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Kasugamycin-Dependent Mutants of *Escherichia coli*

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Kasugamycin-dependent mutants have been isolated from *Escherichia coli* B. They were obtained through mutagenesis with ethyl methane sulfonate or nitrosoguanidine in conjunction with an antibiotic underlay technique. In the case of nitrosoguanidine, dependent mutants were obtained at a frequency of about 3% of survivors growing up in the selection. In the case of ethyl methane sulfonate, the corresponding value was 1%. Nineteen mutants showing a kasugamycin-dependent phenotype were studied. In terms of response to various temperatures and antibiotic concentrations, they were very heterogeneous, although most fell into two general classes. Genetic analysis indicated that in at least some cases, the kasugamycin-dependent phenotype was the product of two mutations. Two-dimensional gel electropherograms revealed alterations in the ribosomal proteins of seven mutants. One mutant had an alteration in protein S13, and one had an alteration in protein L14. Three showed changes in protein S9. Each of two mutants had changes in two proteins, S18 and L11. Three of these mutants additionally had protein S18 occurring in a partly altered, partly unaltered form.

The use of antibiotics has been a major approach to obtain mutationally altered ribosomes. Ribosomally targeted antibiotics which have been studied in some detail include, among others, streptomycin and related antibiotics (e.g., bluensomycin and dihydrostreptomycin), spectinomycin, and kasugamycin. Mutants of *Escherichia coli* resistant to streptomycin have been shown to have an alteration in small-subunit protein S12 (18). Mutants resistant to spectinomycin have been found to be altered in protein S5 (3). Distinct from these is kasugamycin, where resistance has been shown to be due to a change in the RNA moiety rather than in the protein moiety of the ribosome (11). When antibiotic-dependent mutants are obtained, they can give further insight concerning functional interactions between ribosomal components since from them antibiotic-independent secondary mutants can be obtained which, in the cases studied, are mutants with changes in a component(s) other than that originally altered. The best known case involves dependence on streptomycin, where the lesion due to the protein S12 alteration is relieved by alterations in protein S4 or S5 (10).

Mutants dependent on other antibiotics, such as spectinomycin or kasugamycin, are not obtained when cells of *E. coli* are spread on antibiotic-containing plates and incubated. In the case of streptomycin, when the underlay technique (2) is used for bringing the cells into contact with antibiotic more gradually, 90 to 95% of survivors are streptomycin dependent, versus 20 to 50% when direct spreading on antibiotic-containing plates is used. The technique has been tried with another antibiotic, and the successful isolation of spectinomycin-dependent (Spc^d) mutants of *E. coli* has recently been reported (5).

Mutants of *E. coli* resistant to kasugamycin have lost activity of a methylase that acts on two adjacent adenine residues near the 3′ terminus of the 16S RNA (11). This part of the RNA is essential to ribosomal function (4) and is near a functional site of the 30S subunit during initiation complex formation (22). Kasugamycin specifically inhibits initiation (20). The factor IF-3 (23) and ribosomal proteins S1 (15) and S18 and S21 (21) are in the vicinity of this part of the 16S RNA. As a further probe of this ribosomal neighborhood, it seemed worthwhile to attempt to isolate kasugamycin-dependent (Ksg^d) mutants.

Ksg^d mutants have in fact been obtained as 3 to 4% of the survivors under appropriate conditions. The properties of these mutants are described in this paper.

MATERIALS AND METHODS

Bacterial strains. Strain L44 (*E. coli* B *argF leu* from L. Gorini) and its derivatives were used throughout this work. LR was a spontaneous rifampin-resistant (Rif^r) mutant of L44. LT was a *thr* derivative of L44 obtained by ethyl methane sulfonate (EMS) mutagenesis and penicillin selection. LTK was a sponta-

neous kasugamycin-resistant (Ksg^r) mutant of LT.

Several F$^-$ strains of *E. coli* K-12 were also used in an attempt to isolate Ksg^d mutants, since genetic analysis is easier in such strains than in *E. coli* B strains. Such attempts were unsuccessful.

Media. The media used were as described in reference 6. Rich medium used in the preparation of P1 lysates, and for transductions, contained 3 g of NaCl per liter instead of buffering salts, since these salts included sodium citrate.

EMS mutagenesis was carried out by washing exponentially growing cells of strain L44 in buffer, suspending them in buffer at a concentration of about 5×10^8 cells per ml in the presence of 1% EMS (vol/vol), and incubating them at 37°C for 60 min. Afterwards, cells were washed in buffer and then used in the selection as described below. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) mutagenesis was done by washing exponentially growing cells in tris(hydroxymethyl)aminomethane-maleate buffer at pH 6.2 (1), suspending them at a concentration of about 5×10^8 in the same buffer, and then adding 0.1 volume of a solution of 2 mg of NTG per ml to give a final concentration of 200 μg of NTG per ml. The suspension was incubated for 10 min at 37°C, after which it was cooled in an ice-water slurry. Cells were washed twice in pH 7.0 buffer and then used in selection. EMS was obtained from Sigma Chemical Co., and NTG was from EGA Chemie KG. Kasugamycin sulfate was obtained from Boehringer Mannheim GmbH.

Selection of Ksg^d mutants. A variation of the streptomycin underlay technique (2) was used in which kasugamycin was employed instead of streptomycin. Cells suspended at about 5×10^8/ml in buffer, either with or without prior mutagenesis, were spread in a series of dilutions on rich plates. A 0.1-ml portion of suspension was spread per plate, and the plates were incubated for 3 h at 37°C. A 0.15-ml amount of a stock solution of kasugamycin containing 200 mg of antibiotic per ml was introduced under the agar, using a sterile spatula. The plates were stored for 36 to 48 h at 4°C to allow diffusion of the antibiotic. Since plates held about 30 ml of medium, the final kasugamycin concentration in the plates was about 1 mg/ml. The plates were returned to 37°C and incubated for 4 to 5 days. Colonies appearing were patched onto plates containing 1 mg of kasugamycin per ml, incubated at 37°C for 2 days, and then streaked for single colonies on plates also containing 1 mg of kasugamycin per ml. A large and a small colony from the streak of each mutant was selected and spotted on plates containing 0, 50, 100, 400, and 1,000 μg of kasugamycin per ml. These plates were incubated at 30, 37, and 42°C. Putative Ksg^d mutants, designated MV1 through MV35, were restreaked and spot-tested again.

Transduction. Transductions were carried out with phage P1*vir* essentially according to the method of Lennox (16). For strains with a generation time of about 20 min, plate lysates were incubated for 6 h at 37°C. For slower growing strains, plates were incubated proportionally longer according to their generation time. Afterwards, plates were selected which had complete lysis but the lowest concentration of input phage. P1 lysates of Ksg^d strains were dialyzed against 2×50 volumes of medium to remove antibiotic. Introduction of antibiotic resistance markers into a strain required preincubation of recipient cells to allow phenotypic expression before they were exposed to the antibiotic. Recipients with growth rates of about 20 min were preincubated for 2 h, this time being prolonged proportional to the generation time. Antibiotic was then introduced under the agar, and a procedure the same as that described above was followed.

Ribosomal proteins. Mutants were grown in 40 ml of rich medium to stationary phase. Ribosomal proteins were prepared from frozen cells as described in reference 7. Samples of 0.6 to 1.0 mg of protein were run on a scaled-down version of the two-dimensional polyacrylamide gel system described by Kaltschmidt and Wittmann (14). The first dimension was 9.5 cm, and the second was 9.0 cm. Both dimensions were run at 4°C for 18 h at 80 V and 16 h at 80 V, respectively.

RESULTS

Isolation of mutants. Selection of Ksg^d mutants is shown in Table 1. The best conditions for selection of mutants involved an incubation temperature of 37 or 42°C, with NTG as mutagen. Attempts to isolate Ksg^d mutants spontaneously were unsuccessful. EMS mutagenesis could be used but was less effective than NTG mutagenesis. Further selections have been subsequently done; they also resulted in about 1% Ksg^d mutants for EMS and between 3 and 4% for NTG.

Phenotype of mutants. Mutants were tested for their antibiotic response at various kasugamycin concentrations and various temperatures (30, 37, and 42°C). The L44 parent strain was able to grow at 100 μg, but not at 300 μg, of kasugamycin per ml at all three temperatures.

Most mutants fell into one of two classes. In mutants of class I (mutants MV 3, 7, 8, 11, 12, 21, 22, and 27), the maximum and minimum concentrations of kasugamycin at which cells would grow were independent of temperature. In mutants of class II (mutants MV 4, 10, 15, 16, 17, 34, and 35), these parameters correlated strongly with temperature. Class II mutants grew at 30°C in the absence of kasugamycin but were inhibited by the presence of 300 μg of kasugamycin per ml. At 42°C, they required 200 μg of kasugamycin per ml to grow, and they also grew on 1,000 μg/ml. The situation at 37°C varied from mutant to mutant. Some mutants were Ksg^r at this temperature, and others were Ksg^d. Even within each class, the mutants were heterogeneous in terms of exact response to temperature and antibiotic concentration.

Four mutants could not be placed in either class I or class II. Mutants MV2 and MV29 grew at neither 30 nor 42°C, whatever the kasugamycin concentration. Mutant MV1 grew only at 30°C or below 37°C and was Ksg^d within this range. Mutant MV9 was Ksg^d at 30°C and Ksg^r

at 42°C. For a more precise idea of the growth response of Ksg^d mutants, the growth rates of two mutants were determined at a variety of antibiotic concentrations (Fig. 1).

Ribosomal proteins of Ksg^d mutants. The ribosomal protein patterns of the 19 Ksg^d mutant strains were analyzed by using two-dimensional polyacrylamide gel electrophoresis (14). Seven mutants showed reproducible alterations. Portions of the electropherograms of the ribosomal proteins of these mutants are shown in Fig. 2. The acidic half of the gels is not shown since no mutants showed alterations in proteins located in this region. The electropherograms of the proteins of these mutants can be compared with a control 70S pattern in which all the proteins are identified, as shown in reference 12. Electropherograms of ribosomal proteins of *E. coli* K-12, as shown in reference 12, are the same as for *E. coli* B (of which L44 is a derivative), except for two proteins. Protein S5 is more basic in *E. coli* B and, hence, comigrates with protein L6 and not L11. Protein S7 is smaller in *E. coli* B and is located on the gel at a position below that seen in electropherograms of ribosomal proteins of *E. coli* K-12. Electropherograms of proteins from 30S and 50S subunits of ribosomes of *E. coli* B are shown in reference 24. The protein alterations of mutants MV2 and MV29 were indistinguishable. The alteration in mutant MV9 appeared to be the same as that in mutant MV35. Therefore, mutants MV29 and MV35 are not illustrated.

Mutants MV2 and MV29 had two alterations

TABLE 1. *Isolation of Ksg^d mutants under varying conditions*

Pretreatment	Plate incubation temp (°C)	No. of colonies tested	Ksg^d [a]	Mutants	% Ksg^d
None	37	94	0		0
None	42	112	0		0
EMS	37	120	2	MV7, 15, 17, 21	1.3
EMS	42	155	2		
NTG	30	169	0		0
NTG	37	205	7	MV1, 2, 3, 4, 8, 9, 10, 11, 12, 16, 22, 27, 29, 34, 35	3.7
NTG	42	202	8		

[a] Ksg^d refers herein to a mutant having a kasugamycin-dependent phenotype at at least one of the three temperatures tested. Pooled results from two separate experiments.

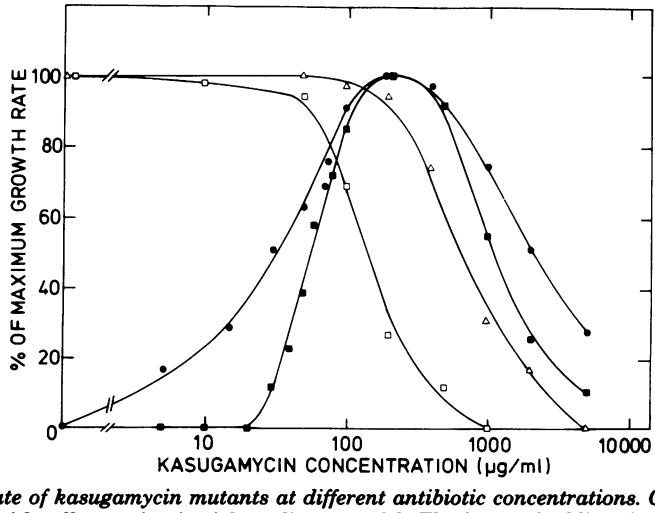

FIG. 1. *Growth rate of kasugamycin mutants at different antibiotic concentrations. Growth rate determinations were made with cells growing in rich medium at 37°C. The fastest doubling times (all at 37°C) were: for MV10, 65 min; for MV29, 60 min; for MV200, 35 min; for L44, 22 min. These values are taken as 100% for each mutant. Growth rates of mutants were measured by optical density of cultures at 650 nm. Ksg^d mutants were pregrown in rich medium containing 200 µg of kasugamycin per ml, washed twice in buffer, and then allowed to grow for 90 min in new medium before readings were taken to allow for adjustment of endogenous antibiotic level. Symbols: Ksg^d strains MV10 (●) and MV29 (■); Ksg^r mutant MV200 from the same selection (△); L44 (Ksg^s parental strain) (□).*

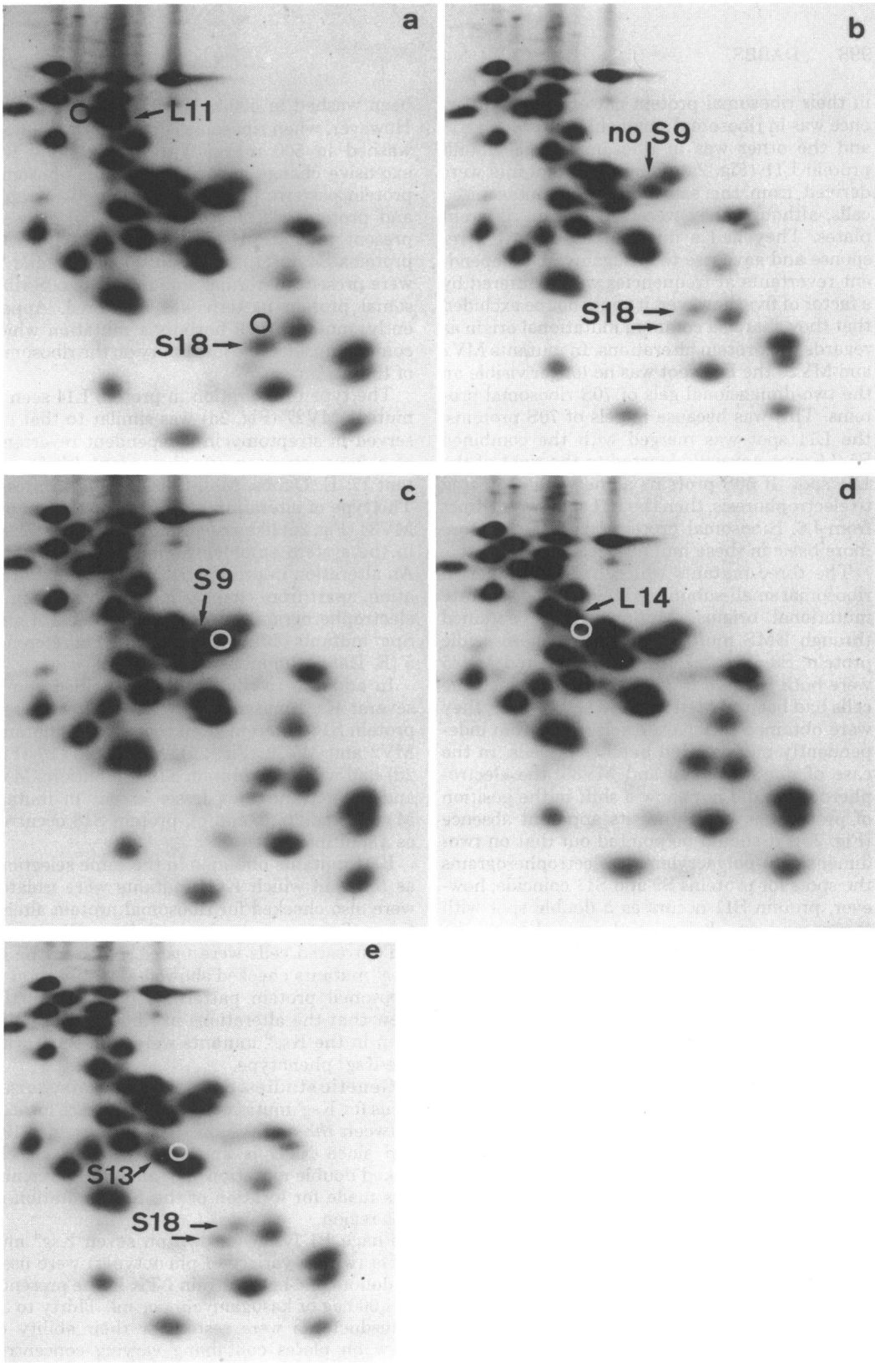

FIG. 2. *Portions of two-dimensional gel electropherograms of 70S ribosomal proteins of Ksg^d mutants of E. coli. Arrows indicate the proteins altered by mutation; the circle indicates the position of the wild-type protein. (a) MV2 (similar to MV29); (b) MV9 (similar to MV35); (c) MV21; (d) MV27; (e) MV34.*

in their ribosomal protein patterns. One difference was in ribosomal small-subunit protein S18 and the other was in ribosomal large-subunit protein L11 (Fig. 2a). These two mutants were derived from the same batch of mutagenized cells, although they were selected on different plates. They had a different kasugamycin response and gave rise to kasugamycin-independent revertants at frequencies which differed by a factor of five; however, it could not be excluded that they shared a common mutational origin as regards the protein alterations. In mutants MV2 and MV29 the L11 spot was no longer visible on the two-dimensional gels of 70S ribosomal proteins. This was because in gels of 70S proteins, the L11 spot was merged with the combined S5/L6 spot normally located to the right of the L11 spot. If 50S proteins alone were subjected to electrophoresis, then the L11 spot was distinct from L6. Ribosomal protein L11 was therefore more basic in these mutants.

The three mutants which had alterations in ribosomal small-subunit protein S9 had separate mutational origins. Mutant MV21, obtained through EMS mutagenesis, had a more acidic protein, S9 (Fig. 2c). Mutants MV9 and MV35 were both isolated from selections in which the cells had been treated with NTG; however, they were obtained in separate selections from independently mutagenized batches of cells. In the case of mutants MV9 and MV35, the electropherograms did not show a shift in the position of protein S9, but rather its apparent absence (Fig. 2b). It should be pointed out that on two-dimensional polyacrylamide electropherograms the spots for proteins S9 and S11 coincide; however, protein S11 occurs as a double spot with the second one below, and somewhat to the right, of the S9–S11 spot (25).

In mutants MV9 (Fig. 2b) and MV35, the composite spot of proteins S9 and S11—normally very prominent—was much fainter. However, the lower spot of protein S11 was present as usual, indicating that it was protein S9 that was absent. 30S subunit electropherograms failed to show an extra spot, excluding the possibility that protein S9 has moved so that its position was masked by a 50S subunit protein, nor were any 30S protein spots more strongly staining. Electropherograms of whole-cell extract showed the relatively faint S9–S11 spot also, eliminating the possibility that protein S9 was more loosely bound and was washed off during ribosome preparation.

There was one difference between the ribosomes of mutants MV9 and MV35. Electropherograms of the ribosomal proteins of mutant MV35 were similar, whether the ribosomes had been washed in 500 mM NH_4Cl buffer or not. However, when ribosomes of mutant MV9 were washed in 500 mM NH_4Cl buffer there was extensive change in the 30S subunit ribosomal protein pattern: protein S14 was undetectable, and proteins S2, 3, 5, 7, 10, 13, and 21 were present in much reduced amounts. However, proteins S4, 8, 11, 12, 15, 16, 17, 18, 19, and 20 were present in normal amounts. The 50S ribosomal protein pattern was unaltered. Apparently, mutant MV9 harbors a mutation which confers considerable instability on the ribosomes of this strain.

The type of alteration in protein L14 seen in mutant MV27 (Fig. 2d) was similar to that observed in streptomycin-independent revertants of a novel streptomycin-dependent (Str^d) mutant (7; E. Dabbs, Mol. Gen. Genet., in press). The type of alteration in protein S13 in mutant MV34 (Fig. 2e) likewise resembled that obtained in the system employing a novel Str^d mutant. An alteration in protein S13 was the only alteration, apart from changes in protein S5, seen in electropherograms of ribosomal proteins of nine Spc^d mutants obtained as described in reference 5 (E. Dabbs, unpublished data).

In addition to the changes described above, several Ksg^d mutants showed an alteration in protein S18 different from that seen in mutants MV2 and MV29 (Fig. 2a). Mutant MV27 (Fig. 2d) had wild-type protein S18. In mutants MV9 and MV34, and to a lesser extent in mutant MV21 (Fig. 2b, e, and c), protein S18 occurred as a multiple spot.

Ksg^r mutants obtained in the same selections as those in which Ksg^d mutants were isolated were also checked for ribosomal protein alterations. Twenty each obtained from EMS- and NTG-treated cells were tested. None of the 40 Ksg^r mutants checked showed any alteration in ribosomal protein pattern. This supports the view that the alterations in ribosomal proteins seen in the Ksg^d mutants were connected with the Ksg^d phenotype.

Genetic studies. Since the best characterized locus for Ksg^r mutants is the *ksgA* locus, located between *thr* and *leu* on the chromosome (19), and since NTG is known to produce closely linked double mutations (9), an initial screening was made for location of the Ksg^d mutation in this region.

Phage P1 lysates grown on seven Ksg^d mutants (with a variety of phenotypes) were used as donors of Thr^+ to strain LTK in the presence of 1,000 μg of kasugamycin per ml. Thirty to 36 transductants were tested for their ability to grow on plates containing varying concentrations of antibiotic. No transductants showed the

Ksg^d phenotype at any temperature.

Several of the mutants gave transductants that were heterogeneous in morphology and kasugamycin response, however. Since, in the case of Spc^d mutants (5), all mutants analyzed showed the combination of a spectinomycin-resistant (Spc^r) mutation and a genetically separable second mutation conferring dependence, transductions were done to determine if the Ksg^r mutation of the *ksgA* locus was present. P1 lysates of six Ksg^d mutant strains were used as donors of Thr$^+$ to strain LT, and the transductants were screened for their response to kasugamycin. Mutants MV17 and MV29 gave Ksg^r transductants (resistant to 1,000 μg of antibiotic per ml versus 100 μg/ml for the kasugamycin-sensitive [Ksg^s] parent) as 9 and 16% of the total (48 transductants tested per mutant). Mutants MV9, MV10, MV11, and MV21 gave no Ksg^r transductants. Subsequently, it became clear that although MV9 and MV11 required kasugamycin to grow, the maximum kasugamycin concentration at which they would grow was similar to that of the Ksg^s parent. Mutants MV10 and MV21, on the other hand, were capable of growing on 1,000 μg of antibiotic per ml. The possibility that there was a lesion located between *thr* and *ksgA* in mutants MV10 and MV21 that prevented cotransduction between these markers could not be excluded on the basis of experiments done in this work.

In an Spc^d mutant previously studied (5), the locus of the mutation causing the Spc^d phenotype was located near *rif* on the *E. coli* chromosome. Two Ksg^d mutants, MV2 and MV29, showed an alteration in protein L11. Since the gene for protein L11 is near *rif* (17), whether the altered protein L11 was responsible for the Ksg^d phenotype was determined. Several other Ksg^d mutants were also tested at the same time. Six Ksg^d strains were used as recipients, and an Rif^r derivative of strain L44, LR, was used to donate the Rif^r mutation into them. In the case of mutant strains MV9, MV10, MV11, and MV27, no alteration was found in the kasugamycin phenotype of the transductants compared with the strains from which the transductants were derived. Mutant MV29, with the altered protein L11, also kept its Ksg^d phenotype when the altered protein L11 was transduced out but became able to grow at 30°C as well as at 37°C. Only in the case of mutant MV21 were the transductants predominantly Ksg^r rather than Ksg^d in phenotype (20 of 20 and 16 of 20 in two different experiments), indicating that the Ksg^d phenotype in this mutant involved a mutation near the *rif* locus. A lysate of an Rif^r transductant of mutant MV21, in which the Ksg^d phenotype was still present, was used to donate the Rif^r marker to strain LTK (spontaneous Ksg^r derivative of L44). Kasugamycin was present in the medium. A majority of the transductants (9 of 12 and 24 of 40 in two different experiments) were Ksg^d. The Ksg^d phenotype of the transductants was similar to, but not identical with, that of mutant MV21. Therefore, the Ksg^d phenotype can be due to the combination of a Ksg^r mutation and a genetically separable second mutation conferring dependence.

Two strains showed alterations in proteins, S13 and L14, whose genes are located in the main cluster of ribosomal genes (13). To determine if alterations in these proteins were involved in the mutant phenotype, transductions were attempted using a spontaneous streptomycin-resistant (Str^r) (*rpsL*) derivative of L44. However, no transductants could be obtained when attempts were made to introduce this mutation into Ksg^d strains. Possibly this observation was connected with the fact that an attempt to isolate Ksg^d mutants from strain L1-401 (an Str^r derivative of strain L1, the strain from which the strain L44 used in this work is derived) was unsuccessful (228 colonies tested). It may be that an Str^r mutation and a Ksg^d mutation cannot coexist in a strain.

The involvement of the ribosomal protein S9 alterations in the kasugamycin phenotype of the strains could not be determined since the location of the gene for S9 is not yet known; however, the independent occurrence of such mutations in three separate selections strongly points to a connection.

DISCUSSION

Ksg^d mutants were not difficult to isolate under appropriate conditions. They resembled Spc^d mutants and differed from Str^d mutants in that genetic analysis indicated that in at least a sizable fraction of the mutants the antibiotic phenotype was the product of more than one mutation. The phenotype of Ksg^d mutants was the result of an antibiotic resistance mutation (which in some cases was likely to be at the *ksgA* locus) together with a further mutation(s) conferring dependence.

Two other loci for mutations altering the kasugamycin response of *E. coli*, *ksgB* (19) and *ksgC* (27), have been described. Ribosomes from mutants harboring a *ksgB* mutation do not show kasugamycin resistance in vitro, in contrast to what is found with ribosomes from mutants harboring a *ksgA* mutation (19). It may be that *ksgB* mutants have a mutation altering the permeability of cells to kasugamycin, and they are probably distinct from the mutants with

altered ribosomal proteins described here. The *ksgC* mutation has been linked to an alteration in the electrophoretic mobility on gels of ribosomal protein S2; no alterations in protein S2 were detected in any of the Ksg^d mutants described here.

Dependent mutants arose as 1 to 4% of all Ksg^r strains after mutagenesis. The Ksg^r mutations present in some strains could be transferred to another genetic background, and the resulting strains grew well. In the two cases amenable to study, the mutation causing an altered protein L11 in mutant MV29 and the mutation causing kasugamycin dependence in MV21, the Rif^r marker could be used to transfer these lesions back into strain L44. Most Rif^r colonies obtained in such selections grew poorly and showed faster growing secondary mutants arising within the colonies. Thus, it might be that mutations induced in genes for ribosomal components by the mutagenesis were stabilized by further mutations, for example, to kasugamycin or spectinomycin resistance, but that these combinations were only viable within a certain range of antibiotic concentrations.

Since in the case of both Spc^d and Ksg^d mutants, mutations near *rif* could confer dependence on a strain containing a resistance allele, it would be interesting to interchange these mutations and see whether they confer dependence on an antibiotic other than that involved in their isolation. Since mutations altering the streptomycin phenotype of an Str^d strain have also been mapped close to *rif* (6), a cross-comparison would also be worthwhile in this case.

It was interesting that several of these Ksg^d mutants showed alterations in ribosomal protein S18. This is a protein that other work (21) has indicated is near the adenine residues that are the target of the *ksgA* methylase. The multiple forms of ribosomal protein S18 in mutants MV9 and MV34 may be due to an incomplete acetylation of the N-terminal amino acid (26; K. Isono, personal communication), and the altered S18 in mutants MV2 and MV29 may represent an unacetylated S18. Further work is necessary to see if the S18 alterations are connected with the Ksg^d phenotype of these mutants.

The reason why mutations affecting ribosomal protein S9 were seen several times among these Ksg^d mutants will have to await a better understanding of the ribosome. One explanation would follow from the knowledge that the methylase, whose activity is impaired in *ksgA* mutant cells, acts late in assembly of the ribosome; it cannot use naked 16S RNA as a substrate (22). It may be that the ribosomal environment of the 3'-end of the 16S RNA is altered enough by the absence of protein S9 from the ribosome that the methylase fails to recognize its usual substrate. It would be worthwhile to study methylase activity and the degree of methylation of its usual substrate in these mutants.

Since several Ksg^d mutants showed alterations in protein S9 or S18, and no Ksg^r mutants showed such alterations, it would seem very likely that the alterations in these proteins were connected with the Ksg^d phenotype.

Kasugamycin-independent secondary mutants could readily be obtained from all Ksg^d mutants. A preliminary screening of 20 spontaneously isolated kasugamycin-independent mutants obtained from each of six Ksg^d strains was done, using two-dimensional polyacrylamide gel electrophoresis of ribosomal proteins. Several antibiotic-independent mutants obtained from strains MV2 and MV29 showed further changes in protein L11. This would suggest that the alteration in protein L11 was involved in the Ksg^d phenotype. Several kasugamycin-independent mutants isolated from mutant MV17 in separate selections showed alterations in protein L23.

There were clear indications from these mutants that the phenotype of *E. coli* mutants with respect to kasugamycin (whose site of action is the 30S subunit) was modified by mutations leading to alterations in components of the 50S subunit. This would point to the close functional interaction between the subunits.

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