# THE NUMBER OF rRNA GENES IN *ESCHERICHIA COLI*

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## 1. Introduction

The three different ribosomal RNA species of *Escherichia coli* (5 S, 16 S and 23 S) originate from a single 30 S precursor RNA. The DNA coding for this 30 S rRNA is called an rRNA gene set (*rrn*). It has long been known that these genes are redundant in bacteria, their number has been estimated on the basis of saturation hybridization experiments as being 5–10 (for a review see [1]). The most reliable measurements suggested the existence of six *rrn* genes [2]. Plasmids and transducing phages helped to map unambiguously five of these, which are now designated *rrn*A (85 min), *rrn*B (88 min), *rrn*C (83 min), *rrn*D (71 min) and *rrn*E (89 min) [3]. In this paper we present evidence strongly suggesting that the actual number of rRNA gene sets is seven in *E. coli* K12.

## 2. Materials and methods

*Bam*HI and *Sal*I restriction endonucleases were purified from *Bacillus amyloliquefaciens* H and *Streptomyces albus* G by established protocols [4].

High molecular weight *E. coli* DNA was prepared from JC5466 (K12) as described earlier [5] and λ*rif*d18 DNA as described in [6].

Restriction enzyme digestions were performed in mixtures containing 10 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 50 mM NaCl, 0.5 μg λ*rif*d18 DNA or 8 μg bacterial DNA and 2–5 μl *Bam*HI or *Sal*I enzymes in 30 μl vol. The samples were incubated at 37°C for appropriate length of time to obtain complete digestion.

Restriction fragments were electrophoresed in 1% agarose (Sigma) gels according to [7] at 30 V for 15 h. The gels were stained with ethidium bromide and photographed in order to locate *Bam*HI and *Sal*I fragments of λ*rif*d18 DNA which were used as molecular weight standards [8]. The DNA fragments were then transferred from the gels to nitrocellulose filters (Sartorius) according to [9]. Filters were hybridized either to $^{32}$P-labeled 16 S rRNA (0.5 μg/ml, 1 750 000 cpm/μg) or $^{32}$P-labeled 23 S rRNA (1 μg/ml, 350 000 cpm/μg) in the presence of cold 23 S rRNA (3.3 μg/ml) and 16 S rRNA (1 μg/ml) respectively. rRNAs were prepared as described in [5].

Filters were radioautographed on Kodak Xomat R film. Densitometric scanning of the autoradiograms was done in a Gilford 250 spectrophotometer equipped with an automatic scanning device.

## 3. Results and discussion

Our experimental approach was to make limit digest of total *E. coli* DNA with the restriction endonuclease *Bam*HI, or *Sal*I. The digests were electrophoresed on agarose gels and then transferred to nitrocellulose filters. The filters were hybridized with purified $^{32}$P-labeled 16 S or 23 S rRNA and the hybridizing fragments were located by autoradiography and their molecular weights were determined by appropriate molecular weight standards.

It has been established that the *rrn*B gene which is carried by the transducing bacteriophage λ*rif*d18 [10], does not contain any *Bam*HI cleavage site, but it contains two *Sal*I sites, one within the 16 S, the other within the 23 S region [8]. If all the *rrn* genes were identical, but in different surroundings with respect to restriction enzyme cleavage sites (a likely but unproven assumption), one would expect that all

*Bam*I fragments hybridizing with 16 S rRNA would also hybridize with 23 S rRNA and that the number of such fragments would be the same as the number of rRNA genes. On the other hand, the *Sal*I digestion would create an identical fragment from all *rrn* genes, hybridizing with both probes, and two different sets of fragments hybridizing either with 16 S, or with 23 S rRNA.

As the results on fig.1 show, the *Bam*I pattern is indeed identical with both rRNA probes, and the number of bands is seven (17.0, 9.0, 8.4, 7.8, 6.75, 5.0 and 4.2 Mdalton—in lack of appropriate standards the molecular weights of the four larger fragments are only approximate values), suggesting the existence of seven *rrn* gene copies. Although it is possible that two or more *rrn* genes are located on different *Bam*HI fragments of the same size, or one of the largest fragments contains two rRNA gene sets (in these cases seven would be only a minimum estimate), but the approximately equal intensity of the bands make this possibility unlikely (fig.2). This conclusion is also supported by the *Sal*I pattern. In this case one very strong band is common to both autoradiograms, an 1.56 Mdalton fragment hybridizes both with 23 S and with 16 S rRNA and this must contain the spacer region. On closer examination this band appears to

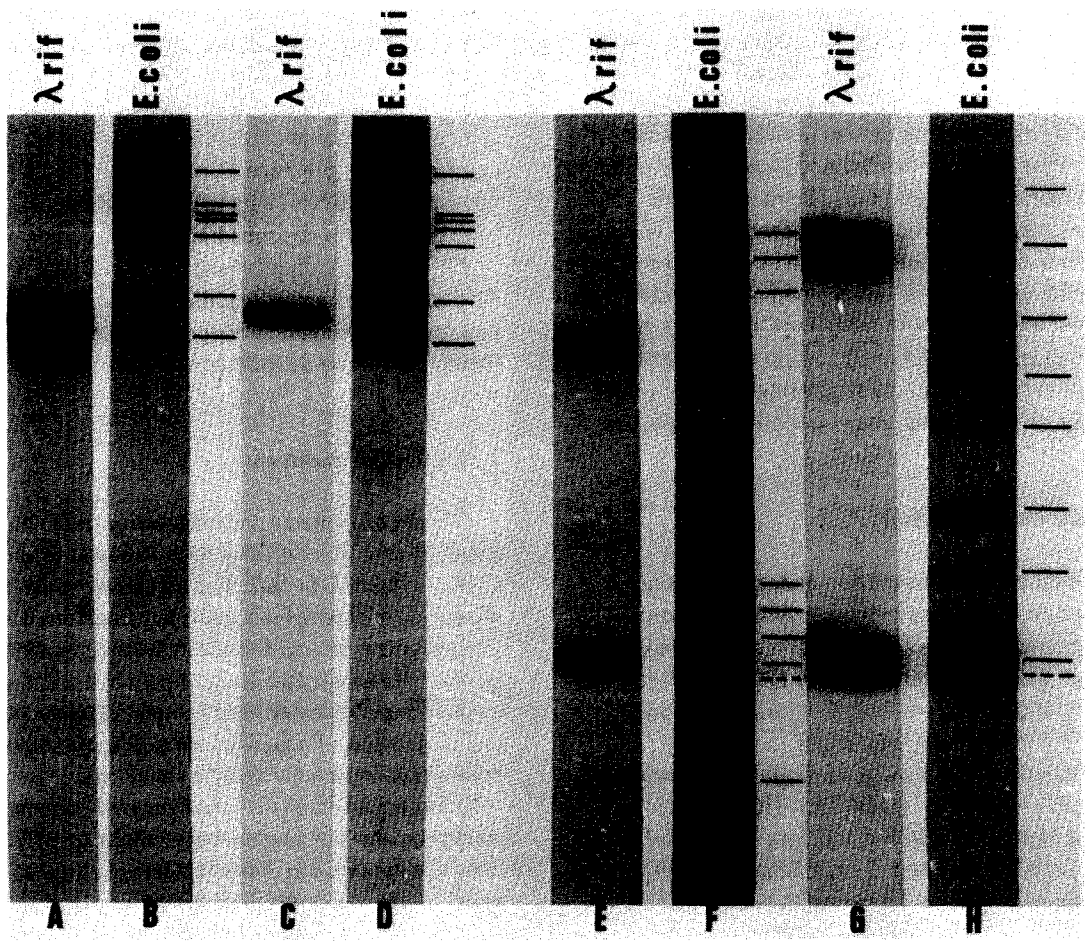


Fig.1. Autoradiograms of λ*rif*d18 and *E. coli* DNA digested with *Bam*HI (A–D) and Sal I (E-H). ABEF panels show hybridization to 16 S rRNA and CDGH panels to 23 S rRNA. In this figure autoradiograms resulting from our different experiments are shown.

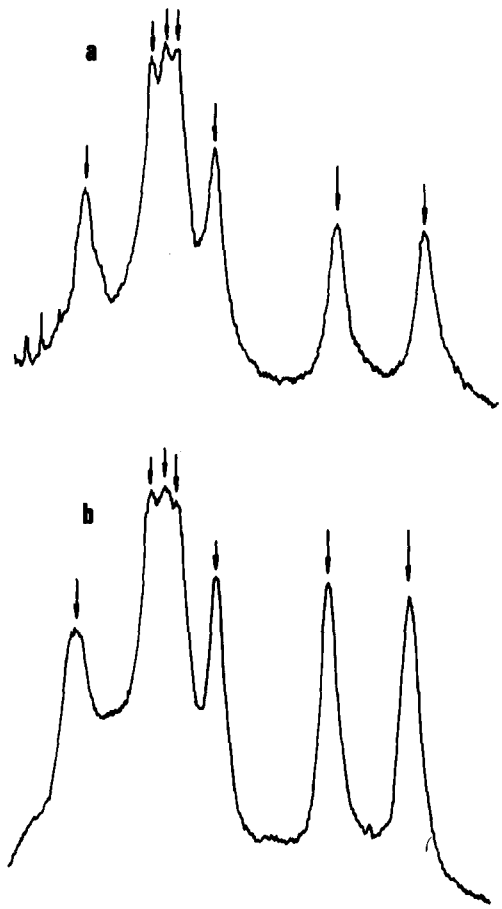


Fig.2. Densitometric scanning of the two autoradiograms made with the *Bam*HI digests of *E. coli* DNA. (a) Hybridized to 16 S rRNA. (b) Hybridized to 23 S rRNA.

be split, in front of the strong 1.56 Mdalton band a faint 1.50 Mdalton band can be seen. The most likely interpretation is the internal heterogeneity of the spacer region. Possibly in one of the *rrn* gene copies this region is approximately 100 base pairs shorter than in the other six copies. The heterogeneity of the spacer region has already been demonstrated by other authors [11,12]. Apart from this common double band both autoradiograms show 7 different fragments (8.4, 7.0, 6.0, 2.0, 1.82, 1.7, 1.1 Mdalton hybridizing with 16 S and 11.9, 7.0, 4.9, 3.8, 3.1, 2.4, 2.0 Mdalton hybridizing with 23 S) confirming the conclusion drawn from the *Bam*HI digestion pattern.

On the basis of these results we believe that the number of *rrn* gene sets is seven in *E. coli* K12, and an intense search for the localization of the missing *rrn*F and *rrn*G is justified.

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