JOURNAL OF BACTERIOLOGY, Feb. 1983, p. 709–715
0021-9193/83/020709-07$02.00/0

Vol. 153, No. 2

Escherichia coli Kasugamycin Dependence Arising from Mutation at the *rpsI* Locus

ERIC R. DABBS

Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, D-1000 Berlin 33 (Dahlem), Federal Republic of Germany

Received 16 February 1982/Accepted 26 October 1982

Escherichia coli mutants with alterations in the electrophoretic mobility of ribosomal protein S9 were used to locate *rpsI*, the gene for this protein, on the linkage map. *rpsI* was located at about 70 min, roughly halfway between *argG* and *fabE*. It was very close to the gene for ribosomal protein L13, *rplM*. Another mutation at the *rpsI* locus gave rise to a phenotype of kasugamycin dependence and resistance. In this mutant, dependence on antibiotic came from kasugamycin being necessary to slow the rate of protein synthesis.

The gene loci of all ribosomal proteins of *Escherichia coli* except S9 and L20 have been mapped. Apart from the two main clusters at 72 and 89 min (1), the pinpointing of ribosomal protein gene loci has mainly been done by using mutants with electrophoretic alterations in the protein. A number of mutants with alterations in protein S9 have been described (2, 3). However, these were either mutants of *E. coli* B (3) or of a derivative of *E. coli* K-12 which had lost the Hfr ability to mobilize its chromosome, although it maintained the surface exclusion properties of an Hfr strain (2). Such factors, plus the paucity of markers in these strains, precluded their use as recipients in crosses with Hfr strains to locate *rpsI*, the gene for protein S9.

Therefore, I developed a selection which gave rise to a mutant with an alteration in protein S9, starting from an F^- strain, and I used this mutant to locate *rpsI*. I then investigated the involvement of protein S9 in the phenotype of kasugamycin dependence, for which there were several lines of evidence (see below).

MATERIALS AND METHODS

The materials and methods used were essentially those previously described (3). Strains used in this work are listed in Table 1. Strain TA44 was derived from *E. coli* B strain L44 by crossing it with Hfr strain AT2472. leu^+ recombinants were tested for acquisition of K-12 host specificity by determining the plating efficiency of P1 phage grown on strains L44 and K-12 strain CP78. The *argF* mutation was eliminated by transduction, and an *argG* mutation was introduced as described previously (4). A *thr* mutation was also introduced by auxotroph enrichment.

Strain TA128 was a faster-growing derivative of strain VT628 (2) in which the ribosomal protein mutations were the same. The *fabE argR* strain TA130 was derived from *argR* strain MA1030. *aroE* was introduced into strain MA1030 by using a nearby spectinomycin resistance mutation selected in Hfr strain AT2472. The *aroE argR* strain was then transduced to $aroE^+$ with a lysate of *fabE* strain L8; TA130 was a *fabE argR* transductant from this selection.

The Tn*10* insertion between *argG* and *rpsI* (see Fig. 2 for markers in this region of the chromosome) was obtained from a mixed population of insertions in strain NF915. Lambda NK370 (*b*221 *c*1857 *c*1171::Tn*10* *O*uga261), provided by B. Mygind, Copenhagen University, was used to select about 1,000 tetracycline-resistant (Tc^r) clones of separate origin. A P1 lysate of a pool of these strains was used to select $fabE^+$ Tc^r transductants of strain L8. A lysate of a pool of these transductants was used to select $argG^+$ Tc^r into strain JC411. Strain TA129, in which the transposon was mapped and found to be between *argG* and *rpsI*, was constructed from one of the $argG^+$ Tc^r transductants. Kasugamycin-dependent (Ksg^d) mutants were selected as previously described (3). Mutants MV101 and MV102 were isolated after ethyl methane sulfonate mutagenesis of strain TA44. They were Ksg^d at 37°C and above but not at 30°C; they were also kasugamycin resistant at 37°C and above. Mutant MV103 was isolated after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis; its dependent phenotype was clearer at 30°C than at 37°C, and it did not grow at all at 42°C.

Wild-type forms of ribosomal proteins S9 and S11 migrate to the same position on two-dimensional gels (e.g., Fig. 1A). Distinguishing between alterations in these proteins was possible on the basis of transduction experiments, since the gene for protein S11 maps close to *aroE* (1). Additionally, the spot of protein S9 is considerably stronger than that of protein S11 (see, for example, Fig. 1B). There is a second, weaker spot of S11 (not visible in these photographs) that also has altered mobility in S11 mutants.

RESULTS

Isolation of F^- *rpsI* mutant. The selection used in earlier work to give rise to strains with alterations in any of all ribosomal proteins ame-

TABLE 1. Strains used in this work

Strain	Genotype	Source or reference
AT2472	Hfr *aroE24 thi-1 relA1*	L. Gorini
CP78	F⁻ *thr-1 leuB6 his-65 argH46 thi-1 gal-3 malA1 xyl-7 mtl-2 tonA2 supE44*	K. Isono
JC411	F⁻ *leu-6 his-1 argG6 metB1 lacY1 gal-6 xyl-7 mtl-2 malA1 rpsL104 tonA2 supE44*	K. Isono
KL14	Hfr *thi-1 relA1*	K. Isono
KL228	Hfr *thi-1 leu-6 gal-6 lacY1 supE44*	K. Isono
KM25-1	as strain TA10, *rpsL rplM1 argH*⁺ *argG*	(5)
L8	F⁻ *gltA5 fabE22 lct-1 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL20 tsx-57 tfr-5 supE44*	B. Bachmann
L44	F⁻ *argF40 argR11 leu*, B strain	L. Gorini
MA1030	Hfr *argR46 thi-1 purF1*	B. Bachmann
NF915	as strain CP78, λˢ	N. Fiil
PB67	as strain TA10, *rpoB ridA1*	(6)
TA10	as strain CP78, *aroE24 rpsE gyrA*	(6)
VT385	Hfr *metB1 relA1 rna-19 rpsL rplM4*	(2)
VT597	Hfr *metB1 relA1 rna-19 rpsL rpsI3*	(2)
KM100	as strain TA10, *rpsL rplN*	This work
KM140	as strain KM100, *rpsI1*	This work
MV101	as strain TA44, *rpsI4*	This work
MV102	as strain TA44, *ksgA*	This work
MV103	as strain TA44, Ksgᵈ	This work
TA44	as strain L44, *hsd* of K12	This work
TA128	Hfr *metB1 relA1 rna-19 rpsL rpsI2 rpmE2*	This work
TA129	as strain TA128, *zgi*::Tn*10*	This work
TA130	as strain MA1030, *fabE22 rpsE*	This work

nable to analysis on two-dimensional gels had employed strain VT (2), a streptomycin-dependent mutant of unusual phenotype. Therefore, streptomycin-dependent mutants were isolated from F⁻ strain TA10. I chose mutant KM100 from the candidates because it resembled strain VT in phenotype. Mutant KM100 gave rise to antibiotic-independent revertants with alterations in any of a number of ribosomal proteins. One of 40 revertants analyzed on gels showed a reproducible change in the electrophoretic mobility of protein S9. In this revertant, KM140, the protein migrated faster to the cathode than wild-type, i.e., it was more basic (Fig. 1A and B). I mapped the *rpsI1* mutation.

Mapping of *rpsI*. *rpsI* was between the points of origin of Hfr strains KL14 (PO68) and KL228 (PO13). Gradient of transmission experiments placed the lesion between PO68 and *aroE*, i.e., between 66 and 72 min. Further localization was made by transduction with phage P1*vir*.

The genes for four other ribosomal proteins have been found in this segment of the linkage map. These are the genes for proteins S15, L21, L27, and L13 (5, 8). Loci for the first three are about 80% cotransducible with *argG* and the fourth is about 10% cotransducible. I used a lysate of mutant KM140 as donor of *argG*⁺ to strain JC411, but no cotransduction of *rpsI* with this marker was found (0 of 40). No cotransduction with *aroE* (which would be located to the right of *fabE* in Fig. 2) found either. However, when a *fabE rpsI* strain was transduced to *fabE*⁺, 2 of 10 transductants had the wild-type form of protein S9. Therefore, the mutation responsible was between *argG* and *fabE*.

Since mutant KM140 grew very poorly, further pinpointing of the *rpsI* locus was done by using other strains with alterations in protein S9, especially strain TA128 (this had the same type of S9 alteration as strain KM140). Strain TA128 was used as donor of *fabE*⁺ to strain L8, and 3 of 20 transductants had acquired the altered form of protein S9. To determine the side of *argR* on which the *rpsI* gene lay, I selected *fabE*⁺ from strain TA128 into strain TA130 and scored for *rpsI* and *argR*. The results (Table 2) indicated that the ribosomal gene was between *argR* and *argG* rather than between *fabE* and *argR*. This was also where the genes for ribosomal proteins L13, L21, and L27 were located (5, 8). When strain TA128 was donor of *argG*⁺ to strain JC411, 8 of 40 transductants acquired the altered form of protein S9. The earlier failure to obtain a similar result with strain KM140, which was reproducible, was due to the presence of a lesion between *argG* and *rpsI* that presumably arose from the *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis used to obtain the mutant.

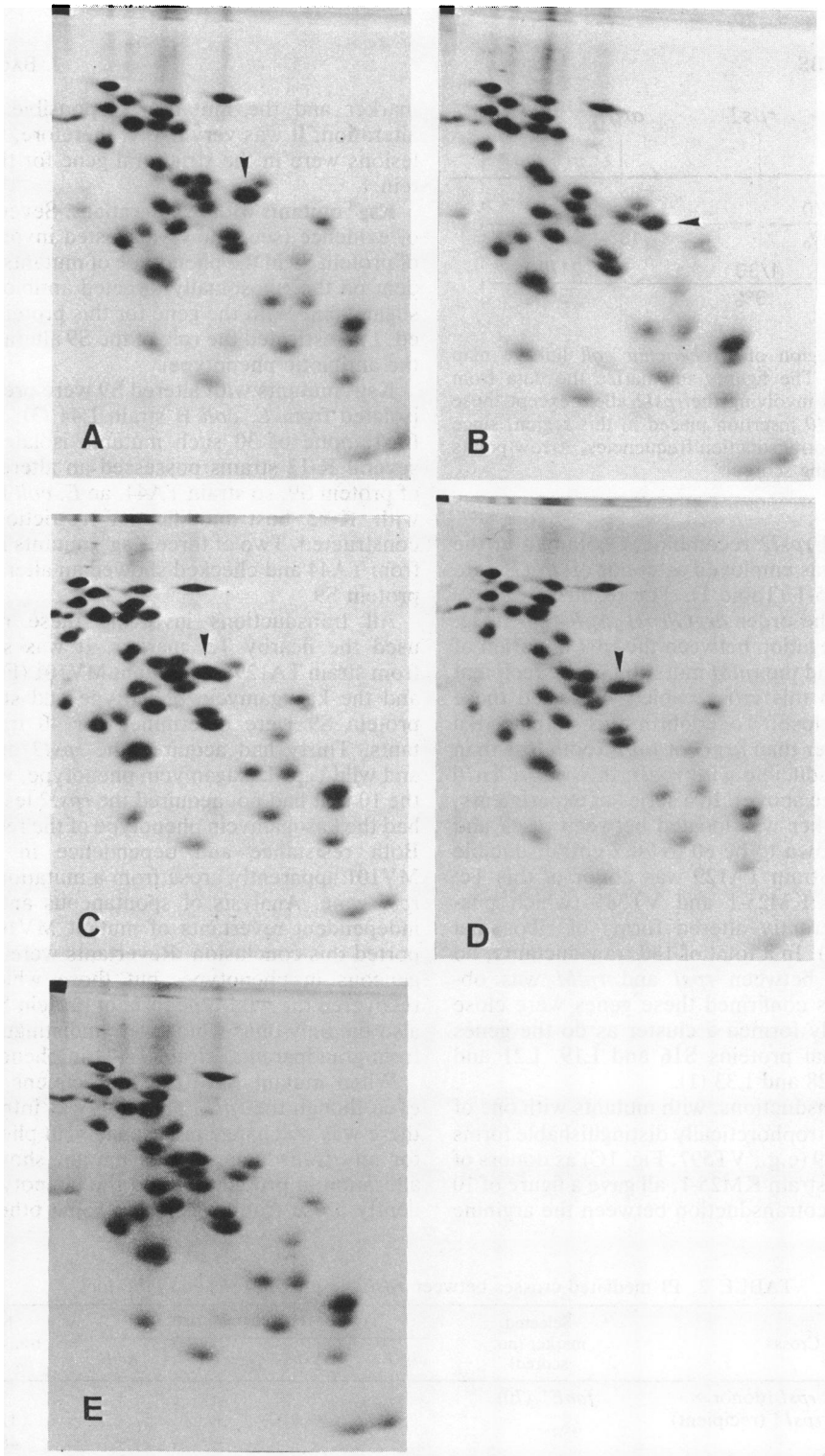

FIG. 1. Two-dimensional gel electropherograms of 70S ribosomal proteins of the strains used in this work. The spot of protein S9 is arrowed. In wild-type strains, the spots of proteins S9 and S11 comigrate (see the text). (A) CP78 (wild type), (B) KM140, (C) VT597, (D) MV101, (E) MV103. Changes in other protein spots in (B) and (C) reflect mutations in other ribosomal protein genes which arose during original selections. Differences seen in (D) and (E), apart from S9, reflect differences in ribosomal proteins between *E. coli* B strains compared with *E. coli* K-12 strains [as seen in (A)]. First dimension runs from left to right, with cathode to the right. Second dimension runs from top to bottom, with cathode on the bottom.

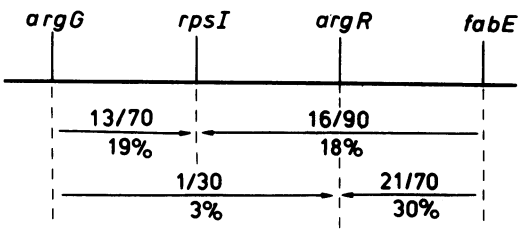

FIG. 2. Region of *Escherichia coli* linkage map around *rpsI*. The figures summarize the data from transductions involving the *rpsI2* allele except those using the Tn*10* insertion placed in this region, since this altered cotransduction frequencies. Arrow points to marker being scored.

The *argR rpsI2* recombinant obtained in the first cross was employed as donor of *argG*$^+$ into strain KM25-1 (Table 1). The results (Table 2) confirmed the order *argG-rpsI-argR-fabE* (Fig. 2). No segregation between the *rpsI* mutation of the donor and the *rplM* mutation of the recipient was seen in this cross, which suggested these loci were close. To confirm this, I placed a marker closer than *argG* or *fabE* (both less than 20% cotransducible with *rpsI*); this was a Tn*10* insertion (see above). In a series of experiments, the Tc^r marker was located between *argG* and *rpsI* and shown to be 60 to 70% cotransducible with *rpsI*. Strain TA129 was donor of this Tc^r into strains KM25-1 and VT385 (which possessed differently altered forms of ribosomal protein L13). In a total of 140 transductants, no segregation between *rpsI* and *rplM* was observed. This confirmed these genes were close and probably formed a cluster as do the genes for ribosomal proteins S16 and L19, L21 and L27, and L28 and L33 (1).

Other transductions, with mutants with one of several electrophoretically distinguishable forms of protein S9 (e.g., VT597; Fig. 1C) as donors of *argG*$^+$ into strain KM25-1, all gave a figure of 10 to 20% for cotransduction between the arginine marker and the mutation responsible for S9 alteration. It was very likely, therefore, that the lesions were in the structural gene for this protein.

Ksg^d mutants with S9 alterations. Several lines of evidence (see below) suggested involvement of protein S9 in the phenotype of mutants dependent on the ribosomally targeted antibiotic, kasugamycin. With the gene for this protein located, I investigated the role of the S9 alterations in the antibiotic phenotype.

Ksg^d mutants with altered S9 were previously isolated from *E. coli* B strain L44 (3). In contrast, none of 30 such mutants isolated from several K-12 strains possessed an altered form of protein S9, so strain TA44, an *E. coli* B strain with K-12 host modification-restriction, was constructed. Two of three Ksg^d mutants isolated from TA44 and checked showed an alteration in protein S9.

All transductions involving these mutants used the nearby Tc^r marker. It was selected from strain TA129 into mutant MV101 (Fig. 1D), and the kasugamycin phenotype and status of protein S9 were determined for 40 transductants. Thirty had acquired the *rpsI2* mutation and wild-type kasugamycin phenotype, whereas the 10 that had not acquired the *rpsI2* lesion still had the kasugamycin phenotype of the recipient. Both resistance and dependence in mutant MV101 apparently arose from a mutation in the *rpsI* gene. Analysis of spontaneous antibiotic-independent revertants of mutant MV101 supported this conclusion. Revertants were heterogeneous in phenotype, but those which had recovered the wild-type form of protein S9 were also the only ones which were indistinguishable from grandparental strain TA44 in phenotype.

When mutant MV102 was recipient to Tc^r, even though the *rpsI2* mutation was introduced there was no change in kasugamycin phenotype for any transductant. This mutant showed no alteration in protein S9, and the phenotype evidently arose from a lesion in some other ribo-

TABLE 2. P1-mediated crosses between *rpsI* and *argG*, *argR*, and *fabE* loci

Cross	Selected marker (no. scored)	Transductant characteristics[a]				No. of transductants (% of total)
		argG	*rpsI*	*argR*	*fabE*	
fabE$^+$ *argR*$^+$ *rpsI* (donor × *fabE argR rpsI*$^+$ (recipient)	*fabE*$^+$ (70)		+	+	+	9 (13)
			−	+	+	12 (17)
			+	−	+	48 (69)
			−	−	+	1 (1)
argG$^+$ *rpsI argR* (donor) × *argG rpsI*$^+$ *argR*$^+$ (recipient)	*argG*$^+$ (30)	+	+	+		25 (83)
		+	+	−		0 (0)
		+	−	+		4 (13)
		+	−	−		1 (3)

[a] +, wild-type allele; −, mutant allele.

somal component. Mutant MV103 (Fig. 1E) resembled a number of Ksg^d mutants isolated earlier (described in reference 3) in that electropherograms showed protein S9 apparently absent from the ribosome. However, cellulose acetate gel electropherograms of these mutants' ribosomal proteins revealed a band of S9 cross-reacting material much reduced in strength and much altered in mobility in comparison with the wild type (G. Stöffler and E. Dabbs, unpublished data). Tc^r transductants of this mutant all maintained the phenotype of the recipient, and none recovered a spot corresponding to protein S9. When the same selection was made with a donor strain that possessed the *rplM* mutation of KM25-1, 7 of 10 transductants analyzed acquired the *rplM1* lesion, but all remained Ksg^d and failed to show a spot of protein S9 on gels. Thus, the mutation in mutant MV103 responsible for the drastic alteration in S9 was not in the structural gene of the protein.

Response of MV101 to antibiotic deprivation. Earlier, the ribosomes of Ksg^d mutants were characterized in terms of their response to kasugamycin (unpublished data) (7). In every case, there was no in vitro dependence on kasugamycin for polypeptide synthesis of fMet-tRNA$_f^{Met}$ binding. All strains with a mutational alteration in ribosomal protein S9 had ribosomes that were hypersensitive to kasugamycin, although the strains were dependent on—and often resistant to—antibiotic in vivo. The ribosomes of no other strain were hypersensitive to kasugamycin. It was necessary to reconcile the apparently divergent in vivo and in vitro responses to antibiotic, so to understand better the nature of kasugamycin dependence, I monitored the response of cultures of mutant MV101 to antibiotic removal.

A strain with a Ksg^d phenotype known to result from mutation at a locus other than *rpsI* was run in parallel in some experiments. Strain PB67 was used; dependence in this mutant results from a lesion at the *ridA* locus, about 1 min from *rpsI* on the linkage map (6).

Figure 3 shows the effects on cell density and viable cell counts of kasugamycin deprivation. The first response of mutant MV101 to this treatment was an increase in both parameters in comparison with the culture maintained in the presence of antibiotic. In contrast, mutant PB67 showed no surge of absorption or cell number upon deprivation. Increase in cell number for mutant MV101 slowed even as a substantial increase in optical density was still occurring; a plateau in optical density was attained only at a time when irreversible changes had led to loss of viability in over half the population of cells. Later stages of the response to deprivation were qualitatively the same for mutants MV101 and PB67 in that removal of kasugamycin from the medium was bactericidal. It was the stimulatory effect of deprivation that was absent with strain PB67.

The result of deprivation on rates of nucleic acid and protein synthesis are shown for mutant MV101 in Fig. 4. As expected for a ribosomally targeted antibiotic, the initial effect was on protein synthesis and again it was one of stimulation. Only when the protein synthesis rate slowed was a change in nucleic acid synthesis rate apparent; it too was a slowing, and there was no stimulatory effect on this synthesis rate due to deprivation.

These data presented a consistent picture. They showed that mutant MV101 was dependent on kasugamycin because the antibiotic was needed to depress the level of protein synthesis. Removal led to enhanced ribosome activity, which was reflected in increased cell mass and number. This enhancement presumably caused a disequilibrium in the cell, manifested first by a

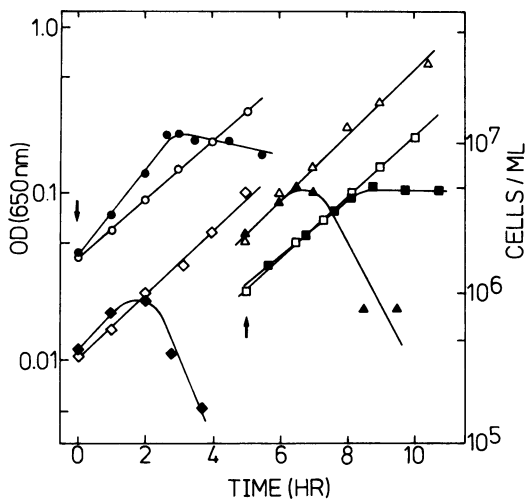

FIG. 3. Growth curves of mutants MV101 and PB67 monitored at 650 nm. Cells of MV101 growing in kasugamycin-containing medium (○) and in medium without kasugamycin (●); cells of PB67 growing in kasugamycin-containing medium (□) and in medium without kasugamycin (■). Viable counts of MV101 in kasugamycin-containing medium (◇) and in medium without kasugamycin (◆). Viable counts of PB67 in medium with kasugamycin (△) and in medium without kasugamycin (▲). When present, kasugamycin was at a concentration of 50 μg/ml. Arrow indicates the time at which cells in late-log phase were transferred to fresh medium at the start of the experiment. The large difference in viable counts of MV101 and PB67 for a particular optical density at 650 nm (OD_{650}) presumably reflected the fact that mutants selected for Ksg^d phenotype were elongated to a filamentous morphology (K. Looman and E. Dabbs, unpublished data), whereas PB67 (which was not selected for Ksg^d phenotype) showed normal morphology.

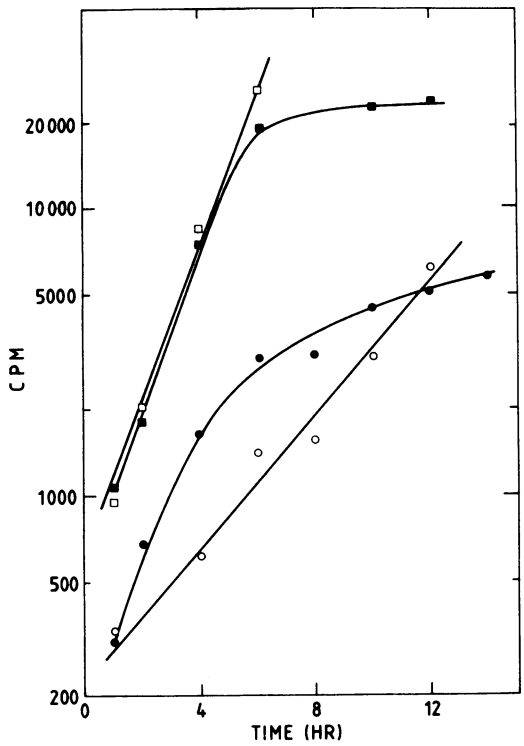

FIG. 4. Protein and nucleic acid synthesis of mutant MV101 growing in minimal medium, with or without kasugamycin. Protein synthesis in the presence (○) and absence (●) of kasugamycin; nucleic acid synthesis in the presence (□) and absence (■) of kasugamycin. Arginine, threonine, leucine, and uridine were present at 40 μg per ml. [^{3}H]uridine was present at 0.2 μCi/ml, and [^{14}C]leucine was present at 0.05 μCi/ml. Cells were suspended in fresh medium with or without antibiotic at time zero.

slowing of cell division and then by a reduction in RNA and protein synthesis activity. This disequilibrium was rapidly lethal to the cell. My genetic analysis showed that this enhancement of ribosome activity in mutant MV101 was due to mutational alteration in ribosomal protein S9.

Kasugamycin exerts its effect on the initiation step of protein synthesis (11); protein S9 is part of the ribosomal domain involved in initiation (for example, see reference 10). Due to the alteration in protein S9, protein synthesis (probably initiation of protein synthesis) was not constrained to act in harmony with other cellular processes in mutant MV101. A constraint could be imposed by the presence of kasugamycin, and hence the mutant needed this antibiotic to be present in the medium for sustained growth.

Resistance can also be explained. Wild-type *E. coli* B strains grew in the presence of 0 to 200 μg of kasugamycin per ml. If one posited a similar range for mutant MV101, which needed a minimum of 100 μg of kasugamycin per ml to grow (at 37°C and above), then the expected phenotype was not far from that observed: mutant MV101 grew in media containing between 100 and 400 μg of antibiotic per ml.

In terms of the alteration in protein S9 resulting in hypersensitivity of ribosomes to kasugamycin, it was not surprising that a mutationally altered protein affected the antibiotic response properties of a ribosomal domain of which the protein was a part and upon which the antibiotic acted. S9 mutants with ribosomes of decreased sensitivity to kasugamycin might also be anticipated; that such mutants have not been found among Ksg^d strains may be because decreased sensitivity would be counterproductive to the proposed "rescue" effect (i.e., partial ribosomal inhibition) of kasugamycin in Ksg^d strains. Hypersensitivity of ribosomes would aid this rescue effect.

DISCUSSION

I used mutants with alterations in small subunit ribosomal protein S9 to locate *rpsI*, the gene for this protein, at about 70 min on the *E. coli* chromosomal linkage map. *rpsI* was roughly halfway between *argG* and *fabE*, and was close to *rplM*, the gene for large subunit protein L13. The genes for these two proteins may form a cluster.

Inserting a Tn*10* transposon near *rpsI*, I investigated the involvement of protein S9 alterations in the phenotype of Ksg^d mutants. Several lines of evidence suggested such an involvement. S9 mutants are comparatively very rare (unpublished data), and yet a number of Ksg^d strains (reference 3; this work) possessed alterations in this protein. Spontaneous antibiotic-independent revertants of mutant MV9 (3), which had a drastically altered form of S9, had a form of this protein which migrated to the same position on cellulose acetate gels as the wild-type protein (G. Stöffler and E. Dabbs, unpublished data). There was also the fact that ribosomes of all S9 Ksg^d mutants showed altered response to kasugamycin in vitro, whereas ribosomes from all other strains did not (7). This suggested involvement of S9 in ribosome response to kasugamycin, albeit that it was hard to reconcile dependent and resistant behavior of growing cells with ribosomes independent of and hypersensitive to this antibiotic.

My genetic experiments showed that in mutant MV101 both dependence on and resistance to kasugamycin were the result of a lesion at the *rpsI* locus. Monitoring the response of cultures of this mutant to antibiotic deprivation revealed that kasugamycin was necessary to slow protein synthesis and presumably prevent a lethal disequilibrium in the cell. This allowed me to offer

an explanation reconciling the in vivo and in vitro responses to kasugamycin.

A puzzling fact was the absence of protein S9 alterations in Ksg^d mutants isolated from several *E. coli* K-12 strains. To investigate this, I used the Tc^r marker near *rpsI* to transfer the lesion of mutant MV101 into K-12 strain CP78. Mutant MV101 (see above) was dependent at 37°C and above and resistant at all temperatures. In the K-12 background, the *rpsI* mutation conferred resistance at 30°C, and the strain did not grow at 37°C and above. However, in contrast to the B background, this inability to grow was not reversed by the presence of kasugamycin in the medium. Two of three phenotypic attributes of the *rpsI* mutation of mutant MV101 were maintained upon transfer from *E. coli* B to *E. coli* K-12, which suggested a rather subtle difference in the initiation domains of ribosomes of the two strains. One candidate for the ribosomal component mediating such a difference was ribosomal protein S1, which is electrophoretically distinguishable in different strains of *E. coli* (9) and is different in *E. coli* B and K-12 (G. Stöffler and E. Dabbs, unpublished data).

In conclusion, it is clear that protein S9 is one of the small number of ribosomal proteins in which mutational alteration can alter phenotype. The change in electrophoretic mobility of S9 in mutant MV101 was fully consistent with a single-charge change in the protein, yet it was sufficient—in the absence of appropriate concentrations of the right antibiotic—to kill the cell. Protein S9 must be considered an important ribosomal protein.

LITERATURE CITED

1. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
2. **Dabbs, E. R.** 1978. Mutational alterations in 50 proteins of the *Escherichia coli* ribosome. Mol. Gen. Genet. **165**:73–78.
3. **Dabbs, E. R.** 1978. Kasugamycin-dependent mutants of *Escherichia coli*. J. Bacteriol. **136**:994–1001.
4. **Dabbs, E. R.** 1980. The gene for ribosomal protein S21, *rpsU*, maps close to *dnaG* at 66.5 min on the *Escherichia coli* chromosomal linkage map. J. Bacteriol. **144**:603–607.
5. **Dabbs, E. R.** 1982. The gene for ribosomal protein L13, *rplM*, is located near *argR*, at about 70 minutes on the *Escherichia coli* chromosomal linkage map. J. Bacteriol. **149**:779–782.
6. **Dabbs, E. R., and K. Looman.** 1981. An antibiotic dependent conditional lethal mutant with a mutation affecting transcription and translation. Mol. Gen. Genet. **184**:224–229.
7. **Dabbs, E. R., B. Poldermans, H. Bakke, and P. H. Van Knippenberg.** 1980. Biochemical characterisation of ribosomes of kasugamycin dependent mutants of *Escherichia coli*. FEBS Lett. **117**:164–166.
8. **Kitakawa, M., E. R. Dabbs, and K. Isono.** 1979. Genes coding for ribosomal proteins S15, L21, and L27 map near *argG* in *Escherichia coli*. J. Bacteriol. **138**:832–838.
9. **Laughrea, M., and P. B. Moore.** 1978. On the relationship between the binding of ribosomal protein S1 to the 30S subunit of *Escherichia coli* and the 3′ terminus of the 16S RNA. J. Mol. Biol. **121**:411–430.
10. **Sillers, I. Y., and P. B. Moore.** 1981. Position of protein S1 in the 30S ribosomal subunit of *Escherichia coli*. J. Mol. Biol. **153**:761–780.
11. **Tai, P. C., B. J. Wallace, and B. D. Davis.** 1973. Actions of aurintricarboxylate, kasugamycin, and pactamycin on *Escherichia coli* polysomes. Biochemistry **12**:616–620.