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Deletion of a Ribosomal Ribonucleic Acid Operon in *Escherichia coli*†

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One (*rrnE*) of the seven operons which codes for ribosomal ribonucleic acid in *Escherichia coli* was deleted. No significant change in phenotype was observed even under maximum laboratory growth conditions.

Genes which code for rRNA in bacteria are unusual in that they are redundant and noncontiguous about the chromosome. In *Escherichia coli,* the seven copies of the rRNA genes are organized in similar transcription units (here called operons) comprised of genes for 16S, 23S, and 5S rRNA's and several tRNA's (Fig. 1; for a review, see reference 19). Each has been isolated on plasmids, transducing phages, or both (5, 10-12, 15, 23-25). Calculations indicate that multiple rRNA operons are probably necessary to provide sufficient rRNA for rapidly growing cells, but the purpose of having seven is not clear. In this paper, we demonstrate that at least one rRNA operon can be deleted from *E. coli* without obvious deleterious effects.

Strain TX135 (a kind gift of J. Smith) contains a temperature-inducible Mu phage (Mu cts62 *K*am1010) inserted in *purD,* which is close to *rrnE* and *metA* (Fig. 1). Lysogens are killed at 42°C but cells bearing deletions which encompass Mu survive. Survivors of TX135 were selected at 42°C and screened for methionine auxotrophy. Derivatives of the lysogen which were temperature resistant and Met^- were screened for the intervening genes *purJ,H.* Cells which lacked *purJ,H* were presumed to contain deletions extending through *rrnE.* Mutants TXΔ1, Δ7, Δ11, and Δ101 were isolated independently.

Since the seven rRNA operons are at discrete locations on the chromosome, the flanking sequences are different from each other. The unique fragments of all the rRNA operons generated by *Sal*I or *Bam*HI digestion (Fig. 1) can be detected by hybridization of radioactive rRNA to Southern blots of restriction enzyme-digestion chromosomal DNA which has been subjected to agarose gel electrophoresis (14). The fragments generated from the putative deletion mutants were compared with those of the parent strain. Figure 2 shows the results of digestion with the enzyme *Sal*I and hybridization of the DNA with ^{32}P-labeled 23S rRNA. *Sal*I cleaves each rRNA operon once in the 16S region and once in the 23S region (2, 14). Lane 1 shows the eight bands obtained from the parent. In agreement with Kiss et al. (14), we conclude that band S represents a mixture of fragments containing the spacer region (the 2-kilobase *Sal*I fragment common to all operons; Fig. 1) and that the remaining seven bands represent the unique promoter-distal portions of the 23S rRNA genes from each of the operons. Lanes 2 and 3 indicate that deletion mutant TXΔ101 lacked the largest band (band E). Similar results for the other mutants are not shown. Strain TXΔ200, which is a temperature-resistant and *metA purJ*$^+$,*H*$^+$ mutant contained band E (lane 4). These results indicate that band E represents *rrnE* and that the presumptive deletion strains of *rrnE* are indeed lacking this fragment. This conclusion is not entirely without ambiguity since this fragment is the largest of the *Sal*I fragments bearing 23S rRNA genes and its efficiency of transfer to nitrocellulose was less than that of smaller bands.

We found that a unique fragment from *rrnE* was generated by digestion of the chromosome with *Eco*RI, *Sal*I, and *Bam*HI simultaneously. The autoradiogram obtained after hybridization of ^{32}P-labeled 23S rRNA to the chromosome digests (Fig. 3) shows that the deletion strains (lanes 3-6) were clearly lacking band 2, which is 4 kilobases in size. That this fragment was derived from *rrnE* is shown in lane 1. A 12.2-kilobase *Eco*RI fragment was purified from λ *metA*20, which carries *rrnE* (Fig. 1; see reference 24), and was subsequently digested with *Sal*I and *Bam*HI. The fragment which hybridized 23S rRNA was equal in size to band 2. It is clear that the deletions in the mutants Δ1, Δ7, Δ11, and Δ101 encompassed *rrnE.*

The autoradiogram in Fig. 3 also indicates that the *rrnE*-deleted strains did not contain any novel fragments which hybridized 23S rRNA, nor did they show any significant change

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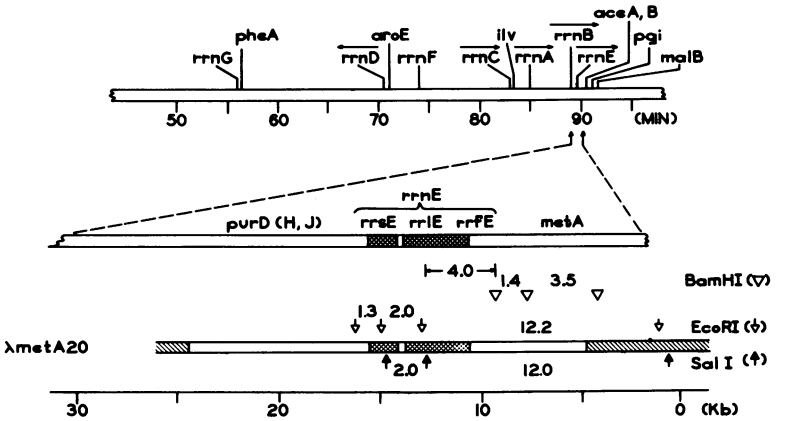

FIG. 1. *Arrangement of rRNA genes on the E. coli chromosome. The region surrounding rrnE is shown in an expanded scale, together with the structure of a part of λ metA20 which carries this region. The open bars represent DNA chromosomal in origin, the hatched bars represent λ DNA, and the crosshatched bars represent rRNA genes. Horizontal arrows indicate the direction of transcription of rRNA operons where known. Restriction sites for BamHI, EcoRI, and SalI are shown for λ metA20, and distances are indicated in kilobases (1,000 base pairs). The purH and purJ genes are located between purD and rrnE (20, 22; J. Smith, personal communication). It should be noted that the growth of purD mutants can be satisfied either by adenine of 4-amino-5-imidazole carboxamide, whereas that of purH and purJ mutants can be satisfied by adenine but not by 4-amino-5-imidazole carboxamide. Deletions of rrnE were obtained as follows. Strain TX135, which carries Mu cts62 Kam1010 inserted in purD was grown at 30°C in broth and then shifted to 42°C to induce the Mu prophage (9). After incubation for 60 min, survivors were plated at 42°C on minimal AB medium (4) containing 2 mg of glucose per ml, 40 μg of adenine per ml, and 200 μg of nutrient broth (Difco Laboratories) per ml. Auxotrophs were able to form only tiny colonies on this medium. They were transferred onto a master tryptone agar plate and replicated onto AB-glucose plates containing 40 μg of adenine per ml, adenine plus 50 μg of methionine per ml, or methionine plus 200 μg of 4-amino-5-imidazole carboxamide per ml. Those colonies that were Met⁻ and purJ,H were selected as presumptive deletion strains and named TXΔ1, Δ7, Δ11, and Δ101. The methionine requirement of each of these strains could be relieved by lysogenization with γ metA20. As described in the text, these deletion strains had the same doubling time as the control strain in minimal AB-glucose medium, except Δ1, which grew more slowly than the control. This slow growth was probably due to the deletion of the pgi gene. This deletion mutant is Mal⁻ and fails to grow on acetate. Therefore, Δ1 appears to carry a large deletion extending at least from purD to malB and encompassing, in addition to rrnE and metA, aceA,B, and pgi. Growth tests on glucose, acetate, and maltose indicated that mutant Δ101 probably carries a deletion including aceA,B but not pgi and that Δ7 and Δ11 deletions terminate before aceA,B.*

in the relative intensity of the existing radioactive bands. Thus, the deletion of *rrnE* did not result in duplication of other rRNA operons or formation of a new one to compensate for its loss. The deletion strains contained only six operons for rRNA.

Since the deletion mutants were selected as normal colony formers, it is apparent that the deletion of *rrnE* was not grossly detrimental. We investigated the growth rates of the mutants more closely in various liquid media. We expected that the loss of an operon would be more apparent at high growth rates; therefore, we replaced the temperature-sensitive parent TX135 with a temperature-resistant derivative which showed no novel auxotrophy (and retains *rrnE*) as a control. Growth rates of all the *rrnE* deletion mutants were identical to that of the control in rich medium (L broth; doubling time, 25 min) and in minimal glucose medium (doubling time 45 min, except TXΔ1 which probably carries a *pgi* mutation and had a doubling time of 58 min [Fig. 1]). Mixed cultures of a deletion strain (TXΔ11) with a control strain also showed that it grew as fast as the control in logarithmic growth in L broth over 100 generations. Measurements of the rates of RNA synthesis after nutritional shift-up did not show any significant difference between deletion and control strains (data not shown). Thus, we were not able to observe any significant change in phenotype resulting from the deletion of *rrnE*.

Although six operons are apparently sufficient to attain maximum rRNA synthesis rate under laboratory conditions *E. coli* K-12 strains have retained seven under long laboratory maintenance, and B and C strains also have seven rRNA operons (our unpublished data). We presume that the multiplicity has a selective advantage, but it is possible that the present organization is a relic of evolutionary history and that some rRNA operons are a luxury to modern *E.*

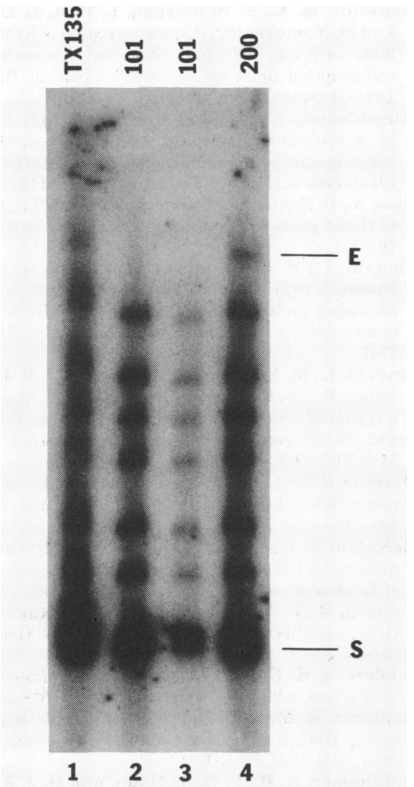

FIG. 2. *Autoradiogram of SalI-digested chromosomal DNA hybridized to ^{32}P-labeled 23S rRNA. Total chromosomal DNA was prepared according to a modification of Marmur (17) which differs in that after lysis of cells the mixture was treated overnight with 10 μg of RNase A (Sigma Chemical Co.) per ml and 1 mg of pronase (B grade, Calbiochem) per ml followed by phenol extraction and ethanol precipitation. Approximately 10 μg of DNA was digested with SalI (New England BioLabs) in 10 mM Tris-hydrochloride (pH 7.4)–5 mM $MgCl_2$–1 mM dithiothreitol–50 mM NaCl and subjected to electrophoresis on 0.7% agarose at 40 V for 4 h in 40 mM Tris-hydrochloride (pH 8.0)–20 mM NaCl–20 mM sodium acetate–2 mM EDTA (7). Gels were stained with ethidium bromide, denatured by alkali, neutralized, and blotted onto nitrocellulose filters (HAWP30, Millipore Corp.) by the procedure of Southern (21). The filters were incubated with ^{32}P-labeled 23S rRNA (2×10^7 cpm, prepared by phenol extraction of purified 50S ribosomal subunits) for 18 h at 67°C in approximately 30 ml of 2x SSC (0.3 M NaCl plus 0.03 M sodium citrate). Lane 1, TX135 (parent); lanes 2 and 3, TXΔ101; lane 4, TXΔ200.*

coli. In "higher" organisms, rRNA genes exist in tandem multiple copies (for a review, see reference 6), of which some reduction in number can be tolerated (3, 18). Although alterations in copy number are known to take place, presumably by unequal sister strand exchange, the degree of redundancy seems to be maintained by unknown genetic mechanisms (6). *E. coli* (8) and *Salmonella typhimurium* (1) have also been observed to duplicate regions of the chromosome by unequal crossover at rRNA operons, resulting in an increase in the number of rRNA operons. These duplications are unstable and must be maintained by selection. Deletion of an rRNA operon by the same mechanism has not been reported, undoubtedly because the deletion of intervening essential genes would be lethal.

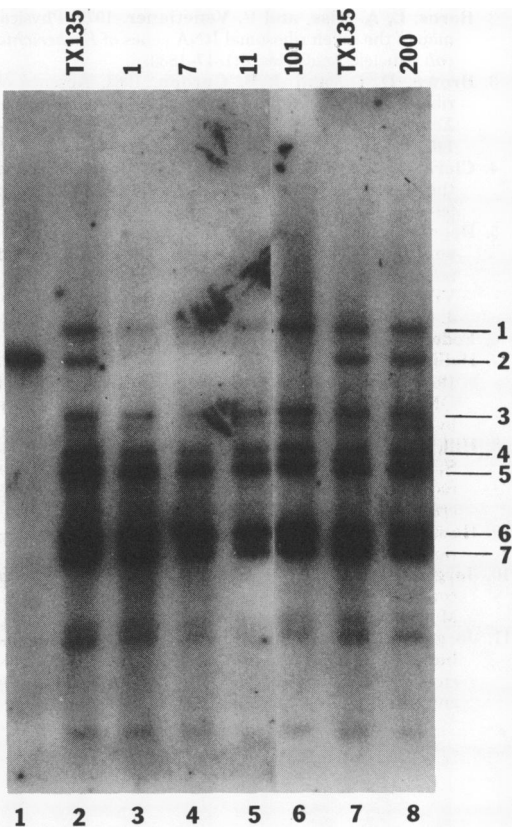

FIG. 3. *Hybridization of ^{32}P-labeled 23S rRNA to chromosomal DNA simultaneously digested with EcoRI, SalI, and BamHI. Experimental procedures were the same as those described in the legend to Fig. 2, except that the DNA was simultaneously digested with EcoRI, SalI, and BamHI, and the agarose gel concentration was 1%. An autoradiogram of the agarose gel is shown. Lane 1, the 12.2-kilobase EcoRI fragment from λ metA20 (Fig. 1) subsequently digested with SalI and BamHI; lanes 2 and 7, TX135 (parent); lane 8, TXΔ200; lanes 3 through 6 deletion strains TXΔ1, Δ7, Δ11, and Δ101, respectively. EcoRI digestion products of λ rif^{d}18 (13, 16) provided size standards (data not shown). Bands 1 through 7 corresponded to the sizes approximately 5, 4, 3, 2.6, 2.3, 1.8, and 1.7 kilobases, respectively.*

Thus, the organization of the present-day *E. coli* genome might ensure the conservation of seven copies of the rRNA genes.

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