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Location of a Gene (*ssrA*) for a Small, Stable RNA (10Sa RNA) in the *Escherichia coli* Chromosome

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The gene for 10Sa RNA, which is a major small, stable RNA in *Escherichia coli*, is a unique gene in the *E. coli* chromosome. The 10Sa RNA gene (*ssrA*) has been located between 2,760 and 2,761 kilobases on the *E. coli* genome.

In *Escherichia coli* at present, four small, stable RNAs are known: 4.5S, 6S, 10Sa, and 10Sb (M-1 RNA) RNAs (5–7, 9, 11, 13, 15). 10Sa RNA is a major small RNA that occurs in about 1,000 copies per haploid genome (11). 10Sa RNA and 10Sb RNA (the RNA of RNase P) almost comigrate in polyacrylamide gels, but they are structurally unrelated (9). The gene encoding 10Sa RNA (*ssrA*) was cloned into a multicopy plasmid (16) and sequenced (2); it is a monocistronic transcription unit of 362 nucleotides, with its own promoter and terminator (2).

To locate the *ssrA* (small stable RNA) gene in the chromosome of *E. coli*, a DNA fragment from pSR207 (16) that contains the 10Sa RNA gene was labeled with radioactive ^{32}P and hybridized to a set of λ bacteriophage clones covering almost the entire *E. coli* genome (10). The results of this experiment indicated that the *ssrA* gene is carried by λ phage clones 439 and 440. However, when we compared the restriction maps of pSR207 and pSR205 (2, 16), both of which contain the *ssrA* gene, with the Kohara map (10), we found discrepancies with respect to some of the restriction sites. Therefore, we considered the possibility that there might be another *ssrA* gene in the *E. coli* genome. We showed this not to be the case by probing with ^{32}P-labeled 10Sa RNA, plasmids, and chromosomal digests. The pattern of hybridization is identical for the *E. coli* and plasmid DNAs (Fig. 1), indicating that there is only a single *ssrA* (10Sa RNA) gene in the *E. coli* genome.

To evaluate the discrepancy between the *E. coli* physical restriction map (10) and the pSR205 map further, we subcloned the *Bam*HI-*Eco*RI fragment from λ 439 into plasmid pIE (E. Yaskowiak, M. Cotoras, G. Lee, and P. E. March, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, H32, p. 150), designating the new plasmid pSR212, and constructed a restriction map. The restriction map of pSR212 is different to some extent from the *E. coli* map (Fig. 2) and is identical to the pSR205 map (data not shown). The main discrepancies concern the restriction enzymes *Eco*RI, *Eco*RV, and *Hin*dIII. These results also indicate that there is not restriction site polymorphism between strains W3110 (used to construct the *E. coli* physical restriction map) and the strain from which plasmid pSR205 was prepared.

It is worth mentioning that strains that carry plasmid pSR212 overproduce 10Sa RNA, as do strains that carry plasmids pSR205 and pSR207 (2, 16), a further indication that the fragment of DNA cloned in these three plasmids is identical.

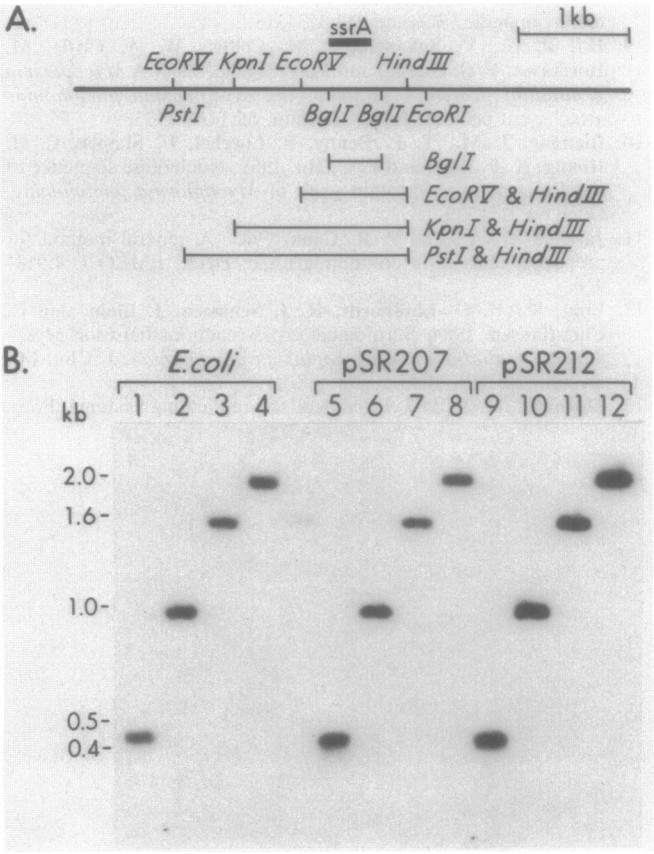

FIG. 1. Expected sizes of DNA fragments that should hybridize with labeled 10Sa RNA in various restriction enzyme digests of *E. coli* DNA (A) and comparison of DNA fragments containing the 10Sa RNA gene in plasmids and in the whole chromosome of *E. coli* (B). *E. coli* chromosomal DNA (12) from *E. coli* D10 (*met rna* [RNase I$^-$]) and plasmid DNA (1, 8) were prepared. Equivalent amounts of DNA from *E. coli* and plasmids pSR207 and pSR212 were digested with a number of restriction enzymes and separated in 1.0% (wt/vol) agarose gel. A Southern hybridization (14) was carried out with Magnagraph nylon membrane with ^{32}P-labeled 10Sa RNA purified by the procedures described by Gegenheimer et al. (4). ^{32}P-labeled 10Sa RNA (2×10^5 cpm) was used as a probe. The membrane was air dried and exposed to X-ray film at −70°C for 10 days. Lanes: 1 to 4, 8 μg of *E. coli* DNA; 5 to 8, 10 ng of pSR207 DNA; 9 to 12, 17 ng of pSR212 DNA. Restriction enzyme used: 1, 5, 9, *Bgl*I; 2, 6, 10, *Eco*RV and *Hin*dIII; 3, 7, 11, *Kpn*I and *Hin*dIII; 4, 8, 12, *Pst*I and *Hin*dIII. kb, Kilobases.

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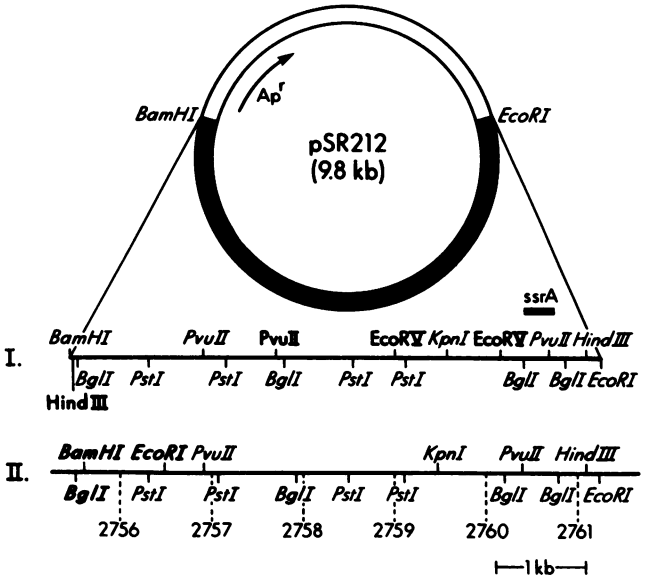

FIG. 2. Comparison of restriction maps of a plasmid and the relevant part of the *E. coli* genome which contains the *ssrA* (10Sa RNA) gene. (I) pSR212. An *Eco*RI-*Bam*HI DNA fragment, containing the 10Sa RNA gene from lambda phage 439 (10), was cloned into the same sites of plasmid pIE (Yaskowiak et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). Preparation of DNA bacteriophage clones was done as described by Davis et al. (3). (II) Part of the published physical map of the *E. coli* genome (10). The restriction sites found in pSR212 (I) but not indicated on the Kohara map are shown in boldface letters. The restriction sites drawn in boldfaced italicized letters in part II are *Eco*RI, which was not detected in pSR212, and *Bgl*I and *Bam*HI, the order of which is reversed in pSR212. kb, Kilobases.

In summary, the 10Sa RNA gene (*ssrA*) is a unique gene in the chromosome of *E. coli* located between 2,760 and 2,761 kilobases.

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