JOURNAL OF BACTERIOLOGY, Feb. 1973, p.704 - 710

Vol. 113, No. 2
Printed in U.S.A.

Two Genetic Loci for Resistance to Kasugamycin in *Escherichia coli*

P. FREDERICK SPARLING, YOSHIKO IKEYA, AND DONNA ELLIOT
University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

Received for publication 5 September 1972

There are two loci for resistance to the antibiotic kasugamycin (Ksg) in *Escherichia coli*. Mutations at *ksgA* resulted in 30*S* ribosomal subunit resistance to Ksg. The map location of *ksgA* was near minute 0.5: *ksgA* was 95% cotransducible with *pdxA*, and the apparent gene order was *thr* ... *ksgA* ... *pdxA*. Studies in stable *ksgA*/*ksgA*$^+$ merodiploids showed that sensitivity was dominant over resistance. Mutations at a second gene (*ksgB*), located between minutes 25 and 39, resulted in phenotypic Ksg^R indistinguishable from *ksgA* mutations, but ribosomes from *ksgB* strains were sensitive to the drug in vitro. Spontaneous and induced mutations to Ksg^R were usually of the *ksgA* (ribosomal) type.

Mutations which affect the structure of bacterial ribosomes are of interest because they provide a convenient tool for studying the relationship between ribosomal structure and function. We have been interested in mutations to resistance to the aminoglycoside antibiotic *kasugamycin* (*Ksg*), *because 30S* subunits from Ksg-resistant (Ksg^R) strains are resistant to Ksg in vitro (11), and because the locus for ribosomal Ksg^R was shown earlier to be quite distant from the cluster of genes which affect 30*S* and 50*S* ribosome structure (11). This suggested that Ksg might act on a different structural part of the 30*S* ribosome than does streptomycin or spectinomycin, which are thought to interact with specific (and different) 30*S* ribosomal proteins (4, 10). This inference was confirmed by the recent report of Helser et al., that 16*S* ribonucleic acid (RNA) and not ribosomal protein determined the response of reconstituted 30*S* subunits to Ksg (6). The same authors subsequently found that 16*S* RNA from Ksg^R 30*S* subunits was undermethylated, and that this was the consequence of lack in Ksg^R strains of an adenine dimethylase which was present in Ksg^S strains (7). We have recently confirmed essentially all of these findings (Zimmermann, Ikeya, and Sparling, *in press*).

In this communication we report studies which more precisely localize the gene (*ksgA*) for 30*S* ribosomal resistence to Ksg. In addition, we have found a second locus for Ksg^R (*ksgB*), which although phenotypically indistinguishable from *ksgA*, does not result in ribosomes which are Ksg^R in vitro.

MATERIALS AND METHODS

Media and growth. Minimal medium A was that of Davis and Mingioli (3). Amino acids and purines were added to 50 μg/ml, and carbohydrates to 0.2%. L broth for conjugation experiments was described by Lennox (9). When quantitative antibiotic sensitivities were to be tested, L medium containing 1.5% agar but without added NaCl (L-S agar) was used at *p*H 7.2. The inhibitory activity of Ksg was antagonized by addition of salts or by decrease in *p*H. Maximal activity of Ksg was achieved at *p*H 8.0, but routine adjustment of the *p*H to 8.0 was not necessary for reproducible results. Sensitivity determinations were performed by inoculating 10^4 to 10^6 colony-forming units onto L-S agar plates containing doubling dilutions of antibiotic. The MIC (minimum inhibitory concentration) was the least concentration of drug preventing visible growth at 24 hr of incubation. All incubations were at 37 C.

Bacterial strains. The parent *Escherichia coli* K-12 strains and the most important derivatives used in these studies are shown in Tables 1 and 2. Mutagenesis with *N*-methyl-*N*-nitrosoguanidine (1) was performed to a survival of 15 to 25% of exposed cells. Transductions were performed with phage P1*kc* (9). Conjugations employed a density of 2.5×10^7 to 5.0×10^7 Hfr and 2×10^8 F$^-$ bacteria per ml in a total volume of 10 ml in 125-ml flasks, with gentle rotation at 37 C in a gyratory shaker. Matings were interrupted by vigorous agitation for 60 sec with a Vortex mixer before dilution and plating. All recombinants or transductants were purified once on selective plates before their phenotypes were scored by replica-plating or by use of an inocula-replicator. Ksg

phenotypes were ordinarily scored on L-S agar plates containing 125 or 250 μg of Ksg per ml (for low-level Ksg^R), and on the same plates containing 2,000 μg of Ksg per ml (for high-level resistance). Genetic symbols and linkage map of *E. coli* were from Taylor (13).

Preparation of cell extracts and assay for in vitro polypeptide synthesis. Methods described previously (12) were used without important modification. Sensitivity of ribosomes to Ksg was determined by measuring inhibition of Ksg of ^{14}C-valine incorporation directed by polyuridylic-guanylic (1:1) acid or MS 2-RNA. This was determined both with crude S-30 extracts, and with once-purified 70*S* ribosomes and S-100 fraction from either a Ksg^R or Ksg^R strain.

Chemicals. MS2-RNA was prepared as described by Gesteland and Boedtker (5). Kasugamycin was a gift of Bristol Laboratories. Streptomycin was obtained from E. R. Squibb. ^{14}C-valine was from New England Nuclear. Polyuridylic-guanylic (1:1) acid was from Miles Chemical Co. *N*-methyl-*N*-nitrosoguanidine was from Aldrich Chemical.

TABLE 1. *Parent strains of E. coli K-12 used in studies of ksg loci*

Strain	Source	Description
JC12	W. K. Maas	Hfr; *purC, met, lac, xyl, mtl,* λ^-
χ408	G. Jacoby	Hfr; *proA, thi*
AB311	CGSC[a]	Hfr; *thi-1, thr-1, leu-6, lacZ4*
B7	CGSC	Hfr; *metB1,* λ^R, λ^-
MA1079	K. B. Low	Hfr; *thi, ser, recA1,* λ^-
JC411	G. Jacoby	F^-; *argG6, met-1, leu-2, his-1, malA1, xyl, mtl, lac, str,* λ^-
χ478	K. B. Low	F^-; *leu, proC, purE, trp, metE, lysA, lacZ, ara, xyl, azi, str,* $T_1T_5T_6^R$
AT2365	A. L. Taylor	F^-; *thr-4, leu-8, pdxA1, ara-14, proA2, lacY1, galK2, xyl-5, mtl-1, thi, strA20*
Q13	M. Nomura	*thi, met, tyr, pnp*

[a] Coli Genetic Stock Center, Yale University, New Haven, Conn.

RESULTS

Selection of Ksg mutants. Spontaneous mutants of strains JC12, Q13, or JC411 resistant to at least 250 μg of Ksg per ml were rare, occurring with a frequency of 1×10^{-9} to 1×10^{-10} (Q13 and JC411) or 1×10^{-10} to 1×10^{-11} (JC12). None of the spontaneous mutants of these (or other) strains was resistant to more than 500 μg of Ksg per ml; this represented only a four- to eightfold increase in resistance as compared to the wild-type strains, which required 60 to 125 μg of Ksg per ml to inhibit growth. There were marked strain differences in the frequency of spontaneous mutations to Ksg^R. In strains χ408 and Hfr C, for instance, spontaneous mutants resistant to 250 to 500 μg of Ksg per ml were found with a frequency of 1×10^{-7} to 1×10^{-8}. The reason for the wide variation in frequencies of spontaneous mutations to low-level Ksg^R in different *E. coli* K-12 strains was not clear.

Nitrosoguanidine mutagenesis was used to isolate stable mutants resistant to at least 1,000 μg of Ksg per ml for purposes of precisely localizing the gene for ribosomal Ksg^R on the *E. coli* chromosome. Levels of Ksg^R greater than 2,000 μg/ml were extremely rare, even after extensive nitrosoguanidine mutagenesis. Only one such mutant (FS157) was obtained.

None of over 100 Ksg^R mutants was dependent on Ksg. No cross-resistance to other antibiotics was observed in any of 40 tested Ksg^R mutants; antibiotics tested for possible

TABLE 2. *Derivative strains of E. coli K-12 used in characterization of ksg loci*

Strain	Source	Description
FS131	NGN[a]	As JC12, but *ksgA19*
FS157	NGN	As JC12, but *ksgA23 ksgB1*
FS173	Recombinant from FS157 × JC411	As JC411, but *his*$^+$ *ksgB1*
FS174	Recombinant from FS157 × JC411	As JC411, but *his*$^+$ *mal*$^+$ *ksgA23 ksgB1*
FS215	Recombinant from FS157 × JC411	As JC411, but *his*$^+$ *mal*$^+$ *ksgA23*
FS232	Transductant of JC411 from FS131	As JC411, but *leu*$^+$ *ksgA19*
FS233	Transductant of JC411 from FS157	As JC411, but *leu*$^+$ *ksgA23*
FS224	NGN	As χ478, but *his-2*
FS227	Transductant of χ408 from FS173	As χ408, but *ksgB1*
FS163	NGN	As JC411, but *ksgA26*
FS223	Recombinant from MA1079 × FS163	As FS163, but *his*$^+$ *recA ser*
FS226	"Recombinant" from χ408 × FS223	Stable merodiploid: as FS223, but *leu-2 ksgA26*/F *leu*$^+$ *ksgA*$^+$
FS240	Spontaneous	As Q13, but *ksgA30*

[a] *N*-methyl-*N*-nitrosoguanidine.

cross-resistance included gentamicin, kanamycin, streptomycin, spectinomycin, paromomycin, erythromycin, lincomycin, chloramphenicol, and tetracycline. Pleiotropic effects of mutations to kasugamycin resistance were rarely observed. Generation times in minimal or rich medium at 30, 37, or 42 C were essentially identical in JC411 and its *leu*$^+$ *ksgA* transductants FS232 and FS233.

Two loci for Ksg^R. Five independently isolated Ksg^R mutants of the Hfr strains JC12 and χ408 (both *leu*$^+$ *str*$^+$) were mated with F$^-$ Ksg^S recipient JC411 (*leu str*). In each instance, approximately 70% of recombinants selected for the donor Leu$^+$ phenotype were also Ksg^R, whereas if the selection was for other donor markers (*argG*$^+$, *lac*$^+$, *his*$^+$), less than 50% of recombinants were Ksg^R. Thus, as reported earlier (11), Ksg^R is linked to *leu*, near minute 1.0 on the chromosome. In every mating but one, the phenotypic level of resistance acquired by the recipient was equal to that of the donor.

When the highly resistant donor FS157 was mated with sensitive recipient JC411, however, recombinants exhibited not only the Ksg phenotype of each parent, but also a new, intermediate type (Table 3). In this mating, 53% of recombinants selected for the donor Leu$^+$ character became Ksg^R, but all were low-level Ksg^R (500 μg/ml). When the more distal donor marker His$^+$ was selected (His$^+$ PurC$^+$ recombinants), three classes of Ksg phenotype resulted: sensitive, low-level resistance (500 μg/ml), and high-level-resistance equal to that of the donor (8,000 μg/ml). This suggested that FS157 was a double mutant, with one locus for Ksg^R near *leu*, and a second somewhere between *leu* and *his*. This unusually high level of resistance exhibited by FS157 might thus be explained as the sum of two independent mutations, and the rarity of such high-level Ksg^R mutants would be explained by the necessity for two mutations.

TABLE 3. *Separation of two ksg loci by conjugation and recombinant analysis*

Hfr FS157 (Ksg^R > 8,000)[a] *argG*$^+$ *leu*$^+$ *his*$^+$ *purC*$^-$ ←

F$^-$ JC411 (Ksg^S)[a] *argG*$^-$ *leu*$^-$ *his*$^-$ *purC*$^+$

Selected class of recombinant	No.	Phenotype		
		Ksg^S	Ksg^R (500)[a]	Ksg^R (>8,000)
Arg$^+$ PurC$^+$	103	102	1	0
Leu$^+$ PurC$^+$	102	39	63	0
His$^+$ PurC$^+$	185	137	17	31

[a] Ksg^S, Ksg^R(500) and Ksg^R(>8,000) indicate (respectively) sensitivity to Ksg at 125 μg/ml, resistance at 500 μg/ml but not at 1,000 μg/ml, and resistance to more than 8,000 μg of Ksg/ml.

The presence of two *ksg* loci was confirmed in several ways. Several Ksg^R His$^+$ PurC$^+$ recombinants were retained from the mating FS157 × JC411. Some (FS215, FS173) were low-level Ksg^R (500 μg/ml), whereas another (FS174) was highly Ksg^R (>8,000 μg/ml); all were *leu*$^-$ and *str*. These were used as recipients in brief interrupted matings with Ksg^S strain χ408, which donates *leu*$^+$ as an early marker and *str*$^+$ as a late marker (see Fig. 1 for origins and orientation of transfer of Hfr strains). When χ408 was mated with the low-level Ksg^R strain FS215, 70% of 100 Leu$^+$ Str^R recombinants acquired the donor Ksg^S phenotype, whereas 30% remained low-level Ksg^R (500 μg/ml). In contrast, when FS173, which is also low-level Ksg^R, was the recipient, none of 124 Leu$^+$ Str^R recombinants was Ksg^S. When the highly Ksg^R (>8,000 μg/ml) strain FS174 was recipient, 84% of Leu$^+$ Str^R recombinants became low-level Ksg^R (500 μg/ml), 16% remained high-level Ksg^R, and none became sensitive to Ksg. On this basis, FS215 was considered to have a locus for Ksg^R (*ksgA23*) near *leu*, whereas the Ksg^R locus (*ksgB1*) in FS173 was considerably distant from *leu*, and FS174 had both loci (*ksgA23*, *ksgB1*).

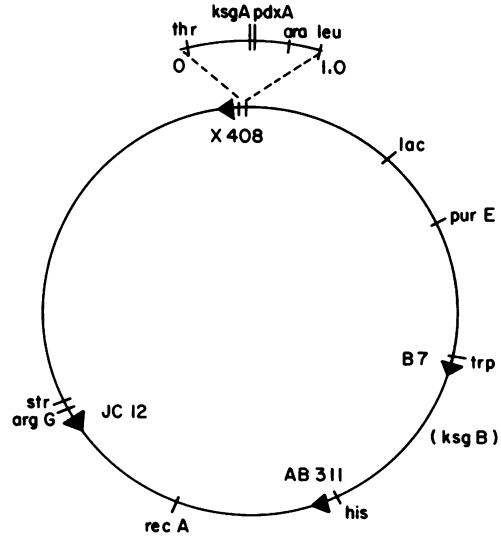

FIG. 1. *The location of ksgA and approximate location of ksgB on the E. coli map. Origins and orientation of transfer of relevant Hfr strains are indicated by arrowheads. Genetic map adapted from Taylor (13).*

Other evidence for two distinct loci for Ksg^R was provided by the frequent appearance of high-level Ksg^R progeny in crosses between a *ksgA* donor and a *ksgB* recipient, but not in similar crosses between two *ksgA* strains or two *ksgB* strains. For instance, mating Hfr strain FS131 (*ksgA19 leu*$^+$ *str*$^+$) with FS173 (F^-, *ksgB1 leu*$^-$ *str-1*) with selection for Leu^+ Str^R recombinants resulted in 86% which were high-level Ksg^R (*ksgA ksgB*), 14% which were low-level Ksg^R (*ksgA* or *ksgB*), and none which was Ksg^S. Moreover, *ksgA* alleles from several strains were 25 to 40% cotransducible with *leu*$^+$, but no cotransduction of *ksgB1* from FS173 was noted when selection was for any of several markers near to *leu* (*thr*$^+$, *pdxA*$^+$, *azi*, or *argF*). Finally, although single-step mutation to high-level Ksg^R (selection at 3,000 μg of Ksg per ml) was not observed from Ksg^S or *ksgA* strains (frequency less than 1×10^{-11}), such mutants were obtained spontaneously with a frequency of 1×10^{-8} from *ksgB1* strain FS173. Thus, there are two loci for Ksg^R, and mutations at *ksgB* are apparently much more rare than those at *ksgA*.

Analysis of ksgA locus by transduction. The chromosomal location of several *ksgA* alleles was better defined by transduction, by using as the recipient strain AT2365, which has several auxotrophic markers near *leu* (Table 4). Three independently isolated *ksgA* alleles, including *ksgA19* from FS131 and *ksgA23* from FS157, cotransduced 25 to 40% with *leu*$^+$, 4 to 10% with *thr*$^+$, and 92 to 98% with *pdxA*$^+$, placing *ksgA* near minute 0.5.

Precise location of *ksgA* relative to *pdxA* by reciprocal three-point transductions was not possible because of unexplained difficulties in direct selection of Ksg^R recombinants. This problem was encountered after transfer of *ksgA* or *ksgB* alleles by transduction or conjugation, and was not overcome by allowing up to 8 hr for phenotypic expression of Ksg^R before exposing cells to selective pressures of Ksg. Nevertheless, *ksgA* was demonstrated to be immediately counterclockwise to *pdxA*, by recombinant analysis of Leu^+ and Thr^+ transductants (Table 4). Among 510 Leu^+ transductants of AT2365, 8 were Pdx^+ Ksg^S, but none was Pdx^- Ksg^R; the former class requires only two crossovers, but the latter requires four crossovers, if *ksgA23* is counterclockwise to *pdxA*. Among Thr^+ transductants, two were Pdx^- Ksg^R but none was Pdx^+ Ksg^S, in agreement with the prediction if the gene order is *thr* ... *ksgA* ... *pdxA* ... *leu*. Identical experiments with two other *ksgA* alleles revealed similar data.

Phenotypic levels of resistance to Ksg among transductants were reproducibly slightly lower (two- to fourfold) than in the donor strains, or in recombinants of conjugation experiments. The reasons for this are uncertain (Table 5). A more striking example of disparities in levels of resistance among donor and transductant was provided by the experiment shown in Table 4. Donor P1*kc* was prepared on the high-level Ksg^R (>8,000 μg/ml) strain FS157, but transductants of AT2365 selected for Thr^+, Pdx^+, or Leu^+ and scored for Ksg^R were all resistant to a maximum of 500 μg of Ksg per ml. This apparent conflict was resolved when it was learned that FS157 was a double mutant (*ksgA23 ksgB1*), and that only *ksgA23* was cotransducible with *leu* or other nearby markers.

TABLE 4. *Mapping of the ksgA23 allele by transduction and recombinant analysis*

P1*kc*(FS157) *ksgA23 ksgB1* × AT2365 *ksgA*$^+$ *ksgB*$^+$					
Donor FS157	*thr*$^+$	*ksgA23*	*pdxA*$^+$	*ara*$^+$	*leu*$^+$
Recipient AT2365	*thr*$^-$	*ksgA*$^+$	*pdxA*$^-$	*ara*$^-$	*leu*$^-$

Selected class of transductant	No.	Cotransduction of unselected markers (%)				
		Thr$^+$	Ksg^R [a]	Pdx$^+$	Ara$^+$	Leu$^+$
Leu$^+$ [b]	510	1.6	39.8	41.9	68.4	100
Pdx$^+$	347	4.3	98.0	100	74.4	52.2
Thr$^+$ [b]	253	100	10.3	9.5	4.3	2.8

[a] Although FS157 was resistant to >8,000 μg of Ksg per ml, all Ksg^R transductants were resistant to only 500 μg of Ksg per ml.

[b] Among Leu^+ transductants there were eight Pdx^+ Ksg^S but no Pdx^- Ksg^R; among Thr^+ transductants there were no Pdx^+ Ksg^S but two Pdx^- Ksg^R.

TABLE 5. *Phenotypic resistance to kasugamycin in strains carrying various combinations of ksgA and ksgB alleles*

Strains	Genotype		Source	MIC of Ksg (μg/ml)
	ksgA	*ksgB*		
JC12, χ408	+	+		60
JC411	+	+		125
FS131	19	+	NGN[a], from JC12	2,000
FS232	19	+	Transductant of JC411	1,000
FS215	23	+	Conjugation, FS157 × JC411	1,000
FS233	23	+	Transductant of JC411	500
FS173	+	1	Conjugation, FS157 × JC411	1,000
FS227	+	1	Transductant of χ408	250
FS157	23	1	NGN, from JC12	>8,000
FS174	23	1	Conjugation, FS157 × JC411	>8,000

[a] Nitrosoguanidine mutagenesis.

Although *ksgA* is very close to *pdxA*, frequencies of cotransduction similar to those shown in Table 4 were not observed with all *ksgA* alleles. The most important exception was *ksgA30*, which was isolated as a spontaneous mutant in strain FS240. Transduction by P1*kc* from FS240 into AT2365, JC411, a *str*$^+$ derivative of JC411, and a *leu*$^-$ derivative of Q13 revealed in each instance that only 1 to 5% of Leu$^+$ transductants acquired the donor Ksg^R character, as opposed to the usual 30 to 40%. There also was less than 1% cotransduction of *ksgA30* with *thr*$^+$, and 67% with *pdxA*$^+$, as opposed to the usual 4 to 10% and 92 to 98%, respectively (Table 6). Moreover, the number of Pdx$^+$ transductants was reduced by a factor of five or more relative to the Thr$^+$ and Leu$^+$ transductants, and those Thr$^+$, Pdx$^+$, and Leu$^+$ transductants which were also Ksg^R grew very slowly, appearing as small colonies only after 3 or more days of incubation. This appears to be an example of positive interference due to restriction of growth (and viability) secondary to introduction of the *ksgA30* allele, or another undetected mutation near *ksgA30*.

Dominance of ksgA$^+$. A *leu-2 ksgA26*/F *leu*$^+$*ksgA*$^+$ merodiploid (FS226) was constructed by mating *recA1 leu-2 ksgA26 strF*$^-$ strain FS223 with *str*$^+$ Hfr strain χ408 which donated *ksgA*$^+$ *leu*$^+$ as very early markers. "Recombinants" selected for the phenotype Leu$^+$ Str^R were scored for sensitivity to Ksg and for stability of the Leu$^+$ and Ksg phenotypes. All "recombinants" were fully Ksg^S (MIC of FS226 and FS223 was 60 μg of Ksg per ml) and segregated Ksg^R clones with a frequency of 5×10^{-4}. Over 90% of Ksg^R segregants were also Leu$^-$. Additional proof of merodiploidy and of the dominance of *ksgA*$^+$ was provided by growing the Leu$^+$ Ksg^S merodiploids in L broth plus 10 μg of acridine orange per ml (8), after which almost 100% of surviving colonies were Leu$^-$ and Ksg^R.

TABLE 6. *Interference exhibited in transductional crosses with the ksgA30 allele*

P1*kc*(FS240) *ksgA30* × AT2365 *ksgA*$^+$

		thr$^+$ *ksgA30* *pdxA*$^+$ *leu*$^+$
Donor	FS240	
Recipient	AT2365	
		thr$^-$ *ksgA*$^+$ *pdxA*$^-$ *leu*$^-$

Selected class of transductant	No.	Cotransduction of unselected markers			
		Thr$^+$	Ksg^R	Pdx$^+$	Leu$^+$
Leu$^+$	334	1.2	1.2	1.8	100
Pdx$^+$	30	6.7	66.7	100	46.7
Thr$^+$	236	100	0	0.8	0.8

Chromosomal location of ksgB. Strain FS173 (*ksgB1 leu str*) was used as a recipient in matings with several *ksgB*$^+$ *leu*$^+$ *str*$^+$ Hfr strains, including χ408, Hfr C, B7, and a spontaneous *leu*$^+$ derivative of AB311 (Fig. 1). Matings were for 30 min, and selection was for Leu$^+$ Str^R. Only in matings with AB311 did the donor *ksgB*$^+$ marker appear in the recombinants, which localized *ksgB1* between the origins of AB311 and B7, or roughly between *his* (minute 39) and *trp* (minute 25).

A *ksgB1 leu*$^+$ *purE*$^+$ *trp*$^+$ *his*$^+$ Hfr strain (FS227) was constructed by using donor P1*kc* grown on FS173, with direct selection for rare Ksg^R transductants of χ408. Conjugation of FS227 with *ksgB*$^+$ recipient FS224 (*leu*$^-$ *purE*$^-$ *trp*$^-$ *his*$^-$) confirmed the location of *ksgB1* between *trp* and *his* as shown in Table 7. More exact localization of *ksgB* has not been accomplished.

Mechanisms of resistance to Ksg. Cell-free protein synthesis with ribosomes from FS215 (*ksgA*), FS173 (*ksgB*), and FS174 (*ksgA ksgB*) showed that only *ksgA* ribosomes were resistant to Ksg in vitro (Fig. 2). Crude S-30 preparations of FS173 were also fully sensitive to Ksg in vitro, with MS2-RNA used as messenger. Thus, the *ksgB* gene product does not appear to affect the structure of the ribosome. The biochemical mechanism of Ksg^R in *ksgB* strains is not known. No cross-resistance was detected to a variety of other antibiotics, including many other aminoglycosides. Kasugamycin was not inactivated by intact cells or cell extracts of *ksgB* strains. It is possible that the *ksgB* mutation results in altered cell envelope permeability to Ksg, but this could not be tested because of unavailability of labeled Ksg.

DISCUSSION

Helser, Davies, and Dahlberg (6, 7) have shown that the *ksgA* mutation results in failure to dimethylate adjacent adenine residues at the 3′ end of 16*S* RNA, which causes resistance to the antibiotic kasugamycin. We have confirmed their findings by showing that the response to Ksg of 30*S* subunits reconstituted from 16*S* RNA and total 30*S* proteins from strains JC411 and FS232, or Q13 and FS240, was determined solely by the source of the 16*S* RNA. Likewise, we have also shown that *ksgA* strains lack a methylase which is present in *ksgA*$^+$ strains, and that the methylase in *ksgA*$^+$ strains will utilize 23*S* core particles from a *ksgA* but not from a *ksgA*$^+$ strain as substrate

(Zimmermann, Ikeya, and Sparling, *in press*).

The studies reported here precisely localize the gene (*ksgA*) for ribosomal resistance to Ksg and demonstrate that *ksgA*$^+$ is dominant over *ksgA*. The latter finding strongly suggests that the mechanism of Ksg^R in *ksgA* strains is not production of an inhibitor of the 16*S* RNA methylase, for in that case *ksgA* should be dominant over *ksgA*$^+$. Therefore, this is further evidence that *ksgA* is the structural gene for 16*S* RNA adenine dimethylase.

Several questions remain unanswered. Chief among these is why there is genetic interference manifest in transductions with *ksgA30*, but not with other *ksgA* alleles. Perhaps this is related to other observations, some of which are reported in detail in a separate publication (Zimmermann, Ikeya, and Sparling, *in press*) but will be briefly summarized here. Strains FS232 (*ksgA19*) and FS233 (*ksgA23*) have been shown to have altered 30*S* protein S4, by phosphocellulose co-chromatography with *ksgA*$^+$ 30*S* proteins from JC411. This is apparently due to mutation in a locus designated *ramB*, which is closely linked to *ksgA* since it frequently (but not invariably) cotransduces with *ksgA* and *leu*. Strains FS131 and FS232 are therefore *ksgA19 ramB1*, and FS157 and FS233 are *ksgA23 ramB2*, whereas strain FS240, which has no chromatographically identifiable alteration of S4, is *ksgA30 ramB*$^+$ (Zimmermann, Ikeya, and Sparling, *in press*). In other studies, we have demonstrated restriction (2) of misreading of synthetic polynucleotide messengers in vitro by 70*S* ribosomes from several *ksgA* strains including FS240 (*ksgA30*). The effect of *ramB* appears to be opposite to that of *ksgA*, since *ramB ksgA*$^+$ ribosomes exhibit increased levels of misreading in vitro compared to *ramB*$^+$ *ksgA*$^+$ ribosomes (Ikeya and Sparling, *manuscript in preparation*).

TABLE 7. *Mapping of the ksgB1 allele by conjugation and recombinant analysis*

Hfr FS227 *leu*$^+$ *purE*$^+$ *trp*$^+$ *ksgB1* *his*$^+$ *str*$^+$ ←

F$^-$ FS224 —

leu$^-$ *purE*$^-$ *trp*$^-$ *ksgB*$^+$ *his*$^-$ *strA*

Selected class of recombinant	No.	Unselected recombinant markers (%)				
		Leu$^+$	PurE$^+$	Trp$^+$	Ksg^R [a]	His$^+$
Leu$^+$ Str^R	100	100	17	7	7	3
PurE$^+$ Str^R	100	64	100	10	6	1
Trp$^+$ Str^R	100	46	75	100	43	4
His$^+$ Str^R [b]	100	56	50	56	66	100

[a] Ksg^R was scored on L-S plates containing 125 μg of Ksg per ml.

[b] Among His$^+$ Str^R, there were 20 Ksg^R Trp$^-$, but only 8 Ksg^S Trp$^+$.

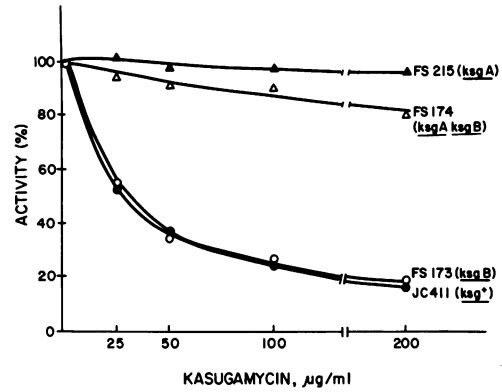

FIG. 2. *Ribosomes from ksgB1 strain FS173 are not resistant to kasugamycin. Sensitivity of MS2-RNA-directed* ^{14}*C-valine incorporation to inhibition by kasugamycin, employing 70S ribosomes from ksgA, ksgB, ksgA ksgB, and ksg*$^+$ *strains, and S-100 (supernatant) fraction from ksg*$^+$ *strain JC411. Concentration of* Mg^{2+} *was 10 mM.*

It is possible, therefore, that *ksgA* and *ramB* have different effects on ribosomal physiology and that introduction of *ksgA* unopposed by *ramB* (Table 6, donor FS240 is *ksgA30 ramB*$^+$) would give results quite unlike those seen when *ksgA* is introduced with the closely linked *ramB* locus (Table 4, donor FS157 is *ksgA23 ramB2*). This explanation will remain tentative until isogenic *ksgA30 ramB*$^+$ and *ksgA30 ramB2* donors can be constructed, which has not yet been possible due to the necessity of scoring for *ramB* by the cumbersome methods of column chromatography. Further studies are in progress to clarify the effects on cellular physiology of undermethylation of 16*S* RNA due to *ksgA*, and alteration of 30*S* protein S4 due to *ramB*.

Another problem was the unexplained difficulty in direct selection of Ksg^R recombinants. This may be due to a combination of factors, especially the relatively poor inhibitory activity of kasugamycin for "sensitive" strains, and perhaps also slow growth of certain *ksgA* recombinants. Thus, during the several hours allowed for phenotypic expression of ribosomal resistance to Ksg, rapid growth of the numerically dominant Ksg^S population occurs and selection of plates containing concentrations of Ksg which allow growth of Ksg^R recombinants no longer provides clean selection against the Ksg^S parent.

The mechanism of Ksg^R in *ksgB* strains is

unknown. Similar examples of separate loci for ribosomal and nonribosomal resistance to the aminoglycoside antibiotics streptomycin and spectinomycin have been described in salmonella, and although it was assumed that nonribosomal resistance to these drugs was due to permeability barriers, direct proof was lacking (14). Nevertheless, *ksgB* may prove a useful marker for purposes of counterselection in genetic experiments, by virtue of its location between *trp* and *his*.

ACKNOWLEDGMENTS

The technical assistance of Eleanor Blackman, and several discussions with R. A. Zimmermann are gratefully acknowledged.

This work was supported by Public Health Service grant AI-09574 from the National Institute of Allergy and Infectious Diseases and a Public Health Service Research Career Development Award to P. F. S.

LITERATURE CITED

1. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788–795.
2. Breckenridge, L., and L. Gorini. 1970. Genetic analysis of streptomycin resistance in *Escherichia coli*. Genetics **65**:9–25.
3. Davis, B. D., and E. S. Mingioli. 1950. Mutants of Escherichia coli requiring methionine or vitamin B12. J. Bacteriol. **60**:17–28.
4. Dekio, S., and R. Takata. 1969. Genetics studies of the ribosomal proteins of *Escherichia coli*. II. Altered 30S ribosomal protein specific to spectinomycin resistant mutants. Mol. Gen. Genet. **105**:219–224.
5. Gesteland, R. F., and H. Boedtker. 1964. Some physical properties of bacteriophage R17 and its ribonucleic acid. J. Mol. Biol. **8**:496–507.
6. Helser, T. L., J. E. Davies, and J. E. Dahlberg. 1971. Change in methylation of 16S ribosomal RNA associated with mutation to kasugamycin resistance in *Escherichia coli*. Nature Biol. **233**:12–14.
7. Helser, T. L., J. E. Davies, and J. E. Dahlberg. 1972. Mechanism of kasugamycin resistance in *Escherichia coli*. Nature Biol. **235**:6–9.
8. Hirota, Y. 1960. The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **46**:57–64.
9. Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
10. Ozaki, M., S. Mizushima, and M. Nomura. 1969. Identification and functional characterization of the protein controlled by the streptomycin-resistant locus in *E. coli*. Nature (London) **222**:333–339.
11. Sparling, P. F. 1970. Kasugamycin resistance: a 30S ribosomal mutation with unusual map location in *E. coli*. Science **167**:56–57.
12. Sparling, P. F., J. Modolell, Y. Takeda, and B. D. Davis. 1968. Ribosomes from *Escherichia coli* merodiploids heterozygous for resistance to streptomycin and to spectinomycin. J. Mol. Biol. **37**:407–421.
13. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155–175.
14. Yamada, T., and J. Davies. 1971. A genetic study of streptomycin and spectinomycin resistance in *Salmonella typhimurium*. Mol. Gen. Genet. **110**:197–210.