The number of ribosomal RNA genes in *Thermus thermophilus* HB8

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ABSTRACT

We have examined the number of rRNA genes in Thermus thermophilus HB8 by hybridization of Bam HI -, Hind III - and Pst I - digests of DNA to 3'-(^{32}P) 23S, 16S and 5S rRNAs according to the Southern procedure. The restriction gels gave two radioactive bands with 23S and 5S rRNA. Furthermore, band positions were indistinguishable from one another when 23S and 5S rRNAs were used as probes to Bam HI and Hind III digests, indicating that each band contains sequences corresponding to the 3'-end of 23S and 5S rRNAs. The Pst I digest also gave two radioactive bands with 23S and 5S rRNAs as probes, where one band position was identical, but the other different. The 16S rRNA did hybridize with two fragments, using a Bam HI, as well as a Bam HI - Hind III double digest. The Hind III digest gave one band using 16S rRNA as a probe.
It is concluded that the Thermus thermophilus HB8 chromosome carries at least two sets of genes for 23S, 16S and 5S rRNAs.

INTRODUCTION

It was shown that Escherichia coli genes for 16S, 23S and 5S rRNAs are arranged as one transcriptional unit (1,2). It has long been known that these genes are redundant in bacteria, their number has been determined as 8 for 5S rRNA, while it is 7 for 16S and 23S rRNAs (3). In each transcriptional unit, the number of each rRNA gene is 1, with the exception of rrnD (71 min), where two 5S rRNA genes are organized tandemly at the end of the operon (4). It was recently shown (5) that Mycoplasma mycoides subsp. capri and Acholeplasma laidlawii reveal two rRNA cistrons, where one rRNA cistron is present in Mycoplasma capricolum. The organization of the rRNA structural genes in the archaebacteria Thermoplasma acidophilum (7) and Halobacterium halobium (6) was investigated. In Thermoplasma acidophilum (7) the structural genes for the rRNA occur once per genome in the order 5' - 23S - 5S - 16S - 3' and in contrast to eubacteria and eukaryotes they appear unlinked. In the Halobacterium haloberium (6) the rRNA cistrons are physically linked in a eubacterial fashion (5' - 16S - 23S - 5S - 3'), nevertheless do they differ from eubacteria in having only one copy of the rRNA gene cluster per

genome. Evidence was presented (8,9) that the copy number of the 5S rRNA genes of Bacillus subtilis is at least ten, since each 5S rRNA gene is closely linked to the 23S rRNA genes and the transcriptional units of the rRNAs is in all probability ten, and it was therefore suggested that no rRNA operon has tandemly repeated 5S rRNA genes.

MATERIALS AND METHODS

Thermus thermophilus HB8 (= ATCC 27634) cells were grown at 75° C as described by Oshima et al. (10). The rRNAs were prepared by the phenol method from the 70S ribosomes or from 50S and 30S ribosomal subunits (11), to avoid a cross contamination between 23S and 16S rRNA, the 23S rRNA was prepared from the 50S ribosomal subunit and the 16S rRNA from the 30S ribosomal subunit and purified by electrophoresis on 3.6 % polyacrylamide gels containing 35.5 mM Tris-phosphate (pH 7.8), 1 mM EDTA and 0.05 % SDS. 5S rRNA was chromatographed on Sephadex G-100 (11), pooled and electrophoresed on a 10 % polyacrylamide gel containing 7M Urea, 50 mM Tris-borate (pH 8.3) and 1 mM EDTA. The intact 16S, 23S and 5S rRNA were eluted from gel slices in 500 mM ammonium acetate 10 mM $Mg(OAc)_2$, 0.1 % SDS and 0.1 mM EDTA at 37° C overnight. The eluate was freed from polyacrylamide by Millipore filtration, ethanol precipitation, dissolved in destilled water, and then used for the 3' - end labelling (12).

For 16S and 23S rRNAs, the labelled rRNA was recovered by phenol extraction (8). In the case of 5S rRNA the reaction mixture was subjected to 10 % polyacrylamide, 7M Urea gel electrophoresis. The radioactive 5S rRNA band was then cut out and eluted as described above. The rRNAs were precipitated by cold ethanol with yeast tRNA as carrier and dissolved in 5 x SSC (1 x SSC: 0.15M NaCl, 0.015M Na-citrate) containing 50 % formamide.

Thermus thermophilus DNA was prepared using a method based on Marmur (13). The digestions of the DNA with the restriction endonucleases, Bam HI, Hind III and Pst I (from P.L. Biochemicals) were carried out as described by the manufacturer. The DNA digests were resolved by 0.8 % agarose gel electrophoresis, subjected to denaturation by alkali treatment and transferred to Millipore filters according to the Southern procedure (14). The labelled rRNA was brought to a final radioactivity of 1.5×10^6 cpm/ml and the filters were wetted with the labelled rRNA solutions (approximately 50 $\mu l/cm^2$). Hybridization was carried out at 37° C for 18 hours. After the incubation, the filter was washed with 2 x SSC (50 ml) for 1 min, followed by a wash with 2

x SSC (20 ml) containing RNase A (10 µg/ml) for 30 min and washed twice with 2 x SSC (50 ml) for 30 min and then dried at room temperature.

RESULTS

Thermus thermophilus DNA was digested with several restriction endonucleases. The digests were resolved by 0.8 % agarose gel electrophoresis, transferred to Millipore filters and hybridized to 3' - end labelled 23S, 16S - and 5S rRNAs by the Southern method (14).

The *Bam* HI digested DNA gel displayed two radioactive bands of 8.5 and 3.8 kb long, equally with 23S and 5S rRNA. Similarly, the *Hind* III digested DNA gels gave two radioactive bands 15 and 8 kb long, equally with 23S and 5S rRNA. In addition, the *Pst* I digested DNA gels showed bands of > 23.7 and 8 kb length, using 5S rRNA as a probe, and two bands 12 and 8 kb long using 23S rRNA as a probe. Finally, the DNA was digested with two endonucleases; the combination *Bam* HI - *Hind* III gave one band 3.8 kb long, equally with 23S and 5S rRNA. Identical experiments using 3' - end labelled 16S rRNA as a probe gave also a specific hybridization pattern with those endonuclease digests. In each digest two radioactive bands could be identified (Table 1). 16S rRNA as well as 5S and 23S rRNA hybridized to *Bam* HI DNA digest fragments of 8.5 kb. In addition, the autoradiogram shows a radioactive band 2.6 kb long which had hybridized to 16S rRNA. The *Hind* III digested DNA gel has one band of 12 kb length, in the *Pst* I digested DNA one band of > 23.7 kb could be identified. The combination *Bam* HI - *Hind* III, resulted in two radioactive bands of 8.5 and 2.6 kb.

Table I

The size (kilobases) of DNA fragments hybridizing with rRNAs

Restriction enzyme fragment	Ribosomal RNA Species hybridized		
	5S rRNA	23S rRNA	16S rRNA
Bam HI	8.5 3.8	8.5 3.8	8.5 2.6
Hind III	15 8	15 8	12
Pst I	>23.7 8	12 8	>23.7
Bam HI *Hind* III	3.8	3.8	8.5 2.6

The sizes (kilobases) of DNA fragments hybridized to rRNAs were measured by co-electrophoresis of *Hind* III of λ DNA (15).

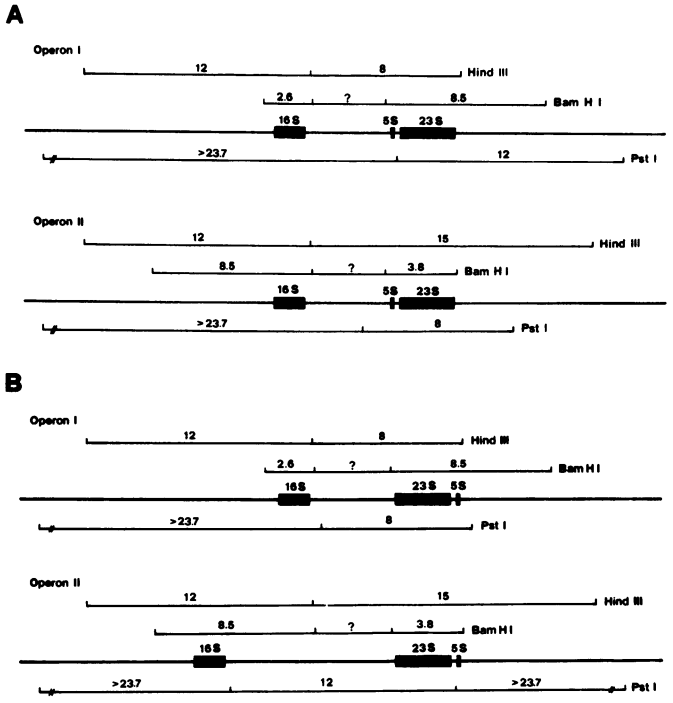

Figure 1. Proposed restriction pattern for Thermus thermophilus. (A) Based on an assumed 3' - 16S - 23S - 5S - 5'gene arrangement. (B) Based on an assumed 3' - 16S - 5S - 23S - 5'gene arrangement.

DISCUSSION

Recently, we have determined the nucleotide sequence of 5S rRNA from Thermus thermophilus (16,17) which showed little heterogeneity in the internal region, an observation which may be of importance in correlating the copy number of 5S rRNA genes to the remaining transcriptional unit. In addition, we often observed that the amount of ribosomes obtained from Thermus thermophilus cells is significantly smaller compared to Escherichia coli, suggesting a correlation between copy number of genes for rRNA and yield of ribosomes. From the inspection of the 5S rRNA sequence, it was determined that the gene for this rRNA should not have a nucleotide sequence which would be recognized by the restriction endonucleases Bam HI, Hind III and Pst I. Therefore, we concluded that a DNA fragment which will hybridize to 3' - end labelled 5S rRNA must represent the whole structural gene for 5S rRNAs.

The 8.5 and 3.8 kb Bam HI fragments which hybridized to 23S and 5S rRNA should include the whole 5S rRNA gene and at least a part of the 3' - end

of the 23S rRNA. We like to suggest that the 8.5 kb Bam HI fragment reacting with 16S rRNA is different. The Hind III digestion resulted in two fragments, 15 and 8 kb in size, which hybridize to 23S and 5S rRNA, and 12 kb fragments which hybridized to 16S rRNA.

The double digest, Bam HI - Hind III, resulted in one band of 3.8 kb fragments, which hybridized with 23S and 5S rRNA. The 16S rRNA did not hybridize with this fragment. We have to assume that a second fragment nearly identical in size is produced by a Hind III cut in the 8.5 kb Bam HI digest, i.e. the 3.8 kb band represents fragments containing the 23S and 5S rRNA genes from both operons. The 16S rRNA did hybridize with two fragments, 8.5 and 2.6 kb, i.e. the double digest is comparable to the Bam HI digest alone.

The band pattern of the Pst I digest shows a difference between 23S and 5S rRNA hybridization. Although one band, 8 kb, could be correlated to both probes the other two bands showed a difference in size, namely > 23.7 and 12 kb, suggesting that one rRNA operon seems to have a Pst I site between the 5S and 23S rRNA gene.

We tried to accomodate the hybridization patterns obtained for the three rRNA types with two restriction patterns which seem to be most reasonable (Fig. 1). Thermus thermophilus is an eubacterium and we tried to fit the data in the classic prokaryote fashion. We feel more comfortable to propose a gene arrangement linked in the order 5' - 16S - 5S - 23S - 3' or 5' - 23S - 5S - 16S - 3' and in contrast to other eubacterial and eukaryotes they appear unlinked, as it was reported for the archaebacterium Thermoplasma acidophilum (7).

It is thus concluded that the chromosome of Thermus thermophilus carries at least two sets of genes, each containing the genes for 16S, 5S and 23S rRNA.

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