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Expression of Ribosomal Protein Genes as Analyzed by Bacteriophage Mu-Induced Mutations*

(*E. coli*/episome/merodiploid/antibiotics/operon)

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ABSTRACT **The organization of ribosomal protein genes and the gene (*fus*) for a protein chain elongation factor, EF G, in *Escherichia coli* were studied with a merodiploid strain that has an episome with genetic markers, *ery*^{r}, *spc*^{r}, *str*^{r}, and *fus*^{r}, and a chromosome with markers *ery*^{s}, *spc*^{s}, *str*^{s}, and *fus*^{s}. The *ery* locus determines a 50S ribosomal protein and the *spc* and *str* loci determine 30S ribosomal proteins. The phenotype of the diploid strain is sensitive to all of the four antibiotics, erythromycin (Ery), spectinomycin (Spc), streptomycin (Str), and fusidic acid (Fus). Analysis of antibiotic-resistant mutants induced by bacteriophage Mu in the diploid strain indicates that these four genes, and probably many other ribosomal protein genes linked to them, are transcribed as a single unit, and the direction of the transcription is in the order of *ery*, *spc*, *str*, and *fus*.**

The number of ribosomes in a bacterial cell is directly proportional to the growth rate of the cells under various culture conditions (1). Thus, the regulation of biosynthesis of ribosomes, as well as individual ribosomal components, is important in connection with the regulation of cellular growth. Bacterial ribosomes contain as many as 50–60 (different) protein components (for a review, see ref. 2). Although it is assumed that all the ribosomal proteins are synthesized coordinately, little information is available on the mechanism of coordinated synthesis and its regulation.

Several known ribosomal protein genes in *Escherichia coli* are clustered near the *str* locus on the chromosome (for a review, see ref. 3). This suggests, but does not prove, that the ribosomal protein genes are coordinately expressed as a group (or several groups), as in the case of the classical operons in bacterial chromosomes. The expression of such operons has been studied by various genetic approaches, such as isolation of nonsense mutants that show polar effects, and isolation of mutants in promoters, operators, or regulatory genes. One of the major obstacles to such genetic approaches in the study of ribosomal protein genes is that many of the ribosome mutations are likely to be lethal, because of the essential requirement of ribosomes for cell growth. Neither delection mutations nor nonsense mutations have been identified in the ribosome genes. The low rates of occurrence of the known ribosomal mutations, such as streptomycin-resistance and spectinomycin-resistance, can also be interpreted on this basis (3).

To overcome this obstacle, we began genetic studies on the organization of ribosomal genes using partial diploid *E. coli* strains heterozygous for the *str–spc* region on the chromosome. This paper describes the analysis of antibiotic-resistant mutants induced by insertion of bacteriophage Mu (*4–7*) in such a diploid strain. The strain used has an episome that has genetic markers, *ery*^{r}, *spc*^{r}, *str*^{r}, and *fus*^{r}, and a chromosome that has markers *ery*^{s}, *spc*^{s}, *str*^{s}, and *fus*^{s}. The *ery* locus determines a 50S ribosomal protein (8), the *spc* and *str* loci determine 30S ribosomal proteins S5 (P4)‡ and S12 (P10), respectively (9, 10). The *fus* locus determines a protein chain elongation factor, EF G, that is known to map close to the *str* locus (11–13). The results obtained indicate that these four genes are transcribed as a single unit, and the direction of the transcription is in the order of *ery*, *spc*, *str*, and *fus*.

MATERIALS AND METHODS

The following strains derived from *E. coli* K12 were used: KL131; F$^-$, *argG*$^-$, *ery*^{s}, *spc*^{s}, *str*^{s}, *fus*^{s}, *aroB*$^-$, *malT*$^-$, *xyl*$^-$, *metB*$^-$, *leu*$^-$, *recA*$^-$ (from Dr. B. Low). RO32; Hfr, *ery*^{s}, *spc*^{r}, *str*^{r}, *fus*^{s}, *purC*$^-$, *metB*$^-$ (injection of the chromosome markers is in the order of *argG*$^+$, *spc*^{r}, *str*^{r}, *aroB*$^+$, etc.) This strain is derived from JC12, which is *spc*^{s} and *str*^{s}. A spontaneous Str-R mutant was first isolated from JC12, and from this mutant, RO32 was obtained as a spontaneous Spc-R mutant. NO863, Hfr, a spontaneous *fus*^{r} mutant obtained from RO32. NO864; Hfr, an *ery*^{r} mutant obtained from NO863 after mutagenesis with ethyl methane sulfonate. Diploid strains used, NO865 (we call this a "2R-diploid") and NO866 (a "4R-diploid"), were constructed by crossing (14) RO32 and NO864 with KL131, respectively. Minimal plates (see below) were used to select and purify the diploid strains. The diploid strains used contained episomes that cover *argG*, *ery*, *spc*, *str*, *fus*, *aroB*, and *mal*, but *not xyl* (see Fig. 1). The diploidy was confirmed by (*a*) high frequency of segregation of haploid cells identical to KL131 in tryptone broth in the presence or absence of acridine orange (10–20 μg/ml), (*b*) ability of the strains to transfer the episomes to other F$^-$ strains with a

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‡ The nomenclature used for *E. coli* ribosomal proteins is that of Wittmann and coworkers (21). For the 30S proteins, the nomenclature used by our laboratory is added in parentheses. The abbreviations used are: Ery; erythromycin, Spc; spectinomycin, Str; streptomycin, Fus; fusidic acid; EF G, elongation factor G.

high frequency. Bacteriophage Mu was obtained from Dr. A. Bukhari. The phage stock was prepared by a plate lysate method.

The following media were used: Minimal basal medium; 63 mM Na_2HPO_4, 40 mM KH_2PO_4, 15 mM $(NH_4)_2SO_4$, 0.8 mM $MgSO_4$, 60 μM $CaCl_2$, 2 μM $FeCl_3$, 2 μM $Fe(NH_4)_2(SO_4)_2 \cdot 6\ H_2O$, and 250 μg/liter thiamine hydrochloride. To grow haploid strains, glucose (0.2%) and amino acids or bases required for growth were added (each at 20 μg/ml). For the diploid strains, the minimal basal medium was supplemented with maltose (0.2%), methionine, and leucine. To maintain or purify the diploid strains, the same minimal medium containing 2% agar was used. Control broth plates (TM plates); 1.3% tryptone (Difco), 0.7% NaCl, 0.5% meat extract (Difco), and 1.2% agar. TM + Ery plates; 300 mg of Ery (in 2 ml alcohol) was added to 1 liter of TM medium. TM + Spc plates; filtered sterile Spc solution was added to TM medium to a final concentration of 100 μg/ml. TM + Str plates; sterile Str solution was added to TM medium to a final concentration of 200 μg/ml. L + Fus plates; 1% tryptone, 0.5% yeast extract (Difco), 1% NaCl, 1.5% agar. Fus (650 mg is 2 ml of alcohol) was added to 1 liter of the above medium.

For isolation of Mu-induced mutants, the diploid strains were grown in minimal synthetic medium containing maltose, methionine, and leucine. Exponentially growing cells were adjusted to a cell density of about 4×10^8/ml. Tryptophan (50 μg/ml), $CaCl_2$ (2.5 mM), and $MgCl_2$ (5 mM) were added. The phage was then added at a multiplicity of about 0.5–1. After 20 min at 37°, the cultures were diluted 10-fold with minimal basal medium, and the cultures were incubated overnight with shaking. The control cultures were treated in the same way, except for the addition of Mu. The cultures were then suitably diluted, and aliquots were spread on antibiotic-containing plates. Str-R and Fus-R mutants were scored (and/or picked up) after overnight incubation at 37°, Spc-R mutants after 2 days of incubation, and Ery-R mutants after 3 days of incubation. The number of total viable cells of the original diploid cultures was also determined, and the frequency of antibiotic-resistant mutants was calculated. The phenotype of purified mutants was scored by spotting a loopful of mutant cell suspension on TM + Str and TM + Spc plates (for Str- and Spc-sensitivity) and by streaking on L + Fus and TM + Ery plates (for Fus- and Ery-sensitivity). Resistant mutants were scored after overnight (for Str) or 2 days (for Spc) of incubation at 37°. Resistance was evidenced by formation of many separated colonies after overnight incubation (on L + Fus plates) and 2–3 days of incubation (on Ery plates), respectively. The sensitive control strains (KL131 and the parental diploid strains) and the resistant strain (NO864) were always included in the tests.

RESULTS

Experimental Design. Taylor has shown that infection of *E. coli* cells with phage Mu leads to a high frequency of mutations among the surviving lysogenized cells (4). The mutations were observed in different genes. Boram and Abelson (5), subsequently, proved the interpretation originally made by Taylor that the mutations are caused by the integration of Mu into the gene. The insertion of the Mu DNA into any bacterial "operons" should interfere with the transcription of the cistrons distal to the initiation site (the "promoter"), but not the cistrons proximal to the "promoter." This supposition has been confirmed experimentally (6, 7). We used this principle to examine the question of whether ribosomal protein cistrons and the EF G gene (the *fus* cistron) are part of the same transcriptional unit ("operon").

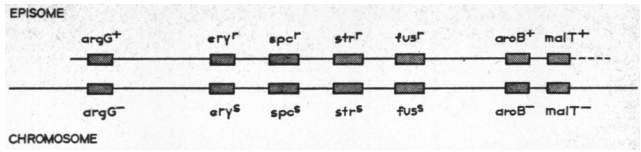

FIG. 1. Genetic structure of the heterozygous chromosome region of merodiploid strain NO866 ("4R-diploid").

Fig. 1 shows the genetic structure of the diploid strain (4R-diploid) used. The episome is *argG*$^+$*ery*^{r}*spc*^{r}*str*^{r}*fus*^{r}-*aroB*$^+$*malT*$^+$. The chromosome is *argG*$^-$*ery*^{s}*spc*^{s}*str*^{s}*fus*^{s}-*aroB*$^-$*malT*$^-$. The order of the loci, *ery*, *spc*, and *str*, has been mapped by P1 transduction (J. Davies, personal communication) with respect to *aroE*, which is between *argG* and *ery*. This order is in accord with the order obtained previously (3). The *fus* locus has been shown, by P1 transduction, to map close to *str*, in agreement with earlier work (11–13), but its exact position relative to *str* and *aroB* has not been determined. However, the analysis of spontaneous *fus*^{r}, as well as *str*^{r}, mutants from several diploid strains has suggested the order shown in Fig. 1. (The data to be described in this paper support this order.) The episome never (or very rarely) recombines with the chromosome, because of the presence of a *recA*$^-$ mutation in the chromosome. The diploid strains are also kept in minimal medium to prevent loss of the episome. Cells that lose any one of *argG*$^+$, *aroB*$^+$, and *malT*$^+$ genes from the episome cannot grow in the medium used.

In the diploid strain, *str*^{s} is dominant over *str*^{r}, and the phenotype is streptomycin sensitive (Str-S) (15, 16). Similarly, *spc*^{s} is dominant over *spc*^{r} (16). We find *ery*^{s} and *fus*^{s} to be dominant over *ery*^{r} and *fus*^{r}, respectively, as judged by cell growth on antibiotic-containing solid media. Thus, the phenotype of the present diploid is Ery-S, Spc-S, Str-S, and Fus-S. Antiobiotic-resistant mutants can be induced in the diploid by inhibition of the functional expression of the antibiotic-sensitive cistrons on the chromosome by the insertion of Mu phage. If the expression of each of four cistrons is independent (model A), Str-R phenotype can be induced by Mu phage only by a direct insertion of Mu in the *str* gene (the *str* cistron and any accessory genes necessary for its expression), and such Mu insertion should not affect the expression of other genes, namely, *ery*^{s}, *spc*^{s}, and fus^s. Thus, if one selects Str-R mutants by plating the diploid cells on plates containing Str, such mutants would be Str-R, but sensitive to Ery, Spc, and Fus [Table 1, model *A*, class (*c*)]. Analogous results would also be exprected with respect to Ery-R, Spc-R, and Fus-R mutants (Table 1, model *A*).

If the four antibiotic-resistant cistrons belong to one large transcriptional unit, two different possibilities can be considered, depending on the direction of the gene expression: (*i*) assume the gene order to be *ery*, *spc*, *str*, and *fus* (model *B*) (*ii*) assume the opposite direction (model *C*). Predictions of the phenotypes of antibiotic-resistant mutants induced by Mu can then be made. For example, according to model *B*, Mu-induced Spc-R mutants isolated by selection with Spc could arise as a result of insertion of Mu at sites anywhere between the hypothetical "promoter" (to the left of *ery* in Fig.

TABLE 1. *Prediction of phenotypes of strong polar, antibiotic-resistant mutants induced by phage Mu in the 4R-diploid strain*

Model	Class	Phenotypic classes expected			
		Ery	Spc	Str	Fus
A. Drug-resistance genes are independent.	(*a*)	R	S	S	S
	(*b*)	S	R	S	S
	(*c*)	S	S	R	S
	(*d*)	S	S	S	R
B. Drug-resistance genes are in an "operon" with "promoter" at left	(*a*)	R	R	R	R
	(*b*)	S	R	R	R
	(*c*)	S	S	R	R
	(*d*)	S	S	S	R
C. Drug-resistance genes are in an "operon" with "promoter" at right	(*a*)	R	S	S	S
	(*b*)	R	R	S	S
	(*c*)	R	R	R	S
	(*d*)	R	R	R	R

R; resistant. *S;* sensitive.

1) and *spc*, including the *spc* cistron itself. If the insertion is in the *ery* cistron, or anywhere between the "promoter" and *ery* cistron, the phenotype of the mutant should be Ery-R, Spc-R, Str-R, Fus-R [Table 1, model *B*, class (*a*)]. On the other hand, if the insertion is somewhere between *ery* and *spc*, or in the *spc* cistron itself, the phenotype would be Ery-S, Spc-R, Str-R, Fus-R [Table 1, model *B*, class (*b*)]. No other phenotype would be expected. If the model *C* is correct, Spc-R could arise by insertion of Mu either within the *spc* cistron or anywhere between *spc* and the hypothetical "promoter", which is located to the right of *fus* (Fig. 1). Thus, all Spc-R mutants selected with Spc would be Ery-R, but could be resistant or sensitive to Str and Fus. Depending on the location of the Mu insertion, three phenotypes would be expected. They are class (*b*), (*c*), and (*d*) shown in Table 1, model *C*. No other phenotype would be expected. Similarly, both models *B* and *C* make specific predictions of the phenotypes of mutants selected with each of the other antibiotics, Ery, Str, and Fus. Table 1 summarizes the predictions.

Of course, one can consider "intermediate" models. For example, one can suppose one transcriptional unit for genes for 50S ribosomal proteins (*ery*), one for 30S ribosomal genes (*spc* and *str*), and one for the EF G gene (*fus*), with the obvious predictions.

Analysis of Mu-Induced Antibiotic-Resistant Mutants from the 4R-Diploid. The 4R-diploid was grown in minimal medium and infected with phage Mu. The cultures were examined for their frequency of antibiotic-resistant mutants. (Comparable experiments were also done with the haploid strain KL131, as as well as the 2R-diploid strain, see below.) Table 2 shows one such experiment. One striking fact is that the frequency of spontaneous antibiotic-resistant mutants is markedly higher in the diploids than in haploid cultures. Also, the Mu-infected culture contained much larger numbers of mutants resistant to any one of four antibiotics examined than did uninfected cultures. The range of increase due to Mu treatment was between 10- and 200-fold in all the experiments.

Altogether, 21 independent cultures of the 4R-diploid were treated with Mu and antiobiotic resistant-mutants were selected with Fus, Str, Spc, and Ery. 21 Independent control cultures without Mu infection were also analyzed. Two colonies (usually one with relatively large and another with relatively small colony size) were picked from each of the antibiotic-containing selection plates and purified by streaking on the broth plates in the absence of any antibiotic. Then their phenotype with respect to resistance to the four antibiotics was examined, and their diploidy (presence of $argG^+$, $aroB^+$, and $malT^+$ genes) was confirmed. The mutants were classified according to their pattern of antiobiotic resistance and the number of independent mutants in each class was scored. When the two resistant mutants derived from the same culture showed the same phenotype, they were counted as one. Mutants that were not diploid were discarded. The results are summarized in Table 3.

It is clear that the phenotypes of the mutants induced by Mu from the 4R-diploid are exactly those predicted by the model *B*. All the predicted phenotypes were observed and no mutant showed any other phenotype. We conclude that the four cistrons examined, *ery*, *spc*, *str*, and *fus*, are transcribed as a unit, and the direction of transcription is in the order of *ery*, *spc*, *str*, and *fus*.

Table 3 also shows data obtained in control experiments without Mu infection. There are several ways by which such antibiotic-resistant mutations might occur: (*a*) point mutations in the pertinent antibiotic-sensitive gene on the chromosome to produce inactive proteins or proteins with the resistant phenotype; (*b*) deletions of a part of the chromosome

TABLE 2. *Increase in the frequency of antibiotic-resistant mutants in cultures of diploid strains, 4R-diploid, 2R-diploid, and haploid strain KL131 treated with phage Mu*

Cultures	Frequency ($\times 10^6$) of mutants resistant to			
	Fus	Str	Spc	Ery
4R-diploid				
Control	2.9	3	0.11	0.35
Mu-infected	140	150	11	13
Ratio	48	50	100	38
2R-diploid				
Control	—*	1.1	0.12	<0.005
Mu-infected	—*	65	21	<0.005
Ratio (Mu/control)	—	59	175	—
KL131				
Control	—*	~0.01	<0.005	<0.005
Mu-infected	—*	~0.01	<0.005	<0.005
Ratio (Mu/control)	—	~1	—	—

* No colonies ($<10^{-8}$) appeared after overnight incubation, whereas Fus-R colonies appeared from 4R-diploid after overnight incubation on the same Fus-containing plates. However, many small colonies (about 10^{-6}) appeared both from 2R-diploid and KL131 after 2 days of incubation. These small colonies were picked, purified, and retested for Fus resistance and diploidy. All of them proved to be Fus-S and haploid. Thus, most of the colonies appearing on the original Fus-containing plates were not true Fus-R mutants. The exact number of true Fus-R mutants could not be determined. It is probable that KL131 cells (and the corresponding haploid cells segregated from 2R-diploid) are phenotypically somewhat resistant to Fus, possibly due to poor permeation of Fus into cells, and give rise to colonies on the Fus-containing plates under certain conditions. Spreading of many cells on the plates may be one such condition.

including the pertinent gene; (c) nonsense mutations on the chromosome in the pertinent gene, or somewhere proximal to the "promoter" in this large transcriptional unit, to cause "polar effects" and decrease the frequency of transcription of the pertinent sensitive chromosomal cistron; (d) some kind of mutation that makes the episomal resistance genes dominant over the chromosomal sensitivity genes. Among the spontaneous mutants, the class most strikingly different from those induced by Mu is the class of mutants selected by Str that is resistant only to Str, but sensitive to all other antibiotics. Ten of 31 independent, spontaneous Str-R mutants obtained by selection with Str, belong to this class. Since we believe that the order of the four genes are *ery, spc, str, fus*, the occurrence of mutants of this class can be best explained by the possibility (a) [and/or (d)] mentioned above. In contrast, none of the 33 Mu-induced, independent Str-R mutants belong to this class. Such differences support our assumption that Mu-induced mutations are strongly polar and strengthen our conclusion that the large "ribosomal protein operon" is arranged as shown in model *B*. Notice that, even among spontaneous mutants (especially those selected by Ery, Spc, and Fus), "nonpolar" mutations are in the minority, and the general pattern is similar to that of Mu-induced resistant mutations. Thus, four classes are found among the spontaneous Fus-R mutants selected by Fus; these are exactly the four classes expected (and actually found) for the Mu-induced mutants based on model *B*. Similarly, a majority of the spontaneous Ery-R mutants selected by Ery are resistant to all four antibiotics. These results suggest that many of the spontaneous resistant mutants obtained from the present diploid involve mutational events that cause a "polar effect." The presence of nonsense mutations with a polar effect to the genes distal to the "promoter" is highly probable among the spontaneous mutants.

Control Experiments with 2R-Diploid. Experiments similar to those described above were repeated with the 2R-diploid. This strain has an episome that is $ery^s spc^r str^r fus^s$; the chromosome is $ery^s spc^s str^s fus^s$. As shown in Table 2, Mu increased the frequency of mutants resistant to Str or Spc by a factor of 50–200. No Ery-R mutants were observed, with or without Mu treatment. [The exact frequency of Fus-R mutants could not be scored (see Table 2).] Altogether 10 independent cultures were infected with Mu. Mutants selected with Str or Spc were then purified, and their sensitivity to other antibiotics was examined. As shown in Table 3, the results are consistent with those predicted from the model involving both *spc* and *str* genes in one transcriptional unit. The direction of transcription is again in the order *spc* → *str*. None of the mutants isolated was resistant to Ery or Fus. Thus, the presence of Ery-R or Fus-R mutants among those selected with Spc or Str from the 4R-diploid in the previous experiments is due to the presence of ery^r and fus^r genes on the episome in the 4R-diploid strain. As discussed before, inhibition of expression of the ery^s (or fus^s) gene on the chromosome by the Mu-induced mutational events must be responsible for the Ery-R (or Fus-R) phenotype observed in experiments with 4R-diploid.

Spontaneous Ery-R, Spc-R, and Str-R mutants from haploid strain KL131 analyzed in other experiments are resistant only to the antibiotics used for the selection. No cross resistance was observed among three antibiotics used.

TABLE 3. *Analysis of antibiotic-resistant mutants induced by Mu from diploid strains 4R-diploid and 2R-diploid*

Selected by	Phenotypes observed				Number of independent mutants from	
	Ery	Spc	Str	Fus	4R-diploid*	2R-diploid*
Ery	R	S	S	S	1 (0)	—
	R	R	R	R	16 (18)	—
Spc	S	R	S	S	1 (0)	0 (0)
	S	R	R	S	2 (0)	8 (10)
	S	R	R	R	12 (9)	0 (0)
	R	R	R	R	13 (19)	0 (0)
Str	S	S	R	S	10 (0)	9 (7)
	S	R	R	S	0 (0)	4 (7)
	S	S	R	R	5 (11)	0 (0)
	S	R	R	R	12 (16)	0 (0)
	R	R	R	R	4 (6)	0 (0)
Fus	S	S	S	R	8 (7)	
	S	S	R	R	4 (4)	
	S	R	R	R	8 (15)	
	R	R	R	R	4 (2)	

* Values without parentheses refer to control cultures and those in parentheses refer to parallel cultures treated with bacteriophage Mu.

Ribosomes from Antibiotic-Resistant Mutants Induced by Mu. The antibiotic sensitivity of ribosomes from the 4R-diploid was examined, and compared to the sensitivity of ribosomes obtained from the parent resistant (NO864) and sensitive strains (KL131), by their capacity to support f2 RNA-dependent polypeptide synthesis (17). The ribosomes from the 4R-diploid were partially resistant to Str and Spc; that is, their sensitivity was between that of resistant and sensitive parents, confirming previous results (16).

Ribosomes from a Mu-induced mutant (M4-30) derived from the 4R-diploid were examined. This mutant was isolated by selection with Spc; its phenotype is Ery-R, Spc-R, Str-R, Fus-R. The ribosomes were resistant to Spc and Str to the same degree as those from the resistant strain (NO864). Similarly, activity of ribosome-free "S-100" fraction (containing EF G) from the mutant M4-30 was more resistant to Fus in poly(U)-dependent polyphenylalanine synthesis *in vitro* (17) than were the corresponding S-100 fractions from the parent 4R-diploid or the sensitive strain (KL131). No difference was observed in the degree of resistance to Fus in the *in vitro* assay between S-100 from the resistant strain (NO864) and that from M4-30. Thus, as predicted, no product of the chromosomal sensitivity genes, spc^s, str^s, and fus^s, was found in any measurable amount in the mutant M4-30. Resistance of the ribosomes to Ery from this mutant has not been analyzed.

DISCUSSION

Prediction of the phenotypes of Mu-induced mutants (Table 1) was made on the basis of the known mechanism of Mu-induced mutation; this phage causes mutations by physical

insertion of its DNA at the site of mutation (5). The conclusion that the four antibiotic-resistance genes studied belong to one transcriptional unit is, however, not dependent on the exact mechanism of Mu-induced mutation. Regardless of the exact mechanism, the results shown in Table 2 are difficult to explain by any model other than model *B*, which assumes that the cistrons *ery*, *spc*, *str*, and *fus* belong to one transcriptional unit, and that the direction of transcription is in the order of *ery*, *spc*, *str*, *fus*. Another possible explanation would be to invoke some kind of chromosomal deletion induced by Mu. But, in view of the observed polarity, this explanation is highly unlikely. In fact, in work on Mu-induced polar effects in late genes of λ phage, the genes inactivated by Mu insertion were shown to be present by their ability to rescue superinfecting defective λ phages (7). In addition, similarity of the polar pattern in the phenotypes of spontaneous mutants to that of Mu-induced mutants suggests that the observed polarity is not unique to Mu, but reflects the more general nature of this genetic system.

Since a number of genes known to code for 50S and 30S ribosomal proteins map in the region studied here [that is, close to the *str* gene (for a review, see ref. 3)], these genes may also belong to the same transcriptional unit. For convenience, we propose to call this transcriptional unit a "ribosomal protein operon", even though the properties of this genetic unit may not be the same as other conventional operons. The ribosomal protein operon contains not only genes for both 30S and 50S ribosomal proteins, but also the gene for elongation factor EF G. It is highly likely that most of the ribosomal protein genes are contained in this operon. Thus, coordinated synthesis of all of the ribosomal proteins under normal conditions can be simply explained by the presence of a single polycistronic mRNA for these proteins. Furthermore, the presence of the gene (*fus*) for EF G in this operon is consistent with reports that the number of EF G molecules per *E. coli* cell is about the same as the number of ribosomes (18, 19). In this connection, EF G could be considered as a ribosomal protein.

The use of stable diploid strains for the study of essential genes or clusters of essential genes may be of great advantage. Scaife and coworkers used such strains to isolate amber mutants in the *rif* gene, which codes for the β-subunit of RNA polymerase in *E. coli* (20). In the present study, diploid strains heterozygous for known ribosomal protein loci were successfully used to examine the question of whether these ribosomal protein cistrons are independently expressed. The frequency of spontaneous antibiotic-resistant mutants in these diploid strains is several orders of magnitude higher than that in haploid strains. Any "lethal" mutations in the ribosomal protein genes could be rescued in diploid strains. Thus, in principle, deletion, insertion, missense, and nonsense mutations in ribosomal protein genes can all be studied. In addition, isolation of polar nonsense mutants or "promoter" mutants would also be possible. At present, the regulatory mechanism for the synthesis of ribosomal proteins is not known. Further genetic studies with the present diploid should give useful information on the initiation site ("promoter") of the ribosomal protein operon, and the regulatory mechanisms controlling the expression of these important genes.

NOTE ADDED IN PROOF

After we had submitted the present paper, we learned that Drs. T. Cabezón, M. Faelen, and A. Bollen (Lab. Génétique, Université libre de Bruxelles, Belgium) have done similar experiments using a diploid strain of *E. coli* that is heterozygous for *spc* and *str* loci, and concluded that these two genes belong to one single transcriptional unit and that the direction of the gene expression is in the order of *aroE*, *spc*, and *str* (personal communication).

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