GENETIC ANALYSIS OF STREPTOMYCIN DEPENDENCE IN *ESCHERICHIA COLI*†

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Received August 28, 1970

IT is classically found (NEWCOMBE and NYHOLM 1950) that by exposing cells of *Escherichia coli* to high levels of streptomycin (Sm‡), two classes of resistant mutants are obtained: those which are indifferent to the drug (Sm^R) and those which are dependent on the presence of Sm for growth under any condition. The two classes of mutants were located at the same chromosomal site, the *strA* locus (HASHIMOTO 1960). The direct demonstration that the Sm^R mutants possess a mutated protein in the 30S ribosomal subunit (TRAUB and NOMURA 1968) was followed by the finding that a mutation in the same *strA* protein is also responsible for the Sm dependence (BIRGE and KURLAND 1969). These discoveries should have settled the genetic aspect of the problem if it were not for the finding that neither the Sm^R nor the Sm-dependent phenotypes constitute a homogeneous class of mutants. Different Sm^R mutations were found to confer different levels of "competence" for phenotypic suppression induced by Sm (GORINI and KATAJA 1964) and different levels of "restriction" of genetic suppression (COUTURIER, DESMET and THOMAS 1964). Four classes of Sm^R mutations have been distinguished on the basis of quantitative differences in the restriction they impose on nonsense suppression (STRIGINI and GORINI 1970) and their mapping within the *strA* cistron has been reported (BRECKENRIDGE and GORINI 1970).

The lack of homogeneity in the Sm-dependent class of mutants is even more striking. It was found in the first place that by selecting survivors to another aminoglucoside antibiotic, paromomycin (Pm), a class of dependent mutants was obtained phenotypically identical to one of those isolated with the Sm selection (GORINI, ROSSET and ZIMMERMANN 1967). Yet no cross-resistance between Sm and Pm was previously found since all Sm^R mutants are Pm^S. Evidently, it is necessary to more critically examine the notion that the *strA* gene product is the specific site of Sm action. Secondly, four classes of dependent mutants were obtained, widely differing in (a) their dependence upon three factors, Sm, Pm, and alcohol (Et); (b) their resistance to three drugs, Sm, Pm, and kanamycin (Km); (c) their reversibility to drug-independence through secondary mutations external to *strA*; and (d) the level of restriction they impose on phenotypic suppression. It is evident that any attempt to reduce all these complex phenotypes

† This work was supported by American Cancer Society grant E-226H and USPHS-National Institute of Allergy and Infectious Diseases grant 5 ROI AI 02011-12.

‡ Abbreviations used: SM = streptomycin; Pm = paromomycin; Km = kanamycin; Et = ethanol; $Drug^D$ = dependent on Sm, Pm, or Et; Sm^DEt^D = dependent on Sm or Et; Sm^D = dependent on Sm only; Pm^DSm^R = dependent on Pm only. These four classes of dependent strains are designated with the prefix D, SE, S, and P, respectively. CSD = conditional Sm dependence. moi = multiplicity of infection.

to a common denominator depends on the demonstration of a genetic homogeneity of all these classes of mutants.

This work is concerned with the genetics of the Sm-, Pm-, and Et-dependent mutants. Having established that all of them are located at the *strA* site, a genetic fine structure of the *strA* cistron is presented.

MATERIALS AND METHODS

1. *Bacterial strains*: All bacterial strains are derivatives of *Escherichia coli* B. They have been derived from a single parent, L1 (JACOBY and GORINI 1969) with the exception of B513. Strain L1 contains the *argF*40 amber mutation (GORINI, GUNDERSEN and BURGER 1961) which is leaky in *strA*+ strains and negative in *strA* mutants (GORINI, JACOBY and BRECKENRIDGE 1966). Furthermore, L1 is derepressed for the arginine biosynthetic pathway, a prerequisite in strain B for the expression of the arg-leaky phenotype (JACOBY and GORINI 1967).

The *strA* mutations E11 and E26 are both Sm^R but they differ by the degree of restriction they impose on nonsense suppression: E11 (like *strA*40) permits phenotypic suppression by Sm (conditional Sm dependence, CSD) of both amber *argF*40 and UGA*leu*2 mutations (arg^{CSD} and leu^{CSD}), while E26 (like *strA*2) is only competent for *leu*2 phenotypic suppression (arg^- and leu^{CSD}) (BRECKENRIDGE and GORINI 1970).

Strain B513 is a spontaneous mutant of wild-type B surviving from exposure to Pm (ROSSET and GORINI, unpublished). It is able to grow in Pm or Pm + Sm, but not in Sm alone or in the absence of any drug; it is thus Pm^DSm^R. This apparently rare class of mutants was not found among the Pm-survivors of L44 in the selection described in section 5 below.

A description of the strains and their mutations is given in Table 1.

2. *Bacteriophages*: Bacteriophage P1, *Kc* (originally from S. LURIA) or virulent (vir) (from J. BECKWITH) were used as indicated in each experiment. The phage was titrated using *shigella Sh* as an indicator strain.

3. The following *culture media* were used:

L medium—Bacto-Tryptone (Difco) 10 g; yeast extract (Difco) 5 g; NaCl 5 g; distilled water to 1000 ml, pH adjusted to 7 with 1 ml NaOH N/liter (LENNOX 1955).

M medium—KH_2PO_4 3 g; K_2HPO_4 7 g; Na_3citrate·3 H_2O 0.5 g; $MgSO_4$·7 H_2O 0.1 g; $(NH_4)_2SO_4$ 1 g; distilled water to 1000 ml, pH 7 (Medium A of DAVIS and MINGIOLI 1950).

L and M media were supplemented with 0.2% of glucose after autoclaving. For plates, 2% agar was also added.

Supplemented media—When necessary, M medium was supplemented with various amino acids at the following concentrations (μg per ml): L-histidine 30; L-arginine HCl 100; L-leucine 40; L-phenylalanine 60; L-tryptophan 25; L-tyrosine 40. Vitamins were in mμg/ml; *p*-aminobenzoic acid 10; *p*-hydroxybenzoic acid 0.5. The mixture of Phe, Try, Tyr, *p*-aminobenzoic and *p*-hydroxybenzoic acid was supplied to satisfy the aro^- requirement (this combination of nutrient will hereafter be referred to as Aro).

Soft agar—Nutrient broth (Difco) 0.8%; NaCl 0.5%; Agar (Difco) 0.65%, pH 7.

Basal agar—Bacto-Tryptone (Difco) 1%; yeast extract (Difco) 0.5%; NaCl 0.5%; agar (Difco) 1%, pH 7. After autoclaving, 0.2% glucose was added. Soft agar and basal agar were supplemented with $CaCl_2$ after autoclaving, at a final concentration of 2×10^{-3}M.

Test plates—L = L medium; M = minimal A medium. To designate the addition of an amino acid to minimal medium the indication M is omitted; thus plates Arg, Leu, Arg Leu, His, Aro mean plates M plus the different amino acids or mixture thereof. To designate the addition of drugs the symbols L, M or amino acid are followed by Sm, Pm, Et or (Sm + Pm): for example, L Sm, or L Et or L Sm Pm, or M Pm, or Arg Sm or Arg Leu Pm. When the amount of drug added is the standard, i.e., 500 μg/ml Sm, 200 μg/ml Pm or 3% Et, the indication of this amount is omitted. Medium M minus $(NH_4)_2SO_4$ (A — N) was used for dilution of bacterial suspensions.

4. *Growth conditions*: All cultures were incubated at 37°C. The volume of liquid cultures

TABLE 1

Description of strains

Bacterial strain	Pertinent genotype							
	argF	argR	leu	his	aroE	spc	strA	mut
L44	40	11	2	+	+	+	+	+
L44-MutS1	40	11	2	+	+	+	+	S1
L44-MutT1	40	11	2	+	+	+	+	T1
LE11	40	11	2	+	+	+	E11	+
LE26	40	11	2	+	+	+	E26	+
L11 His$^-$	40	11	+	11	24	3	+	+
L11 His$^-$E11	40	11	+	11	24	+	E11	+
L11 His$^-$E26	40	11	+	11	24	+	E26	+
B513	+	+	+	+	+	+	P513	+

Mutations:

*argF*40 = amber mutation in ornithine transcarbamylase (GORINI, GUNDERSON and BURGER 1961)
*argR*11 = constitutive mutation in arginine regulation (JACOBY and GORINI 1967)
*leu*2 = UGA mutation in a leucine biosynthetic enzyme (BRECKENRIDGE and GORINI 1970)
*his*11 = mutation in a histidine biosynthetic enzyme (BRECKENRIDGE and GORINI 1970)
*aroE*24 = mutation in dehydroshikimate reductase (PITTARD and WALLACE 1966)
*spc*3 = mutation to spectinomycin resistance (BRECKENRIDGE and GORINI 1970)
*strA*E11 = arg^{CSD} leu^{CSD} of the *strA*40 type (BRECKENRIDGE and GORINI 1970)
*strA*E26 = arg$^-$ leu^{CSD} of the *strA*2 type (BRECKENRIDGE and GORINI 1970)
*strA*P513 Pm^{D}Sm^R (ROSSET and GORINI, unpublished)
*mut*S1 = mutator mutation (Cox, see BRECKENRIDGE and GORINI 1970)
*mut*T1 = mutator mutation (Cox, see BRECKENRIDGE and GORINI 1970)

For more details see BRECKENRIDGE and GORINI (1970)

was 1/10 of that of the container (Erlenmeyer) and the culture was shaken to ensure good aeration. The growth was measured by optical density (OD) at 490 mμ using a Lumetron colorimeter Model No. 401. A population density of 10^8 cells/ml was equal to 2 OD Lumetron units.

5. *Selection of dependent mutants*: It was necessary to ensure that each mutant clone originated from a separate mutational event. For this purpose washed cells of strain L44 growing exponentially in L medium were spread on plates L, 10^8 cells per plate. The plates were incubated for 5 hr at 37°C and then either Sm or Pm was added underneath the agar to reach a final concentration of 50 μg Sm or 200 μg Pm per ml. The plates were immediately refrigerated at 2°C for 24 hr and then reincubated at 37°C. Surviving colonies appeared in increasing num-

TABLE 2

Phage titers in lysate preparations

Donor strain			Phage strain		Drug content (μg/ml lysate)
Name	*strA* allele	Phenotype	Strain	Titer ($\times 10^{10}$/ml)	
LE11	E11	Sm^R	P1	6.3; 4.0; 8.8	none
LE11	E11	Sm^R	P1 vir	9.7	none
LE26	E26	Sm^R	P1	5.9; 7.0; 17.0	none
LE26	E26	Sm^R	P1 vir	7.3	none
3–67	D367	$Drug^D$	P1	4.1	Pm 20
3–85	D385	$Drug^D$	P1	8.3	Pm 20
4–5	D45	$Drug^D$	P1	6.3	Pm 20
4–18	D418	$Drug^D$	P1	7.2	Pm 20
4–26	D426	$Drug^D$	P1	4.8	Pm 20
5–2	D52	$Drug^D$	P1	6.4	Pm 20
5–6	D56	$Drug^D$	P1	5.5	Pm 20
17–7	D177	$Drug^D$	P1	4.4	Pm 20
5–18	S518	Sm^D	P1	1.6	Sm 40
5–21	S521	Sm^D	P1	1.9	Sm 40
3–80	SE380	Sm^DEt^D	P1	1.2	Sm 40
B513	P513	Pm^DSm^R	P1 vir	1.7	Pm 20

B513 was derived from B wild type. All the other donor strains were derivatives of L44 (*argF*40 *argR*11 *leu* 2).

bers between the 2nd and 6th day of incubation. (Totals of 146 and 18 colonies were found in 8 Sm and 8 Pm plates, respectively.) They were stabbed on plates L + 50 μg/ml Sm or L + 200 μg/ml Pm, respectively, and further purified through single-colony isolation. No survivors appeared on controls in which the cells were spread directly on L Sm or L Pm plates, indicating that in 10^8 cells no mutant could be isolated when the experiment was started. The Sm survivors, although found resistant to at least 500 μg/ml Sm, were selected on plates containing only 50 μg/ml Sm because it was observed that in higher concentrations, the drug inhibited growth of several Sm^D clones reducing their number (and conferring a selective advantage of unwanted faster-growing secondary mutations).

6. *Preparation of P1 lysates*: Essentially the basic procedures of ADAMS (1959) were followed for P1 propagation and titration. Bacterial cells were grown on L medium to OD 2(10^8 cells/ml), $CaCl_2$ was added to a final concentration of 5×10^{-3}M and 10 min later 1 ml of the bacterial culture was mixed with 0.1 ml of P1 or P1 (vir) lysate having a titer of 10^7–10^8 phages/ml. After 20 min at 37°C without aeration, 2 ml of soft agar was added and the mixture was poured onto a fresh basal agar plate. In the case of dependent strains, L medium was supplemented with 25 μg/ml of Pm for $Drug^D$ and Pm^D strains, and with 50 μg/ml Sm for Sm^DEt^D and Sm^D strains. Soft agar and basal agar plates were similarly supplemented. Although these low concentrations of antibiotics were only suboptimal for growth, they were necessary for keeping the level of antibiotic during transduction below the amount which could kill or inhibit *strA*+ transductants. After overnight incubation at 37°C, the top agar was scraped and the phages harvested in the presence of a few drops of chloroform. The lysates obtained from the strains grown in the presence of antibiotic contained 20 μg/ml Pm or 40 μg/ml Sm. An attempt to eliminate the drug by dialysis proved to be unfeasible and it was decided to seek phage titers sufficiently high to permit extensive dilution in the suspension during transduction.

Special care has been taken to obtain P1 lysates as pure as possible with respect to each *strA* allele under study. The purity of the donor strain was assured not only by starting each time from a single colony, but also by checking the culture immediately before P1 infection. More-

over, the phages were propagated up to more than 10^7 multiplication by repeating the above procedure two or three times. Thus any transducing particle carrying a different *strA* allele than that contained in the original stock lysate was diluted beyond any appreciable amount in the final lysate.

Table 2 gives the P1 lysates prepared and used in this work.

7. *Transduction*: Transduction was performed following essentially the procedure of LENNOX (1955). The lysates described in Table 2 were diluted with L medium containing 5×10^{-3}M Ca++ to the titer indicated in each experiment (see Table 3). Other modifications of the standard procedure required by the particular purpose of our experiments are also summarized in Table 3 and described in the following two paragraphs.

8. *Introduction of dependent alleles into strain L11 His⁻*: The standard procedure for studying recombination between *strA* alleles was to cross an *aroE*+ *his*+ donor into an *aroE*24 *his*11 recipient, *aroE* being the selective marker cotransducible with *strA* used for obtaining the *strA*+ recombinants and *his* being a reference marker unlinked to *strA*. It was thus necessary to transduce the drug-dependent mutations isolated from L44 into L11 His⁻. Cells of L11 His⁻ strain were infected with each lysate of Table 2 at a moi of approximately 1.5. After 20 min of incubation at 37°C, the cells were spun down, washed and plated on L plates. After 3–3½ hr of preincubation, Pm or Sm was added to 200 μg/ml or 50 μg/ml, respectively, and the same procedure described under section 5 was followed thereafter. In this way, drug-dependent recombinants were selected and one of those retaining the *aroE*24 marker was saved. They were designated as L11 His⁻–D367, etc., corresponding to the denominations given in Table 2.

9. *Procedure for cross experiments*: The procedure used for crosses between Sm^R and drug-dependent strains was basically the same as that already described for $Sm^R \times Sm^R$ crosses (BRECKENRIDGE and GORINI 1970). The donors were always *aroE*+ *his*+ and the recipients *aroE*24 *his*11. Therefore, the dependent derivatives of L44 were used as donors in crosses of drug-dependent into Sm^R strains, while in the reciprocal crosses the dependent derivatives of L11 His⁻ were used as recipients. Some modifications were needed due to the presence of antibiotic either in the donor's lysate or in the growth medium of the recipient. All necessary information is summarized in Table 3. The transduction was carried out at a bacterial density of 4×10^8 cells/ml in a total volume of 5 ml which was reduced after the last washing by resuspending the cells in

TABLE 3

Protocol for transduction procedure

	Procedural steps	$Drug^D$ Sm^R	Sm^R $Drug^D$	Sm^D Sm^R	$Sm^D{}_{Et}{}^D$ Sm^R	Sm^R $Sm^D{}_{Et}{}^D$	Pm^D Sm^R
1) ↓	Medium for growth (4 x 10^8 bact/ml)	L	LPm (25 μg/ml)	L	L	LSm (50 μg/ml)	L
2) ↓	A-N washing before infection	no	once	no	no	once	no
3) ↓	phage used	P1	P1	P1	P1	P1 vir	P1 vir
4) ↓	m o i	1.5	15 to 17	1.5, 3.0	1.5	0.75, 1.5	0.37, 1.5
5) ↓	Drug in lysate (μg/ml final)	≤ 0.3 Pm	none	1.35, 2.7 Sm	2.7 Sm	none	0.17, 0.7 Pm
6) ↓	Post-incubation additions	none	25 μg/ml Pm*	none	none	none	none
7)	A-N washings before plating	2	4	4	4	4	4

* After 20 min of incubation at 37°C the cells were spun down, washed once and resuspended in L + Pm, as indicated. The incubation at 37°C was then continued for 3 hr.

1.1 ml of A — N. This final suspension which, barring killing due to the phage, should contain about 2×10^9 cells/ml was plated on selective media as follows:

a. 0.1 ml of undiluted suspension on His plates for arg$^{\pm}$ aro+ Sm^S recombinants. They appeared within 6–10 days incubation at 37°C. Evaporation was prevented by keeping the plates in plastic bags.

b. 0.1 ml of 10^{-1} and 10^{-2} dilutions on Arg His, Arg His Sm (or Pm) plates for *aroE*+ transductants (various types) and on Arg Aro Sm (or Pm) for *his*+ transductants.

Total cell counts on L or L Sm or L Pm plates and controls without phages were also performed. Revertant colonies were never detected on His plates (they should have arisen from two mutational events, to *aroE*+ and to *strA*+) and the number of revertants to aro+ or his+ detected on plates Arg His or Arg Aro, respectively, was insignificant.

10. *Analysis of colonies appearing on His plates* (check for true arg$^{\pm}$ aro+ Sm^S recombinants): All clones (or at least 20) which appeared on His plates after 10 days incubation were purified through single-colony reisolation and spot-tested on His and His Aro plates for ability to grow in the absence of Arg and on Arg His plus 1, 3, 5, and 500 μg/ml Sm for sensitivity to Sm. Full growth on 2 μg/ml, scanty growth on 3 μg/ml and no growth on 5 μg/ml and 500 μg/ml was a phenotype considered identical to that possessed by a *strA*+ wild-type strain.

11. *Calculation of recombination frequency between* strA *mutant alleles*: The recombination frequencies (Fr) between *strA* alleles are expressed as the ratio of the recombinational events occurring at the *strA* locus (arg$^{\pm}$ aro+ Sm^S recombinants) over the transductants which have received the donor's *aroE*+ *strA* chromosomal segment. Transductants aro+ Sm^R may be selected directly on Arg His plates and scored for Sm^R; therefore, in crosses in which the donor's allele was Sm^R, the value $\mathrm{Fr} = \dfrac{\text{arg}^{\pm}\ \text{aro+}\ \text{Sm}^S}{\text{aro+}\ \text{Sm}^R}$ was directly obtainable. In the reciprocal crosses, however, the number of transductants aro+ Drug^D was not directly obtainable from the difference of total aro+ transductants (resistant + dependent) selected on Arg His Sm (or Pm) plate minus the aro+ Sm^R selected on Arg His plate. It was found in fact that the number of transductants in Arg His Sm (or Pm) selection was always about 40% less than that expected on the basis of the *aroE strA* cotransduction frequency found to be 55% in separate experiments in which *aroE*+ *strAd* was crossed with *aroE*24 *strA*+ (see Table 5). Therefore in crosses Drug^D (donor) × Sm^R (recipient) the aro+ Drug^D transductants were calculated from the aro+ Sm^R selected on Arg His plates, knowing that the ratio (aro+ Drug^D/aro+ Sm^R) should be 55/45. Thus

$$\mathrm{Fr} = \frac{\text{arg}^{\pm}\text{aro+Sm}^S}{\text{aro+}\ \text{Sm}^R \times 1.22}$$

In crosses Drug$^{Di} \times$ Drug^{Dii} (recipient), the aro+ Drug^{Di} transductants were calculated from the total aro+ (Drug^{Di} + Drug^{Dii}) selected on Arg His Sm (or Pm) plates, knowing that those coming from the donor should be 55%. Thus,

$$\mathrm{Fr} = \frac{\text{arg}^{\pm}\ \text{aro+}\ \text{Sm}^S}{\text{total (aro+ Drug}^{Di} + \text{aro+ Drug}^{Dii}) \times 0.55}$$

As an additional control to detect any effect of the dependent or resistant *strA* alleles on the *aroE*+ transduction frequency, in each experiment *his*+ transductants were also selected on Arg Aro plates and their number compared to that of the *aroE*+ transductants. No abnormality was detected in the *aroE*+ transduction frequency in any type of crosses.

RESULTS

1. *Classification of dependent mutants from L44:* Cell suspensions of Sm and Pm survivors were spotted on L plates. If a mutant could grow on an L plate and could be transferred from L to L, it was classified as "resistant" while in the event it could not grow on an L plate (or only poorly) and could not be transferred from L to L, it was classified as "dependent." Further spot tests allowed the subclassification of the dependent clones given in Table 4. If small differences

TABLE 4

Classes of dependent mutants derived from L44

Class	Subclass		Phenotypic Suppression	Clones saved
Among survivors to Sm 50 μg/ml				
28 $Drug^D$	5	$Sm^D\ Pm^D\ Et^D$	$arg^{CSD, CPD};\ leu^{(CSD)(CPD)}$	4-5; 4-18; 5-6
($Sm^D Pm^D Et^D$)	1	$Sm^D\ Pm^{D\pm}$ (30 μg) Et^D	$arg^{CSD, CPD};\ leu^{CSD\ (CPD)}$	3-81
phenotyp. masked	4	Sm^D (50 μg) $Pm^D\ Et^D$	$arg^{CSD, CPD};\ leu^{((CSD))\ ((CPD))}$	3-67; 3-85
42%	2	Sm^D (50 μg) $Pm^D\ Et^D$	$arg^{CSD, CPD};\ leu^{(CSD)\ ((CPD))}$	5-11
	4	Sm^D (50 μg) $Pm^D\ Et^D$	$arg^{CSD, CPD};\ leu^{(CSD)\ (CPD)}$	4-26; 5-2
	12	Sm^D (50 μg) Pm^D (30 μg) $Et^{D\pm}$	$arg^{CSD, CPD};\ leu^{(CSD)(CPD)}$	4-8; 6-4
17 $Sm^D\ Et^D$	17	$Sm^D\ Pm^{inhibit.}\ Et^D$	$arg^{Sm\ incomp.};\ leu^{Sm\ incomp.}$	3-62; 3-80; 4-21
25%				
22 Sm^D	2	$Sm^D\ Pm^S\ Et^O$	$arg^{CSD};\ leu^{(CSD)}$	5-21
33%	19	$Sm^{D\pm}\ Pm^S\ Et^O$	$arg^{CSD};\ leu^{(CSD)}$	5-1; 5-18
	1	$Sm^{D\pm}$ (50 μg) $Pm^S\ Et^O$	$arg^{CSD};\ leu^{(CSD)}$	6-6
Among survivors to Pm 200 μg/ml				
15 $Drug^D$	14	$Pm^D\ Sm^D\ Et^D$	$arg^{CPD\ (CSD)};\ leu^{(CPD)(CSD)}$	17-7
($Sm^D Pm^D Et^D$) phenotypic. masked	1	$Pm^D\ Sm^D\ Et^{D\pm}$	$arg^{CPD\ (CSD)};\ leu^{(CPD)(CSD)}$	17-4

μg in parentheses = μg of drug per ml for optimal growth. When Sm or Pm are not followed by μg in parentheses, this means that the growth is maximal or unchanged with Sm 500 μg and Pm 200 μg/ml.

CSD = +++ in Sm without arg or/and leu. (CSD) = ++ in Sm without arg or/and leu. ((CSD)) = + in Sm without arg or/and leu.

are disregarded, three classes of dependent strains have been found: (1) dependent on Sm, Pm, or Et ($Drug^D$); (2) dependent on Sm or Et ($Sm^D\ Et^D$); and (3) dependent on Sm only (Sm^D). All three classes are found among the Sm survivors while only class (1) is found among the Pm survivors. As already described (GORINI, ROSSET and ZIMMERMANN 1967), the cells of class (1) cannot be transferred directly from L Sm to L Pm or *vice versa* but only after an intermediate growth in L Et. This phenomenon has been called "phenotypic masking."

2. *Phenotypic suppression of nonsense mutations:* Strain L44 contains an amber *argF*40, and a UGA mutation, *leu*2. Different classes of Sm^R mutations have been distinguished by their "competence" to permit Sm to satisfy the arg or leu or both requirements in this strain (BRECKENRIDGE and GORINI 1970). This phenotypic suppression of a mutation by Sm has been designated as CSD (conditional streptomycin dependence). Thus the *strA*E11 allele is competent for arg and leu suppression (arg^{CSD} and leu^{CSD}) while *strA*E26 is arg^- and only leu^{CSD}. This analysis can be extended to the drug-dependent derivatives of L44 by asking the question whether or not the Arg or Leu or both requirements are dispensable in these mutants. The designation CSD (or CPD in the case of Pm) is retained for simplicity, although improperly. As seen in Table 4, it is found that all $Drug^D$ mutants are arg and leu CSD and CPD; all Sm^D mutants are arg and leu CSD while the $Sm^D\ Et^D$ are strictly arg^- and leu^-. Using the terminology

adopted for the Sm^R mutations, it may be said that $Drug^D$ and Sm^D mutants are competent for permitting phenotypic suppression of both amber and UGA nonsense mutations, while Sm^D Et^D are incompetent.

3. *Effect of kanamycin on $Drug^D$ and of Pm on Sm^D Et^D and Sm^D:* Spot-test experiments indicated that kanamycin (Km) 200 μg/ml does not satisfy the drug requirement of dependent mutants of any class. Mutants of $Drug^D$ class, however, grew slightly when Km was added to Pm (not when added to Sm). This suggested that the $Drug^D$ mutants (eight were tested) may be inhibited but not killed by Km. An experiment was performed by adding 200 μg/ml Km to cultures of $Drug^D$ 177 and of its parent L44 exponentially growing in L Et medium. Growth was followed by OD and cell counts performed by withdrawing one ml samples at intervals, spinning down the cells and plating different dilutions of the cell suspension in A—N onto plates L for L44 and L Pm for D177. Figure 1 shows that Km did not interfere with the growth of the $Drug^D$ mutant in L Et; as expected, Km rapidly killed the L44 parent. Consistently, the OD of the $Drug^D$ 177 culture steadily increased even in the presence of Km, while that of the L44 culture stopped immediately upon Km addition.

It is known that the $Drug^D$ mutants are not only sensitive to killing by Sm and Pm when used in combination, but also growth in either drug sensitizes cells to the other (phenotypic masking, GORINI, ROSSET and ZIMMERMAN 1967). It was interesting to know the effect of Pm on the other two classes of dependent mutants isolated from L44: the Sm^D Et^D and the Sm^D. Pm (200 μg/ml) was added to exponentially growing cultures (in L Sm) of a $Drug^D$ (D367), a Sm^D (S521), three Sm^D Et^D (SE362, SE421, and SE380) and the Sm^R LE11 as control. The

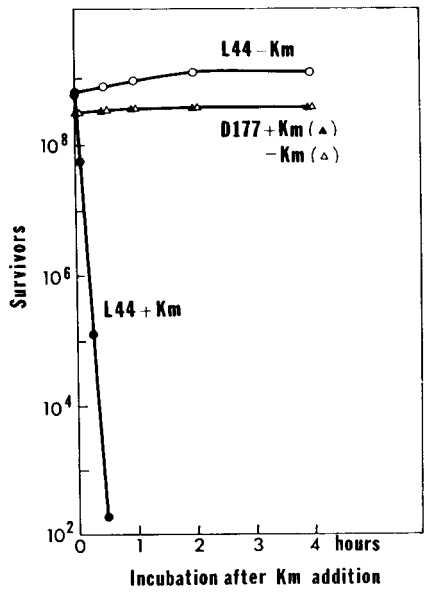

FIGURE 1.—Effect of Km on viability of a $Drug^D$ strain. D177 and L44 strains are growing in L Et. At time zero 200 μg/ml Km is added to ● and ▲.

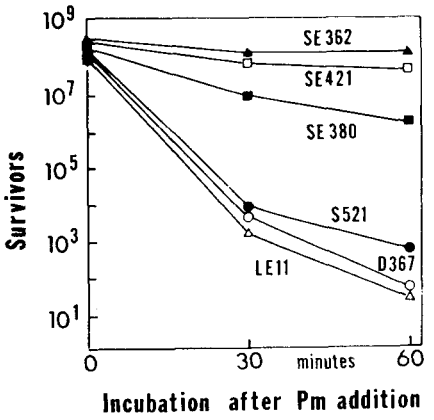

FIGURE 2.—Effect of Pm on viability of Drug^D, Sm^D Et^D, and Sm^D strains. D367, SE362, SE421, SE380, S521 and LE11 (Sm^R) are growing in L Sm. At time zero 200 μg/ml Pm is added.

increase of OD stopped immediately and cell counts were performed by withdrawing 1 ml samples at intervals, spinning down the cells and plating on L Sm plates. Figure 2 shows that the Sm^D Et^D class of mutants is inhibited but not killed by Pm added to Sm, while the Sm^D class is as sensitive to Pm + Sm as is a normal Sm^R or a Drug^D strain.

4. *Reversion of Sm^D mutants to drug independence:* It was found that the Drug^D and Sm^D Et^D classes of dependent mutants spontaneously gave rise to drug-independent "revertants" through a mutation occurring at the *ram* locus (BJARE and GORINI 1971). The Sm^D class does not "revert" spontaneously and moreover a *ram* mutation introduced into the strain has no effect on drug dependence. It is also known that two mutator genes, *mut*S1 and *mut*T1, differ drastically in the types of Sm^R mutations they induce in L44. *mut*T1 induces only arg$^-$ leu^{CSD} or arg$^-$ leu$^-$ mutants while *mut*S1 induces mostly arg^{CSD} leu^{CSD} (BRECKENRIDGE and GORINI 1970). We have repeated the selection of survivors to Sm from L44–MutS1 and L44–MutT1 and confirmed the previous results as far as Sm^R mutants were concerned. In addition, we have found that from L44–MutS1 (but not from L44–MutT1), dependent mutants are also obtained, most are Drug^D and some are Sm^D. From the latter, i.e., Sm^D mutants of L44–MutS1, drug-independent "revertants" induced by *mut*S1 are obtained.

5. *Cotransduction frequencies of dependent alleles with* aroE: Strains *aroE*$^+$ carrying either the different drug-dependent mutations or the *strA* alleles E11 or E26 are crossed into L11 His$^-$ (*aroE*24), selecting for Pm (or Sm) dependent and/or resistant transductants as described under MATERIALS AND METHODS, section 8. They are then scored for *aroE*$^\pm$ as drug-dependent transductants on plates Arg His Aro, Arg His Aro Pm (or Sm), Arg His Pm (or Sm). The result is reported in Table 5 which indicates that all dependent alleles were cotransducible with *aroE* at the frequency of 50–60% similar to that obtained with the *strA* alleles.

TABLE 5

Frequency of cotransduction of dependent alleles with aroE

Allele	Selected on Pm (200 μg/ml)			Selected on Sm (50 μg/ml)		
	Number of transductant tested (a)	Number of *aroE*$^+$ (b)	(b/a) × 100	Number of transductant tested (a)	Number of *aroE*$^+$ (b)	(b/a) × 100
D367	144	82	57	144	78	54
D385	108	56	52	108	60	55
D45	144	84	58	144	75	52
D418	108	64	59	108	54	50
D426	108	57	53	108	62	57
D52	144	81	56	144	84	58
D56	108	54	50	108	66	61
D177	144	77	54	144	78	54
S518	..	..	..	144	75	52
S521	..	..	..	144	84	58
SE380	..	..	..	144	82	57
P513	100	49	49	..	..	..
strA E11	..	..	..	216	108	50
strA E26	..	..	..	216	117	54

6. *Recombination between* strA *alleles:* In the absence of specific suppressors, the translation of nonsense mutations (especially amber and UGA) is strictly lacking in restricted *strA* mutant strains but is leaky in the nonrestricted *strA*$^+$ wild type (STRIGINI and GORINI 1970). If the function affected by the nonsense mutations is very sensitive to a small restoration of activity, this leakiness in translation may be apparent in the phenotype. This is the case for the arg phenotype of the *argF*40 amber mutation which is leaky in *strA*$^+$ strains while it is strictly negative in most of the *strA* mutants (GORINI, JACOBY and BRECKENRIDGE 1966). On the basis of *strA*$^+$ recombinants from crosses between Sm^{R1} and Sm^{R11} alleles could be selected as arg$^\pm$ because the two parents also carried the *argF*40 mutation (BRECKENRIDGE and GORINI 1970). The selection was limited to the arg$^\pm$ transductants which received the donor's *aroE*$^+$, so that all genetic suppressors of *argF*40 not located in the *aroE* region were excluded while the recombinational events occurring in the *strA* gene were cotransduced with *aroE* at a frequency of 55%.

The same principle has been applied in the crosses involving dependent strains performed in the present study. Two major difficulties were encountered: (a) traces of drug introduced with the P1 lysates of drug-dependent strains may phenotypically suppress *argF*40 so that arg$^\pm$ aro$^+$ transductants may appear which are not *strA*$^+$ recombinants but carry the parental *strA* competent allele; (b) Sm caused a substantial reduction in general yield of recombinants, when the recipient was a dependent strain and therefore grown in the presence of this drug. These disturbances were overcome by using Pm instead of Sm whenever possible (as in the case of Drug^D) and by introducing several cell-washings in the general procedure as described in Table 3.

The selection of aro$^+$ transductants was the reference for calculating the recombination frequencies. As an additional precaution, transductants which received the donor's unlinked marker *his*$^+$ were also selected. Although the absolute number of *his*$^+$ transductants was somewhat different from those of *aroE*$^+$ transductants, the ratios (*aroE*$^+$/*his*$^+$) transductants were comparable within a deviation of ±50% throughout all crosses.

Finally, each P1 lysate of the dependent derivatives of L44 was transduced into L11 His$^-$ mutant harboring the identical dependent allele. The washing technique adopted for transductions in which the recipient was a dependent strain (Table 3) was used. Although the expected number of *aroE*$^+$ and *his*$^+$ transductants were found on the Arg His Sm (or Pm) and the Arg Aro Sm (or Pm) selective plates, respectively, no recombinants arg$^\pm$ aro$^+$ Sm^S were found on eight His selective plates. It was concluded that the lysates did not contain an appreciable number of particles transducing *strA* alleles other than those designated (Table 2).

7. Crosses of Drug^D into Sm^R mutants: The L11 His$^-$ strains (*aroE*24 *his*11) harboring Sm^R mutations *strA*E11 and *strA*E26, between which the relative map distance is known (BRECKENRIDGE and GORINI 1970), were used as recipients. Mutants Drug^D of strain L44 (*aroE*$^+$ *his*$^+$) were the donors. Their P1 lysates contained 20 μg/ml Pm coming from the strain's growth medium and it was established experimentally that Pm above 2 μg/ml was toxic for the recipients. To overcome this inconvenience lysates were chosen with titers $\geqslant 4\times 10^{10}$ phages/ml because the drug could be diluted to ≤ 0.3 μg/ml, since the transduction was performed at a concentration of 6×10^8 phages/ml. Lysates of seven Drug^D mutants selected as Sm-survivors, and one selected as a Pm-survivor, were used. However, when the plating technique, which was successfully used in the Sm^R × Sm^R crosses in the absence of Pm (cells spun down and resuspended in 1/5 volume of A—N), was applied to the Drug^D × Sm^R crosses, a large number of tiny colonies appeared on the His selective plates of some crosses and interfered with the growth and scoring of the arg$^\pm$ aro$^+$ Sm^S recombinants. It was further found that (a) these colonies were arg$^-$ aro$^+$ Sm^R and did not grow again when transferred to a new His plate; (b) they appeared only in crosses in which the competent *strA*E11 strain was used; (c) their frequency agreed with that of the total aro$^+$ transductants appearing on an Arg His plate. It was concluded that Pm as low as 0.3 μg/ml might still be able to suppress the arg phenotype. Indeed a model experiment in which an *argF*40 *aroE*$^+$ *strA*E11 strain was plated on His plates ± 0.2 μg/ml Pm confirmed this conclusion because growth appeared in the Pm plates. Two washings with A—N immediately after completion of the 20 min incubation for infection prevented this interference.

Most of the arg$^\pm$ aro$^+$ Sm^S recombinants appeared on the His plates after six to ten days of incubation although the other transductants, aro$^+$ on Arg His or his$^+$ on the Arg Aro plates, appeared after two days. This was expected and is in agreement with the fact that only *argF*40 is leaky and not arg$^+$ in strA$^+$ strains. However, about 10% of the Sm^S recombinants appeared much earlier (after two days), a behavior more consistent with an arg$^+$ than with an arg$^\pm$ strain. A pedigree analysis of several arg$^+$ clones showed that their population was hetero-

geneously composed of arg^+ Sm^S and $arg^\pm$ Sm^S cells. It was concluded that a secondary mutation had occurred in the $arg^\pm$ recombinants, and the arg^+ colonies were included in the category of $arg^\pm$ aro^+ Sm^S recombinants after checking their Sm^S character. The results of this type of cross are given in Table 6; the reported recombination frequencies give the relative distances between $Drug^D$ and the *strA*E11 or *strA*E26 alleles.

8. *Recombination between $Drug^D$ alleles:* Crosses between different $Drug^D$ alleles were carried out with the same technique used for performing crosses between strains carrying identical $Drug^D$ alleles as in section 6 of RESULTS. The recipient cells were cultivated in the presence of 25 μg/ml Pm and washed once before infection and four times after the 20 min standard incubation. Strains L11 His^- carrying the alleles D367 and D52 ($Drug^D$ isolated as Sm survivors) were employed as recipients and P1/D177 lysate (prepared from strain 17–7, one of the L44 $Drug^D$ mutants isolated as a Pm-survivor) was used as donor. No $arg^\pm$ aro^+ Sm^S recombinants were found on eight His selective plates (<0.01 percent recombination in each cross) although the number of *aroE*$^+$ and *his*$^+$ transductants found on Arg His Pm and Arg Aro Pm selective plates, respectively, was normal on the basis of the standard results obtained in the other crosses. It can be concluded that the $Drug^D$ alleles D177, D367, and D52, are closer than 0.01% recombination units or possibly identical.

9. *Crosses of Sm^R into $Drug^D$ mutants:* When $Sm^R \times Drug^D$ crosses were carried out by the same procedure as in $Drug^D \times Drug^D$ crosses (section 8) the frequency of transduction for both aro^+ and his^+ markers was only 1/10 of that obtained in reciprocal crosses $Drug^D \times Sm^R$ (section 7). We were unable to find the reason for this behavior (it happened also when the Sm^R lysates were prepared in the presence of Pm similarly to the $Drug^D$ lysates). Practically, it was found that high levels of moi and an additional 3 hr of incubation at 37°C with 25 μg/ml Pm after the standard 20 min period allowed for infection, overcame this apparent anomaly. During this additional incubation, the increase in cell numbers due to division was only two to three times while the transduction frequency of aro^+ and his^+ markers was brought to normal, i.e., increased ten times. Table 7 gives the frequencies of recombination between the *strA* alleles obtained by applying this modified procedure. They are roughly three times higher than in the reciprocal crosses (Table 6) for which a more conventional procedure was used. A similar relationship was found also in crosses between Sm^R alleles in which case the technique used for cross E11 × E26 and for its reciprocal E26 × E11 was identical (BRECKENRIDGE and GORINI 1970; Table 10). We thus attribute the difference to the relative position of $Drug^D$ and E11 alleles (Figures 3 and 4) rather than to an artifact introduced by the 3 hr incubation technique. It may be inferred that the expression of the *strA*$^+$ recombinant phenotype (Sm^S) is delayed or that the drug does not kill these recombinants under the experimental conditions realized in our experiments.

10. *Crosses of Sm^D Et^D into Sm^R mutants:* The yield in phages obtained from Sm^D Et^D strains was consistently lower than that obtainable with $Drug^D$ strains. Presumably the necessity to grow Sm^D Et^D strains in Sm was the reason for this

TABLE 6

Recombination between Drug^D *and* strA *alleles*

Cross		Number of transductants arg$^\pm$ aro$^+$ Sm^S*	Recombination frequency†
Donor	Recipient		
D367	E11	48	0.121
D385	E11	33	0.060
D45	E11	69	0.125
D418	E11	72	0.142
D426	E11	24	0.111
D52	E11	32	0.114
D56	E11	51	0.096
D177	E11	28	0.086
D367	E26	57	0.214
D385	E26	66	0.163
D45	E26	103	0.216
D418	E26	88	0.224
D426	E26	45	0.278
D52	E26	52	0.236
D56	E26	99	0.251
D177	E26	63	0.268

* Total number selected on eight His plates except for D45 × E11 and D45 × E26 crosses, in which sixteen His plates were used.

† The recombination frequencies are expressed in percent and represent the ratio of the frequency of arg$^\pm$ aro$^+$ Sm^S transductants over the frequency of aro$^+$ Drug^D cotransductants (see MATERIALS AND METHODS).

TABLE 7

Recombination between strA *and Drug^D alleles*

Cross		Number of transductants arg$^\pm$ aro$^+$ Sm^S*	Recombination frequency†
Donor	Recipient		
E11	D177	89	0.28
E11	D367	75	0.34
E11	D52	98	0.35
E26	D177	167	0.58
E26	D367	179	0.63
E26	D52	156	0.67

* Total selected on eight His plates.

† The recombination frequencies are expressed in percent and represent the ratio of the frequency of arg$^\pm$ aro$^+$ Sm^S transductants over the frequency of aro$^+$ Sm^R cotransductants (see MATERIALS AND METHODS).

poor yield, since similar low titers were also obtained with Drug^D strains when grown in Sm instead of Pm. Strain 3–80 gave the highest titer (1.2×10^{10}) and was used for this experiment. The concentration of Sm in the infection mixture could be diluted to only 2.7 μg/ml and four washings after infection were necessary to prevent the appearance of undesirable colonies other than the arg$^\pm$ aro$^+$ Sm^S recombinants. The results of this type of cross are shown in Table 8.

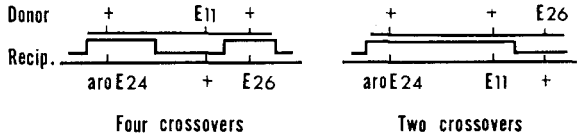

FIGURE 3.—Crossover events postulated to account for different distances in reciprocal crosses.

11. *Crosses of Sm^R into Sm^D Et^D mutants:* To confirm that SE380 is close to E26 and to determine on which side it lies, we performed the reciprocal cross of that just described. Four washings were necessary to avoid undesirable colonies on the selective His plates (section 10). The results are shown in Table 8. It is seen that in this reciprocal cross meaningful figures could be obtained, and that two different experimental conditions (different moi) did not alter the results.

12. *Crosses of Sm^D into Sm^R mutants:* The P1 lysates of Sm^D strains had low titers for reasons analogous to the case for the Sm^D Et^D strains (RESULTS, section 10). Strains 5–18 and 5–12 were selected as representatives of the Sm^D class, because their lysate titer was $> 10^{10}$ phages/ml. The same experimental procedure was applied as in Sm^D Et^D × Sm^R crosses, except that an additional experiment was carried out using a moi of 3 for the S518 × Sm^R crosses. As seen in Table 9, the Sm^D alleles are closely linked to the E26 allele; however, with the S518 allele a meaningful separation is obtained, since recombinants are found.

13. *Crosses of Pm^D Sm^R into Sm^R mutants:* The only strain available in this class of donors was B513. Since the P1 vir lysate titer was low (see Table 2), four washings and two levels of moi, 1.5 and 0.37, were used in the attempt to reduce the concentration of residual Pm in the infection mixture. As shown in Table 9, these two experimental conditions gave almost the same results indicating that Pm^D Sm^R allele (P513) is also closely linked to the E26 allele.

All attempts to perform reciprocal crosses Sm^R into Sm^D and Sm^R into Pm^D Sm^R

TABLE 8

Recombination between Sm^DEt^D and strA *alleles*

Cross		Number of transductants	Recombination frequency†	
Donor	Recipient	$arg^{\pm}$ aro^+ Sm^{S*}		
SE380	E11	27	0.215	
SE380	E26	0	<0.02	
E11	SE380	10	0.076	0.092
E11‡	SE380	8	0.108	
E26	SE380	8	0.089	0.080
E26‡	SE380	4	0.071	

* Total number selected on eight His plates.

† Expressed in percent and represents the ratio of the frequency of $arg^{\pm}$ aro^+ Sm^S transductants over the frequency of aro^+ Sm^D Et^D transductants for Sm^D Et^D × *strA* crosses and of aro^+ Sm^R transductants for *strA* × Sm^D Et^D crosses, respectively.

‡ moi of 0.75 was applied. The others were done with the standard moi of 1.5.

TABLE 9

Recombination between Sm^D; Pm^D Sm^R × strA alleles

Cross		Number of transductants	Recombination frequency†	
Donor	Recipient	arg± aro+ Sm^S*		
S518‡ (Sm^D)	E11	701	0.290	0.295
S518 (Sm^D)	E11	639	0.300	
S518‡ (Sm^D)	E26	7	0.0035	0.004
S518 (Sm^D)	E26	10	0.0041	
S521 (Sm^D)	E11	30	0.234	
S521 (Sm^D)	E26	0	<0.02	
P513 (Pm^D Sm^R)	E11	149	0.304	0.329
P513§ (Pm^D Sm^R)	E11	89	0.354	
P513 (Pm^D Sm^R)	E26	0	<0.004	<0.006
P513§ (Pm^D Sm^R)	E26	0	<0.006	

* Total number selected on eight His plates.
† Expressed in percent and represents the ratio of the frequency of arg± aro+ Sm^S transductants over the frequency of aro+ Sm^D transductants for Sm^D × *strA* crosses and aro+ Pm^D Sm^R transductants for Pm^D Sm^R × *strA* crosses, respectively.
‡ moi of 3.0 was applied.
§ moi of 0.37 was applied.
The others were done with the standard moi of 1.5.

were unsuccessful, even applying the procedure used in crosses Sm^R into $Drug^D$ (RESULTS, section 9).

DISCUSSION

The selection of Sm and Pm survivors from L44 yielded the same classes of dependent mutants obtained with other *E. coli* B strains, i.e., the wild type (ZIMMERMANN, ROSSET and GORINI 1971) and the L1B (*argF*40 *argR*11 *aroE*24) derivative (BJARE and GORINI 1971). The use of 50 μg/ml Sm, instead of 500 μg/ml, did not reveal new types of dependents; thus it might be assumed that the three classes found, the $Drug^D$, the Sm^D Et^D, and the Sm^D, with the much rarer Pm^D Sm^R found only once from the wild-type B, comprise the gamut of mutants dependent upon Sm and/or Pm obtainable from strain B. On the basis of the frequency with which they are cotransduced with *aroE* (Table 5), they are all located at, or extremely near, the *strA* site. This is the same site at which the Sm^R mutants obtained in the same type of Sm selection have been mapped (BRECKENRIDGE and GORINI 1970). The determination of their map distances relative to the Sm^R mutants performed in the present work (Figure 4) allows the conclusion that all dependents we have isolated result from mutations within the *strA* gene.

These mutations simultaneously alter the resistance to three antibiotics, Sm, Pm, and Km (Figures 1 and 2). Km does not satisfy the drug requirement of any

of the dependent mutants in our collection and all our attempts to isolate Km-dependent mutants directly through a Km selection have failed (unpublished results). However, the $Drug^D$ mutants are resistant to Km. A culture of a $Drug^D$ mutant growing in L Et is almost indifferent to Km, is moderately inhibited when growing in L Pm and completely inhibited (but not killed) when the growth medium is L Sm. It is possible that a competition exists between the Km and Pm (or Sm) for reaching the same site of action. Differences in resistance to Sm and Pm used in combination are also very clearcut: the Pm^D Sm^R mutants are indifferent; the Sm^D Et^D mutants are completely inhibited (but not killed); the $Drug^D$ and Sm^D mutants are killed as quickly as wild-type cells by the mixture Sm + Pm. The behavior of the $Drug^D$ mutants is peculiar; when pre-grown in either one drug (Sm or Pm), the cells are sensitized to the other (Pm or Sm) and growth in L Et is required to dilute out the sensitized cells (GORINI, ROSSET and ZIMMERMANN 1967; ZIMMERMANN, ROSSET and GORINI 1971). All these different properties are described here because they are useful phenotypes for distinguishing the different classes of dependent mutants; a more detailed description is beyond the purpose of this work. One point, however, should be stressed; the previous concept that the *strA* ribosomal component is that which is specifically concerned with the action of Sm, is no longer tenable, as has been discussed elsewhere (GORINI 1969).

It is known that *strA* mutations restrict the efficiency of translation in general (GORINI 1969) and suppression of nonsense mutations in particular (STRIGINI and GORINI 1970). Since strain L44 contains an amber (*argF*40) and a UGA (*leu*2) mutation, it was possible to study the degree of restriction which the different classes of dependent *strA* mutations impose on the Sm- or Pm-induced phenotypic suppression of the amber and UGA codons. The *argF*40 and *leu*2 mutations are CSD or CPD or both in all classes of dependent mutants with the only exception being the Sm^D Et^D class in which they are completely restricted (Table 4). (Although not reported in the table, it was also found that the Pm^D Sm^R mutants are arg^{CPD} and leu^{CPD}.) These differences in restriction are relevant to the hypothesis that the drug dependence itself is due to a particular kind of restriction (GORINI, ROSSET and ZIMMERMANN 1967).

It was previously found (BJARE and GORINI 1971) that $Drug^D$ and Sm^D Et^D are reverted to independence by a mutation in the *ram* ribosomal gene. The same is true for the Pm^D Sm^R (ROSSET and GORINI, unpublished). By contrast, the Sm^D do not revert spontaneously nor do they become independent after introduction of a *ram* mutation. It is now found that the genetic mutation *mut*S1 (but not *mut*T1) induces reversions to independence in the Sm^D strains. This indicates that the Sm^D may not necessarily be a multisite mutation as it was provisionally assumed on the basis of its failure to revert. A mechanism similar to that found for the other classes of dependent mutants is conceivable, i.e., a second mutation different from *ram* and not occurring spontaneously but induced by *mut*S1 could be necessary for reversing the Sm^D phenotype to drug independence.

Concerning the mapping experiments it was found previously (BRECKENRIDGE and GORINI 1970) that the distance between *strA*E11 and *strA*E26 is 0.11%

TABLE 10

Summary of recombination frequencies in various crosses

Cross		
Donor	Recipient	Recombination frequency*
E11	E26	0.12
E26	E11	0.31
$Drug^D$	E11	0.11
$Drug^D$	E26	0.23
E11	$Drug^D$	0.32
E26	$Drug^D$	0.63
$Drug^D$	$Drug^D$	<0.02
Sm^D Et^D	E11	0.22
Sm^D Et^D	E26	<0.02
E11	Sm^D Et^D	0.09
E26	Sm^D Et^D	0.08
Sm^D	E11	0.27
Sm^D	E26	0.004† (<0.02)
Pm^D Sm^R	E11	0.33
Pm^D Sm^R	E26	<0.006

* Combined values for every cross of the same category.
† Only the value obtained with S518 was adopted.

when measured in P1-transduction crosses of *aroE*$^+$ *strA*E11 into *aroE*24 *strA*E26 and 0.28% in the reciprocal crosses. Figure 3 gives the proposed relative position of the mutations involved and accounts for the difference: 0.11% is obtained by four crossovers and 0.28% by two crossovers. We have remeasured the distance between E11 and E26 by using the identical procedure adopted in $Drug^D$ × *strA* crosses, i.e., 0.3 μg/ml Pm was intentionally added to the infection mixture and the cells were washed twice after infection. Values of 0.12% and 0.31% were obtained, almost identical to those found with the original technique. On this basis we decided to use the E11 and E26 allele for standard reference for all crosses involving dependent alleles. In Table 10 all data are summarized and average values were taken in every cross of the same category, since the alleles of the same class gave data which could be considered equivalent, taking into account the experimental errors.

Figure 4 presents the proposed fine-structure map of the *strA* locus satisfying all data shown in Table 10, adopting the standard distance between E11 and E26 given above and taking into consideration the number of crossovers in every cross. It is assumed in this reasoning that recombinants carrying the two mutations, resistant (E11 or E26) and dependent (any one of the four classes tested), are counterselected since it was previously found (BRECKENRIDGE and GORINI 1969) that the phenotype of an *argF*40 strain, Sm^R/Sm^D diploid, is Sm-dependent and Arg-negative. In spite of a few small quantitative incongruities between distances, probably due to experimental errors, it is concluded that the $Drug^D$ allele lies close to the E11 allele on the *aroE* side and at least one Sm^D Et^D allele lies close to E26 allele on the E11 side. The Sm^D and Pm^D Sm^R alleles we have

mapped were also found close to E26 but it cannot be determined with confidence on which side of E26 they lie. The A5-dependent strain tentatively mapped previously (BRECKENRIDGE and GORINI 1970) was confirmed to be located at the site of the $Drug^D$ class.

In conclusion, the indication is strong that the different types of dependent mutations we have mapped occurred in the same *strA* cistron as the Sm^R mutations mapped previously. The members of each class which have been tested map so close to each other that they were not separable with our technique. Since small phenotypic differences are found in the mutants within the same class (subclasses in Table 4) it should be concluded that many of these mutants are not genetically identical. Thus, clusters of point mutations, which are individually responsible for the general phenotype characterizing each class, should exist. On the other hand, the classes Sm^D and Pm^D Sm^R are also very close to the Sm^R E26 allele although their phenotypes are very different. Even considering a phenotype which can be quantitated, as, for instance, restriction, one cannot see at present any correlation between the degree of restriction and map position. This lack of correlation over large map distances may reflect the important role of the protein's tertiary structure as a determinant of its properties. However, this rationale is less adequate when one considers very short map distances (corresponding to only a few amino acid residues apart).

The distance between the farthest Sm and Pm mutants is of the order of 0.6%. If one applies the data obtained in mapping the A protein of tryptophan synthetase performed as in our case with P1-transduction (YANOFSKY *et al.* 1964), one could conclude that the region of *strA* presently mapped corresponds to roughly 100 amino acid residues. Recently the *strA* protein has been shown to consist of

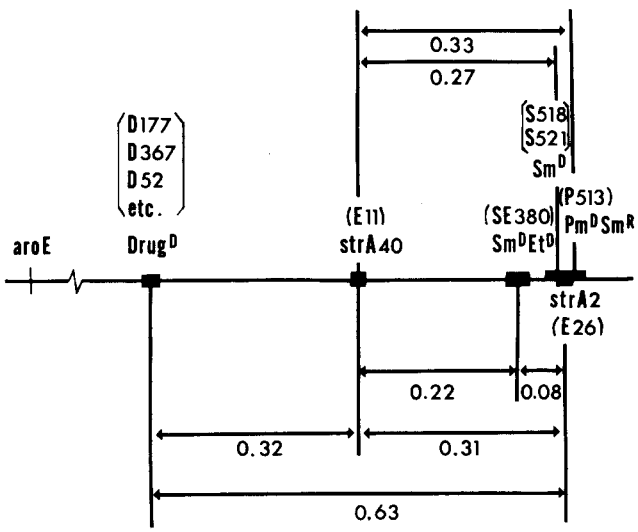

FIGURE 4.—Fine genetic map of the *strA* locus. The map distance represents the average percent recombination between dependent alleles of each group and Sm^R alleles E11 or E26.

approximately 200 amino acid residues (CRAVEN *et al.* 1969). Apparently, all mutations which can be selected by Sm and Pm lie within this stretch of polypeptide chain. They are responsible for at least eight (4 in the Sm^R group and 4 in the dependent group) clearly distinct phenotypes involving resistance to and dependence upon three different aminoglucoside antibiotics.

SUMMARY

Strains dependent on streptomycin (Sm^D) selected as Sm survivors, were tested for their behavior with respect to other drugs, including paromomycin (Pm), kanamycin (Km) and ethanol (Et). Dependent strains were also selected as survivors to Pm. It was found that more than one class of mutants possess the *dependent* phenotype. In *Escherichia coli* B four classes were found, differing (a) by the number of drugs individually able to satisfy their dependence (Sm^D Pm^D Et^D, Sm^D Et^D, Sm^D, and Pm^D); (b) by their resistance to Sm, Pm, and Km; (c) by the restriction they impose to phenotypic suppression; (d) by their ability to revert to drug-independence by secondary mutations.—All four classes of dependent mutants appear to map within the *strA* gene at different distances from two genetically known *strA* alleles of the Sm-resistant (Sm^R) class. A fine structure map of the *strA* cistron is presented. These results contradict the commonly held assumption that the *strA* gene product is specifically the target of the Sm action.

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