
Variable rRNA gene copies in extreme halobacteria

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ABSTRACT

Using PFG electrophoresis techniques, we have examined the organization of rRNA gene in halobacterium species. The results show that the organization of rRNA genes among closely related halobacteria is quite heterogeneous. This contrasts with the high degree of conservation of rRNA sequence (1). The possible mechanism of such rRNA gene amplification and its evolutionary implications are discussed.

INTRODUCTION

A fundamental question regarding archaeobacterial phylogeny is how their genome compares within this group of microorganisms and with eubacteria and eukaryotic organisms. The genomic organization of rRNA is one of the systems used to address this basic question. It was highly surprising to find that the first archaeobacterial species analyzed, H. halobium (2), had only one rRNA gene as opposed to the multiple copies found in eubacteria (seven for E. coli) and the high number of copies in the eukaryotic kingdom. Studies of rRNA gene organization with sulphur dependent archaeobacteria showed that the single-rRNA gene structure is more generalized than previously had been thought (3), although this type of organization is not characteristic of all archaeobacteria. For instance, the methanogenic branch of archaeobacteria showed a more complex structure containing two rRNA genes for Mb. thermoautotrophicum (4) and four genes for Mc. vannielli (5). Large DNA technology and PFG electrophoresis have allowed an efficient and accurate means for analyzing the genomic organization of halobacteria.

MATERIAL AND METHODS

Haloarcula californiae ATCC 33799, "Halobacterium marismortui", Haloferax gibbonsii ATCC 33959, Halococcus morhuae, Halobacterium halobium NCMB 777, Halobacterium salinarium CCM 2148 were used.

Details of the DNA preparation, digestion and electrophoresis are as described in references 6, 7. DNA obtained from each preparation was prerun with 100 second pulses in order to remove plasmids and degraded DNA. Macrorestriction fragments were resolved by PFG electrophoresis in the conditions described in the legends of the figures. The PFG electrophoretic gels were blotted and hybridized (8) with ³²P labeled H. mediterranei 16S and 23S rRNA.

RESULTS AND DISCUSSION

Autoradiography of the blotted PFG electrophoresis of the macrorestriction fragments correspondent to different halobacteria revealed hybridization to various fragments (fig 1 and 2). Table 1 shows that the number of fragments detected is constant for each organism and independent of the restriction enzymes (DraI, NotI, SfiI, BamHI) and probe used (16S or 23S rRNA).

Detection of multiple fragments using rRNA probes is not due to partial digestion of the genomic DNA by the restriction enzymes. Close examination of the ethidium bromide photograph of PFG experiments like those shown in Figure 1 and 2 reveal no evidence of partial digestion and the same number of

TABLE 1. Summary of PFG experiments using chromosomal DNA from different halobacteria, cut with different restriction enzymes and hybridized with 23S and 16S rRNA probes.

| Microorganism | Enzymes used | | | | Minimum gene number |
|--|--------------|------|------|-------|---------------------|
| | NotI | DraI | SfiI | BamHI | |
| <u>Haloarcula californiae</u> ATCC 33799 | * | * | * | * | 4 |
| <u>Haloferax gibbonsii</u> ATCC 33959 | * | * | * | * | 4 |
| <u>Halobacterium halobium</u> NCMB 777 | * | * | * | * | 3 |
| " <u>Halobacterium marismortui</u> " | * | * | * | * | 3 |
| <u>Halococcus morhuae</u> NCMB 757 | * | * | * | * | 2 |
| <u>Halobacterium salinarium</u> CCM 2148 | * | * | * | * | 1 |

hybridization fragments is obtained with the various restriction enzymes (Table 1). The digestion patterns are reproducible and no further digestion is obtained with addition of fresh enzyme and/or longer incubation times.

Detection of multiple fragments using rRNA gene probe is not due to the presence of restriction site in 16S or 23S rDNA because: i) the same hybridization pattern is obtained with both rRNA probes, ii) the highly homologous 16S rRNA sequences of three taxonomically distant halobacteria, *H. halobium*, *H. volcanii* and *H. morrhuae*, lack restriction sites for any of the

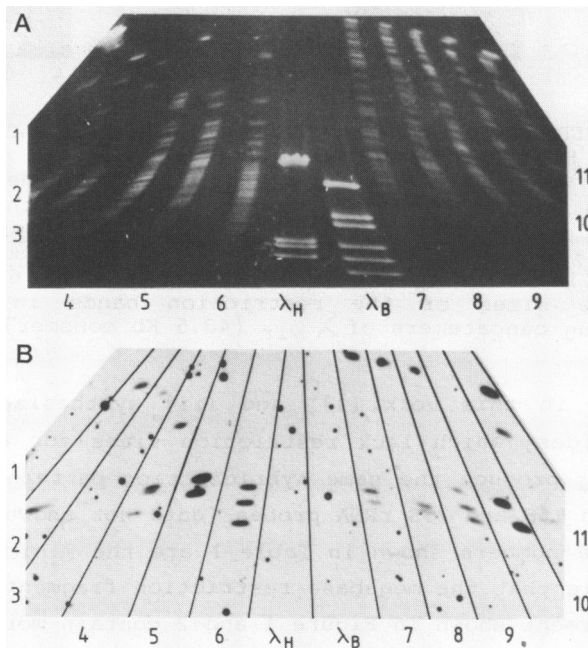


FIG. 1. PULSED FIELD GEL ELECTROPHORESIS OF HALOBACTERIAL DNA (A) AND SOUTHERN HYBRIDIZATION WITH *H. mediterranei* 16S rRNA (B).

Samples were separated in a 55 cm double inhomogeneous PFG apparatus for 72 hours at 500 volts with 5 second pulses between 90° field reorientations. The lanes are: 1 *Saccharomyces cerevisiae*, 2 and 7 *Haloarcula californiae*; 3 and 8 "*Halobacterium marismortui*"; 4 and 9 *Haloferax gibbonsii*; 5 and 10 *Halococcus morrhuae*; 6 and 11 *Halobacterium halobium*, digested with NotI (lanes 2 to 6) and DraI (lanes 7 to 11). λ_H and λ_B are digestions of λ vir (48.5 Kb) with HindIII and EcoRI (λ_H) and BstI (λ_B).

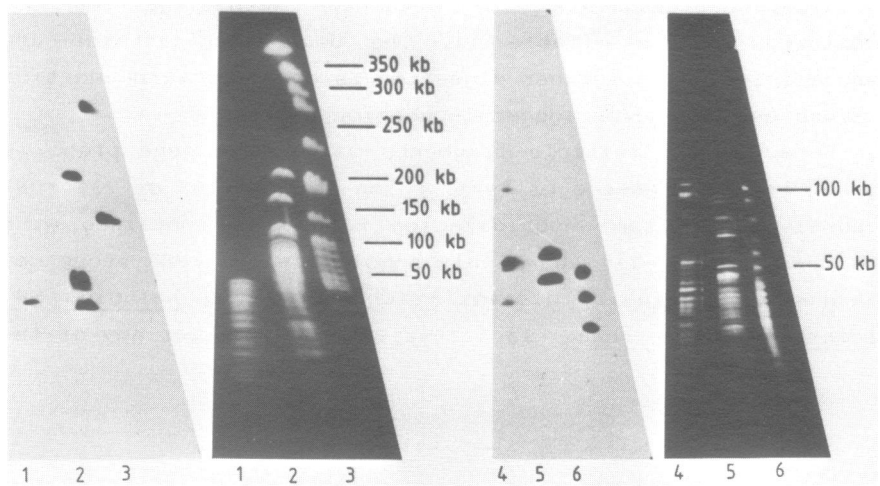


FIG. 2. PULSED FIELD GEL ELECTROPHORESIS OF HALOBACTERIAL DNA and SOUTHERN HYBRIDIZATION WITH *H. mediterranei* 23S rRNA.

Samples were run in the same conditions as described in Fig. 1 with the exception of the pulse time that was 25 seconds. The lanes are: 1, *Halobacterium salinarium*; 2 and 4, *H. gibbonsii*; 3 and 5 *Hc. morrhuae*; 6, *H. halobium*, digested with BamHI (lanes 1 to 3) and NotI (lanes 4 to 6). Numbers indicate the sizes of the restriction bands in kilobases obtained using concatemers of λ_{vir} (48.5 Kb monomer).

enzymes used in this work (1), and iii) synthesized 5S rRNA oligonucleotides, which lack restriction sites for any of the enzymes used, produce the same hybridization patterns as those obtained with 16S and 23S rRNA probes (data not shown).

The gene numbers shown in Table 1 are the minimal values. It is possible that the megabase restriction fragments detected in the experiment shown in Figure 1 and 2 contain more than one copy of rRNA genes, which might explain why in the southern hybridization of Figure 1 some bands do not show equivalent density, suggesting the presence of more than one rRNA gene in some of the bands.

Our results show that there is considerable variation in the number of rRNA genes in Halobacteria. The one rRNA gene detected for *H. salinarium* CCM 2148 in this study is the same as was reported for *H. halobium* strain R1 (2) and *H. cutirubrum* (9) and with the taxonomic concept that all three are indeed the same species (10). Three rRNA genes have been detected for

H. halobium NCMB 777, a different species from the H. halobium R1, which corroborates the taxonomic incongruence of giving the same name (11) to two very different species. There is a reference to the possibility of two rRNA genes for H. volcanii (12), which agrees with the gradient of rRNA genomic organization reported in this work.

Earlier studies showed that the sulfur dependent branch of archaeobacteria is uniform in having only one copy of its rRNA genes, although some differences in their arrangements (3) were reported. The situation is quite different in the methanogenic-halobacterial branch. In contrast to the presence of a single gene in H. halobium R1 and H. cutirubrum, the reported number in the methanogens varies: two for M. thermoautotrophicum and four in Mc. vannielli. It is obvious that the presumed difference in rRNA gene organization between halobacteria and methanogens disappears when the diverse numbers of halobacterial rRNA gene copies are considered. The characteristic differences between rRNA gene organization of the sulfur dependent and the methanogenic-halobacterial branches of archaeobacteria may be of phylogenetic relevance.

It has been reported that halobacteria have high numbers of insertion elements (12), these mobile elements might facilitate chromosome rearrangement.

Current literature contains many references to the evolutionary importance of the gradient of rRNA genes ranging from one in the sulfur dependent archaeobacteria to between one and four in the methanogenic-halobacteria, seven in the eubacterial gram negative E. coli, and several hundred in eukaryotic systems (13). The most frequent explanation attributes this variability to the adjustment of the transcription of rRNA and the translation of the ribosomal proteins needed for the adequate assembly of the ribosomes. So far it has been difficult to correlate this gene amplification with any known advantage other than relaxation of the type of selective pressure which would exist when only one copy of rRNA gene is present. Clearly further studies of halobacterial chromosomal organization are needed to determine the mechanism of rDNA amplification, as well as how stable these multiple

copies are, and the phenotypic difference, if any, that multiple rRNA genes confer on the various species. Currently we are constructing chromosomal physical maps of these organisms in order to investigate these important questions.

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