The AT content in positions -200 to -1 was 72.8% and that in positions 120 to 240 was 71.5%, in contrast to the RNA coding region of which the AT content was 45.4%.

It has been shown that the sequences essential for promotion function in the 5S rRNA gene expression are located within the RNA coding region (14,15). Other lines of evidence suggests that the upstream region also involves in regulation of promoter function (14). In the upstream regions of the RNA coding regions, we could find neither significant homologous sequences nor repeating sequences common to all the four clones. A run of AT residues seems to commonly occur at positions -29 to -23, but their sequences are not unique. We have also compared the sequences with those in the corresponding regions of S. cerevisiae (16,17) and T. utilis (4) but could not detect any significant homology.

The RNA coding sequences were followed by T clusters interrupted by a small number of GC pairs. Such structures are also seen in the corresponding regions of other eucaryotes including the two yeasts, and may be common to the termination sites of the 5S rRNA transcription.

ACKNOWLEDGEMENTS

I wish to thank Dr. M. Takanami for his encouragement through the course of this investigation and his help in preparation of the manuscript.

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