GENETIC ANALYSIS OF STREPTOMYCIN RESISTANCE IN *ESCHERICHIA COLI*[1]

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THE *strA* locus in *Escherichia coli* determines the primary structure of a protein of the 30S ribosomal subunit (TRAUB and NOMURA 1968; OZAKI, MIZUSHIMA and NOMURA 1969). The phenotype classically used for selecting *strA* mutants is survival to the bactericidal action of streptomycin. These survivors either are indifferent to streptomycin ("resistant") or they require the presence of the aminoglycoside for growth ("dependent"). In addition to its bactericidal action, streptomycin has other effects on the wild-type *strA*$^+$ sensitive cell and on the ribosomes extracted from it, including the ability to promote phenotypic suppression *in vivo* (GORINI and KATAJA 1964) and to induce misreading *in vitro* (DAVIES, GILBERT and GORINI 1964). These effects are similarly eliminated or reduced in *strA* mutants.

Presumably more fundamentally related to the role of the *strA* protein in ribosomal function is an impairment in translation efficiency brought about by the *strA* mutation. Such an impairment is evident in mutants, either missense or nonsense, whose phenotype is positive or leaky in *strA*$^+$ cells, but which becomes negative upon the introduction of a *strA* mutation. Since this impairment in translation efficiency might be lethal if not contained within limits compatible with cell growth, it is evident only in mutant strains whose growth is limited by the availability of a tRNA species able to translate properly the defective codon. The limiting tRNA may be either a suppressor that translates the mutated codon by conventional base-pairing or a normal tRNA that translates it by misreading (GORINI 1969).

In addition to the classification as indifferent or dependent, *strA* mutants may be classified by their different patterns of phenotypic suppression. Certain *strA* alleles are competent for permitting phenotypic suppression by streptomycin of *argF40*, an amber mutation in the structural gene for ornithine transcarbamylase, while others are incompetent (GORINI and KATAJA 1964). A third class of *strA* alleles is incompetent for *argF40* suppression but competent for the suppression of certain other mutations (ANDERSON, GORINI and BRECKENRIDGE 1965). The existence of these three classes was confirmed when the *strA* alleles were intro-

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duced into an otherwise isogenic strain carrying two nonsense mutations (GORINI, JACOBY and BRECKENRIDGE 1966).

Another subdivision in classes may be obtained if one measures the restriction of genetic suppression produced by different *strA* alleles (GARTNER and ORIAS 1966; STRIGINI and GORINI 1970). It has been shown that the classification by increasing restriction in translation efficiency is consistent whichever the system of genetic suppression used. Although not necessarily implied, it has been also found that a high level of restriction of genetic suppression is paralleled by a low level of competence for phenotypic suppression (STRIGINI and GORINI 1970).

In the present investigation a large number of spontaneous and mutagenically induced *strA* alleles are examined with respect to the pattern of phenotypic suppression by streptomycin and the structurally related aminoglycoside bluensomycin permitted in an *E. coli* B strain carrying two nonsense mutations, the *argF40* UAG mutation and the *leu-2* UGA mutation. A genetic map of the *strA* gene is then constructed, based on the fact that recombination can be detected in the *strA* locus because the *argF40* amber mutation is phenotypically arginine leaky, i.e., slight growth occurs in the absence of arginine, in *strA*$^+$ sensitive cells but not in *strA* resistant or dependent cells (GORINI, JACOBY and BRECKENRIDGE 1966).

MATERIALS AND METHODS

Bacterial strains: The *E. coli* B derivatives employed are listed in Table 1. All contain the UV-induced *argF40* amber mutation (GORINI, GUNDERSEN and BURGER 1961; GORINI, JACOBY and BRECKENRIDGE 1966), and most are derepressed for the arginine biosynthetic pathway, a prerequisite for the expression of the arginine CSD[3] phenotype (JACOBY and GORINI 1967). *spcA3* is a spontaneous mutation to high level resistance to spectinomycin (Spm); *strA1*, *strA2*, *strA40*, and *strA60* are spontaneous mutations to high level resistance to streptomycin (Sm); and *strA5* is a spontaneous mutation to Sm dependence. The *spcA* and *strA* alleles were transduced from the original strains listed in Table 1 into L1 or L44 as indicated in the text. *leu-2* was induced by nitrosoguanidine (NG) in strain P2 and later transduced into L1; it is most likely a UGA mutation (ROSSET and STRIGINI, unpublished). *his-11* was also NG-induced. *aroE24* was transduced into L1 and AT2472 (PITTARD and WALLACE 1966) by means of cotransduction with *spcA3*. *mutT1* was introduced into L44 from W3110 (*azi-r mutT1*) kindly provided by Dr. E. C. Cox) by transduction with *azi-r*. *mutS1* was introduced into a *cysC* derivative of L44 by cotransduction with *cysC*+ from AZA/R1 (*mutS1*) (also from Dr. E. C. Cox).

Media: Minimal medium A (DAVIS and MINGIOLI 1951) was supplemented with 0.1% glucose and with required growth factors at the final concentrations of (in μg/ml): arginine, 100; cysteine, 20; histidine, 28; leucine, 80; phenylalanine, 60; tryptophan, 25; tyrosine, 40; shikimic acid, 20; thiamine HCl, 1; *para*-aminobenzoic acid, 0.1; and *para*-hydroxybenzoic acid, 0.1. L medium (LENNOX 1955) was supplemented with 0.2% glucose. Solid minimal and complete media contained 0.5% glucose and 2% Difco agar. 500 μg/ml Sm and bluensomycin (Bm) (pure base) and 200 μg/ml Spm (pure base) were used unless otherwise indicated.

Mutagenesis: a) NG mutagenesis was performed according to ADELBERG, MANDEL and CHEN (1965). Cells in late log phase were washed and treated for 30 minutes at 37° with 500 μg/ml NG in tris-maleic buffer (pH 6.0). b) For ethyl methanesulfonate (EMS) mutagenesis a 2 ml

[3] Abbreviations used include Sm, streptomycin; Bm, bluensomycin; Spm, spectinomycin; EMS, ethyl methanesulfonate; NG, nitrosoguanidine; 2-AP, 2-aminopurine; HX, hydroxylamine; OTC, ornithine transcarbamylase; CSD, conditionally Sm dependent; CBD, conditionally Bm dependent; Sm^s, Sm sensitive; and Sm^r, Sm resistant.

TABLE 1

Bacterial Strains

Name	Pertinent genotype						
	argE	*argR*	*aroE*	*spcA*	*strA*	*leu*	*his*
B40	F40	R^B	+	+	+	+	+
B40 Sm^r	F40	R^B	+	+	A40	+	+
P1	F40	R2	+	+	A1	+	+
P2	F40	R2	+	+	A2	+	+
P2-2	F40	R2	+	+	A2	2	+
6-15	F40	R15	+	+	A40	+	+
L1	F40	R11	+	+	+	+	+
LS-1	F40	R11	+	A3	+	+	+
LC-1	F40	R11	+	+	A60	+	+
LD-26	F40	R11	+	+	A5	+	+
L11	F40	R11	E24	A3	+	+	+
L11 His #1	F40	R11	E24	A3	+	+	11
L44	F40	R11	+	+	+	2	+

The genetic symbols are those recommended by TAYLOR and TROTTER (1967): *argF* is the structural gene for ornithine transcarbamylase (OTC), the sixth enzyme in the arginine biosynthetic pathway; *argR* is the regulatory gene for the arginine pathway; *aroE* is the structural gene for dehydroshikimate reductase, the fourth enzyme in the aromatic amino acid biosynthetic pathway; *spcA* and *strA* are genes for 30S ribosomal proteins which affect the cell's response to spectinomycin and streptomycin, respectively; and *leu* and *his* are genes in the leucine and histidine pathways which have not been further identified.

aliquot of an overnight culture was washed, treated with 0.02 ml (or about 0.1 M) EMS at 37° for 60 minutes, centrifuged, washed twice, and plated. c) For hydroxylamine (HX) mutagenesis 5 ml of an overnight culture was washed and resuspended in 5 ml fresh 1M NH_2OH·HCl adjusted to pH 6 with 1 N NaOH immediately before use. The sample was incubated at 37° for 20 minutes, then centrifuged, washed, and plated. d) Nitrous acid (HNO_2) mutagenesis was also performed on overnight cultures: 5 ml samples were washed and concentrated 20 times in 0.25 ml 0.1 M acetate buffer (pH 4.5), to which 0.25 ml 0.1 M $NaNO_2$ was added. The tubes were incubated at 37° for exactly 4 minutes, then diluted and plated immediately. e) 2-aminopurine (2-AP) mutagenesis was performed by inoculating 1 ml aliquots of L medium containing 600 μg/ml 2-AP with about 10^2 cells/ml. Cultures were incubated for 24 or 48 hours until growth occurred.

In order to assure that the *strA* mutants originated from independent clones, following NG, EMS, HX, and HNO_2 mutagenesis cells were plated immediately on L plates and a delayed selection for drug resistance was employed: 0.2 ml Sm (so that the final concentration was 500 μg/ml) was added underneath the plates 2.5 hours after incubation at 37°. The plates were then refrigerated overnight before reincubation to insure diffusion of the drug throughout the agar. Following 2-AP and ICR-191B mutagenesis, samples from multiple tubes were similarly plated and a delayed selection employed, and only one *strA* mutant per sample was analyzed.

Transduction: All genetic transfers were mediated by phage P1. The basic procedures of ADAMS (1959) were followed for P1 propagation and titration, and the procedure of LENNOX (1955) followed for transduction. Following transduction, as following mutagenesis, a delayed selection for drug resistance was employed.

In the intragenic recombination experiments between (*argF40 argR11 aroE+ spcA+ strA-X leu-2 his+*) donors and (*argF40 argR11 aroE24 spcA+ strA-X leu+ his-11*) recipients, Arg± Aro+ recombinants were selected on minimal histidine plates; they appeared after 6 to 10 days incubation at 37°. Initially all colonies were genetically analyzed to demonstrate they were true *strA+* recombinants; i.e., it was demonstrated that they could donate *strA+* into *strA* cells

but not *strA* into *strA*+ cells. In later experiments, all colonies with the expected Arg± Aro+ Sm^s phenotype were assumed to be *strA*+ recombinants, although the possibility that some of these recombinants were actually double mutants (*strA-X strA-Y*) cannot be eliminated, as the phenotype of such a double mutant cannot be predicted. (Colonies with other phenotypes represented less than 5% of the colonies on the minimal plates, except in crosses with the least restrictive *strA60* type allele, where the *argF40* mutation was very slightly leaky, and in crosses with the *strA5* dependent allele, where carryover of Sm permitted some growth of *argF40* cells.) Aro+ transductants were selected on minimal arginine histidine plates and then screened for cotransduction of the donor *strA* allele where possible; the cotransduction frequency was generally 50 to 55%. (Rare Aro+ Arg± Sm^s recombinants were detected among these Aro+ transductants, at the expected low frequency of approximately 1 Aro+ Sm^s/1000 Aro+ Sm^R.) His+ transductants were selected on minimal arginine phenylalanine tyrosine shikimic acid plates.

Enzyme assays: Ornithine transcarbamylase (OTC) was assayed as described by GORINI and GUNDERSEN (1961). Assays were performed on overnight cultures grown in minimal glucose medium with arginine. The OTC values, given as specific activities, are expressed in μmoles of citrulline produced per hour per optical density unit of 1.0 corresponding to about 2×10^9 cells/ml.

Chemicals: Bluensomycin sulfate and spectinomycin sulfate were donated by Dr. G. B. WHITFIELD, Jr. of the Upjohn Company. Penicillin G (potassium salt) and streptomycin sulfate were obtained from Squibb and Sons. N-methyl-N′-nitro-N-nitrosoguanidine was obtained from K & K Laboratories, ethyl methanesulfonate from Eastman Organic Chemicals, 2-aminopurine nitrate from Mann Research Laboratories.

RESULTS

Classification of strA *alleles: Spontaneous* strA *alleles:* Approximately 400 *strA* alleles, isolated at different times in this laboratory as spontaneous mutants on LSm plates, were transduced into L44, an *E. coli* B strain that carries the *argF40* amber mutation and the *leu-2* UGA mutation. The phenotypes of at least ten transductants per cross were examined, as described in the legend of Table 2, for phenotypic suppression by Sm and Bm. Bm differs structurally from Sm in only two respects: 1) one of the two guanido groups (-NHCNHNH$_2$) in the streptamine moiety of Sm is substituted by a carbamoyl group (-OCONH$_2$) in Bm and 2) the -CHO group in the streptose moiety is substituted by -CH$_2$OH in Bm (BANNISTER and ARGOUDELIS 1963). There is complete cross-resistance between Bm and Sm; however, they produce qualitatively different types of phenotypic suppression.

The spontaneous *strA* alleles tested in L44 permitted four distinctive patterns of suppression by Sm and Bm. These are described in Table 2 for the prototype *strA* allele of each class. L44 (carrying *strA*$^+$) is arginine-leaky, CSD (conditionally Sm-dependent, i.e., growth is enhanced by exogenous arginine or by Sm), CBD (conditionally Bm-dependent, similarly defined), and L44 is leucine-leaky although not leucine CSD or CBD. In L44-6 (carrying *strA60*), the *argF40* mutation is dramatically suppressed by Bm and weakly suppressed by Sm, while the leucine phenotype is leaky, CSD, and CBD. The *strA40* allele, on the other hand, permits more dramatic suppression of *argF40* by Sm than by Bm. This strain, L44-4 (carrying *strA40*), is not leucine-leaky, but it is leucine CSD and CBD. L44-2 (carrying *strA2*) is arginine negative and not CSD or CBD. It is leucine CSD, but not CBD, still another distinction between the two drugs. L44-1 (carrying *strA1*) is totally incompetent for phenotypic suppression by Sm or Bm

TABLE 2

Phenotypic suppression in L44 derivatives

Strain Name	*strA* allele	Phenotype Arginine	Leucine
L44	*strA+*	± CSD CBD	±
L44-6	*strA60*	CSD *CBD*	± CSD CBD
L44-4	*strA40*	*CSD* CBD	CSD CBD
L44-2	*strA2*	—	CSD
L44-1	*strA1*	—	—

The *strA60*, *strA40*, *strA2*, and *strA1* alleles were transduced into L44 (*argF40 argR11 leu-2 strA+*), and the phenotypes of at least 100 transductants were examined on minimal plates lacking either or both arginine and leucine and on these same plates supplemented with Sm or Bm. The phenotypes of all transductants carrying the same *strA* allele were identical in each case. In later experiments, therefore, *strA* alleles were routinely classified on the basis of 10 transductants/donor *strA* allele.

The phenotype is described by "±" = slow growth on minimal plates lacking the growth factor; "CSD" = stimulation of growth by 1 μg/ml Sm for *strA+* and 500 μg/ml Sm for *strA* strains; and "CBD" = stimulation of growth by 1 μg/ml or 500 μg/ml Bm. "*CSD*" and "*CBD*" describe particularly rapid stimulation of growth.

for either marker. On the basis of these phenotypes in L44, the *strA* alleles can be tentatively ordered with respect to increasing incompetence for phenotypic suppression as they are listed in Table 2: *strA+*, *strA60*, *strA40*, *strA2*, and *strA1*.

The same order of alleles is suggested when the amount of ornithine transcarbamylase (OTC), the *argF* gene product, is measured in strains carrying this series of *strA* alleles. Table 3 shows that a low level of OTC can be detected in the *strA+* strain and that this amount is increased by Sm or Bm just as these drugs stimulate growth on plates. OTC is suppressed more by Bm in the *strA60* strain than by Sm and slightly more by Sm than by Bm in the *strA40* strain. Table 3 also indicates that when *sulI* is introduced into these strains, *strA60*, *strA40*, *strA2*, and *strA1* restrict to an increasing degree the efficiency of genetic suppression. Sm and Bm relieve this restriction with the same characteristic pattern of interaction with the *strA* alleles as that observed in the *su⁻* strains. (Analogous

TABLE 3

Phenotypic and genetic suppression of argF40

	Ornithine transcarbamylase					
		su⁻			*sulI*	
strA allele	0	Sm	Bm	0	Sm	Bm
strA+	.06	.11	.08	6.8	6.1	. .
strA60	<.01	.03	.12	3.2	5.9	10.
strA40	<.01	.05	.04	1.1	8.8	5.2
strA2	<.01	<.01	. .	.35	1.0	.79
strA1	<.01	<.01	. .	.18	.65	.48

Ornithine transcarbamylase (OTC) levels were measured in derivatives of 6-15-4 (*argF40 argR15 strA+*), into which the *strA* alleles and *sulI* were transduced. 1 μg/ml Sm or Bm was added to *strA+* cultures and 500 μg/ml Sm or Bm to *strA* cultures.

The OTC levels in the comparable *argF+* strains are about 200 units.

studies with the β-galactosidase *lacZy14* mutation support these conclusions (STRIGINI and GORINI 1970). When screened by either criterion, incompetence for phenotypic suppression of *argF40* or restriction of genetic suppression of *argF40*, every spontaneous *strA* allele examined fell into one of the four classes described.

Classification of strA *alleles: Mutagenically induced* strA *alleles:* In a second approach to the isolation of new alleles, *strA* mutations were mutagenically induced in L44 by EMS, 2-AP, HX, and HNO_2. Several thousand resistant mutants were purified, and their arginine and leucine phenotypes examined. (Dependent mutants were induced 10 to 100 times more frequently than resistant mutants). Those *strA* mutants which did not grow on minimal arginine leucine medium and which presumably had additional growth requirements were not further studied. Those *strA* alleles apparently permitting unusual patterns of phenotypic suppression were backcrossed, i.e., reintroduced by transduction, into L44, and the phenotypes of the *strA* transductants similarly examined. All novel patterns of suppression proved upon backcross to be due to mutations outside the *strA* locus. The net result of this approach was, therefore, that every single *strA* resistant mutation induced by EMS, 2-AP, HX, or HNO_2 was identical in phenotype, even in the most subtle respects, to *strA1*, *strA2*, *strA40*, or *strA60*. Thus only four classes of *strA* alleles are distinguishable in L44.

The frequency and distribution of the classes of induced *strA* alleles were markedly different from that of the spontaneous alleles, as illustrated in Table 4, which presents the results of one typical experiment with each mutagen. EMS and 2-AP were both effective in inducing Sm resistant mutations, HX was less effective, while HNO_2 induced mutations only slightly above the background level. While most spontaneous resistant mutants are of the more incompetent or restrictive classes, most or all of the induced mutants were competent for phenotypic suppression regardless of the mutagen employed.

Classification of strA *alleles:* strA *alleles induced by mutator genes.* The nature of the base changes involved in *strA* mutations became evident when the mutator

TABLE 4

Distribution and frequency of strA *mutations induced by chemical mutagens*

Class of mutation	Mutagenic agent				
	EMS %	2-AP %	HX %	HNO_2 %	Spontaneous %
strA60	54	59	37	11	4
strA40	38	29	16	11	0
strA2	2	3	17	35	38
strA1	6	9	30	43	58
Total number in experiment	123	191	266	36	160
Relative frequency (induced/spontaneous)	100×	100×	20×	2×	..

strA mutants resistant to Sm were induced by ethyl methanesulfonate (EMS), 2-aminopurine (2-AP), hydroxylamine (HX), or nitrous acid (HNO_2), as described in MATERIALS AND METHODS. Spontaneous *strA* mutants were isolated on LSm plates (frequency about 2×10^{-9}).

TABLE 5

Distribution and frequency of strA *mutations induced by mutator genes*

Class of mutation	Mutator gene *mutT1* %	Mutator gene *mutS1* %
strA60	0	74
strA40	0	24
strA2	71	1
strA1	29	1
Relative frequency (induced/spontaneous)	1000×	100×

mutT1 and *mutS1* were transduced into L44 as described in MATERIALS AND METHODS. Transductants were grown up overnight in L medium and plated on LSm plates, and the phenotypes of 100 survivors to Sm per strain were examined as in Table 2. The data above represent the average distribution found among ten independent transductants carrying each mutator gene (standard deviation ±11% in each case). Excluded from the above averages because they could not be readily classified were 2% of the *mutT1 strA* strains, which had acquired additional growth requirements, and about 1% of the *mutS1 strA* strains, which had acquired mutations that suppressed the Arg or Leu phenotypes.

genes *mutT1* and *mutS1* were introduced into L44. *mutT1* (TREFFERS, SPINELLI and BELSER 1954), which induces adenine-thymine to cytosine-guanine transversions (AT→CG) (YANOFSKY, COX and HORN 1966; COX and YANOFSKY 1967), induced *strA* mutations in L44 at frequency of about 2×10^{-6}, or approximately a thousand-fold above the spontaneous frequency. These mutants were all resistant to Sm, and all were of the more restrictive *strA2* and *strA1* types, as shown in Table 5. Thus mutants similar to *strA2* and *strA1*, the predominant ones among spontaneous mutations, can arise by transversions.

In contrast, *mutS1* (SIEGEL and BRYSON 1967) which induces adenine to guanine transitions (A→G) (Cox personal communication), induced *strA* resistant mutations at a lower frequency, about 2×10^{-7}, and also induced *strA* dependent mutations. As shown in Table 5, the resistant mutations were all of the *strA60* and *strA40* types, except for a few presumably spontaneous *strA2* and *strA1* type mutations. Thus less restrictive mutations like *strA40* and *strA60*, those induced by the chemical mutagens (Table 4), can arise by transitions.

Mapping of the strA *gene:* The fact that *argF40* is phenotypically leaky ($Arg^{\pm}$) in $strA^+$ sensitive cells but not in *strA* resistant or dependent cells provides a selection for rare wild-type $strA^+$ cells from a population of *strA* cells that carry the *argF40* mutation. Since a variety of other mutations can affect the arginine phenotype, however, the selection for $strA^+$ recombinants was further refined in these transduction experiments by the use of a double selection for arginine leakiness and for the introduction of a closely linked outside marker, such as *aroE*, which is about 40% cotransducible with *strA*. Simultaneous selection for an outside marker prevented the appearance of undesirable suppressor mutants in the recipient, since their appearance would have required two independent events, namely, mutation or transduction to $aroE^+$ and mutation (anywhere on the chromosome) to su^+. In addition, use of an outside marker enabled the $strA^+$

TABLE 6

Mutagenically induced strA *alleles*

Type	Map group	Names
strA60	Ia	E1, E3, E8, E10, E20, T2, T5, H2, N1, N3
strA40	II	E11, E12, E16, E17, E23, T7, T8, H5, H6, N6
strA2	Ib	E26, T12, T13, H10, N8
strA1	Ic	E28, T17, H13, H14, N12

All *strA* alleles were mutagenically induced in L44 as described in MATERIALS AND METHODS. The prefix "E" indicates induction by ethyl methanesulfonate, "T" by 2-aminopurine, "H" by hydroxylamine, and "N" by nitrous acid.

recombination frequency to be expressed relative to the transduction frequency of a nearby marker (such as *aroE*) as well as to that of an unlinked marker (such as *his*).

The *strA* alleles chosen for these recombination experiments included the four spontaneous alleles described in Table 2, the thirty mutagenically induced alleles listed in Table 6, and *strA5*, a mutation conferring dependence on Sm. These 35 alleles were introduced (or reintroduced) into L44 by transduction, and these strains (*argF40 argR11 aroE*$^+$ *spcA*$^+$ *strA-X leu-2 his*$^+$) served in the final crosses as donors into L11 derivatives (*argF40 argR11 aroE24 spcA*$^+$ *strA-X his-11*), into which the same *strA* alleles had been transduced.

Arg$^\pm$ Aro$^+$ recombinants, as well as Aro$^+$ and His$^+$ transductants, were selected and screened as described in MATERIALS AND METHODS. The recombination frequencies are expressed as the ratio of the frequency of Arg$^\pm$ Aro$^+$ Sm^s recombinants over the frequency of Aro$^+$ transductants carrying the donor *strA* alleles; i.e., as the number of recombinational events in the *strA* gene over the number of cells into which the donor *strA* fragment was integrated. As a control to detect any effect of the *strA* alleles on the *aroE*$^+$ transduction frequency, the frequency of Arg$^\pm$ Aro$^+$ Sm^s recombinants was also compared to the frequency of His$^+$ transductants.

strA alleles having the same phenotype were first crossed with one another. As shown in the lower half of Table 7, ten *strA* alleles incompetent for *argF40* suppression failed to recombine with either *strA-E26* (*strA2* type) or *strA-E28* (*strA1* type), or with *strA2* (not shown in the table). Moreover, these incompetent alleles also failed to recombine with an arginine CBD allele, *strA-H2*, of the *strA60* type. Similarly, ten CBD alleles did not recombine with two CBD alleles, *strA-E3* and *strA-T2*, or with two incompetent alleles, *strA-T12* and *strA-T17* (upper half of Table 7). At the same time, ten CSD alleles failed to recombine with either the spontaneous *strA40* mutation or two induced mutations, *strA-E11* and *strA-T8* (Table 8).

In this system, therefore, recombination could not be detected among *strA* alleles with the CSD phenotype (termed Group II alleles) or among alleles with three distinct phenotypes, namely, the CBD *strA60* type, the incompetent *strA2* type, and the incompetent *strA1* type (termed Group Ia, Ib, and Ic alleles,

TABLE 7

Recombination frequency among Group I strA *alleles*

Donor *strA* allele Group	Name	Recipient *strA* allele E3	T2	T12	T17
Ia	E1	<.0014	<.0017	<.0020	<.0020
	E3	<.0014	<.0019	<.0022	<.0020
	E8	<.0015	<.0018	<.0024	<.0020
	E10	<.0017	<.0024	<.0031	<.0025
	E20	<.0030	<.0040	<.0060	<.0044
	T2	<.0007	<.0009	<.0015	<.0017
	T5	<.0012	<.0014	....	....
	H2	<.0008	<.0010	<.0013	<.0011
	N1	<.0010	<.0013	<.0013	<.0011
	N3	<.0011	<.0012	<.0012	<.0012

Group	Name	E26	E28	T2
Ib	E26	<.0029	<.0029	<.0031
	T12	<.0020	<.0018	<.0017
	T13	<.0013	<.0018	<.0014
	H10	<.0013	<.0011	<.0014
	N8	<.0033	<.0024	<.0027
Ic	E28	<.013	<.014	<.011
	T17	<.0086	<.0078	<.0071
	H13	<.0001*	<.0044	<.0038
	H14	<.0040	<.0031	<.0031
	N12	<.010	<.013	<.0091

The recombination frequencies are expressed as percent and represent the ratio of the frequency of Arg$^{\pm}$ Aro^{+} Sm^{s} transductants over the frequency of Aro^{+} Sm^{r} cotransductants.

* The selective conditions were particularly sensitive in this cross.

TABLE 8

Recombination frequency among Group II strA *alleles*

Donor *strA* allele Group	Name	Recipient *strA* allele A40	E11	T8
II	E11	<.0020	<.0014	<.0022
	E12	....	<.0015	<.0019
	E16	....	<.0016	<.0018
	E17	<.0013	<.0012	<.0015
	E23	....	<.0022	<.0028
	T7	....	<.0005	<.0005
	T8	<.0014	<.0010	<.0011
	H5	<.0014	<.0012	<.0015
	H6	....	<.0012	<.0010
	N6	<.0019	<.0013	<.0014

The recombination frequencies are expressed as percent and represent the ratio of the frequency of Arg$^{\pm}$ Aro^{+} Sm^{s} transductants over the frequency of Aro^{+} Sm^{r} cotransductants.

TABLE 9

Recombination frequency between Group I and Group II strA *alleles*

Cross			Transductants Arg± Aro+ Sm^s		
	strA alleles				Recombination
Groups crossed	Donor	Recipient	Number	Frequency ($\times 10^{-8}$)	frequency
Ia × II	E3	E11	232	13.	.22
	E11	E3	78	5.6	.14
	T2	E11	412	18.	.23
	E11	T2	75	9.1	.16
	E3	T8	241	16.	.23
	T8	E3	95	12.	.14
	T2	T8	368	23.	.24
	T8	T2	114	12.	.16
Ib × II	E26	E11	256	19.	.27
	E11	E26	42	6.	.075
	H10	E11	486	37.	.23
	E11	H10	24	6.3	.067
	E26	T8	227	18.	.27
	T8	E26	80	9.1	.084
	H10	A8	458	28.	.36
	A8	H10	81	8.3	.063
Ic × II	E28	E11	75	6.6	.32
	E11	E28	9	.87	.017
	E14	E11	253	21.	.31
	E11	H14	23	3.3	.044
	E28	T8	94	7.6	.40
	T8	E28	36	4.7	.069
	H14	T8	284	28.	.32
	T8	H14	40	4.5	.048

The recombination frequencies are expressed as percent and represent the ratio of the frequency of Arg± Aro+ Sm^s transductants over the frequency of Aro+ Sm^r cotransductants.

The *strA* alleles E3 and T2 represent Group Ia (*strA60* type), E26 and H10 Group Ib (*strA2* type), E28 and H14 Group Ic (*strA1* type), and E11 and T8 represent group II (*strA40* type).

respectively). Even when the system was made ten times more sensitive (by using a higher multiplicity of infection and more plates) no recombination (<0.0001%) was detected between *strA-H13* (*strA1* type) and *strA-E26* (*strA2* type). The failure to detect *strA*+ recombinants was not due to the inability of the *strA*+ allele to be expressed in an *strA* genetic background, since in reconstruction experiments *strA*+ was successfully transduced into each of the four types of *strA* recipients by means of the same selection for the Arg± Aro+ phenotype; moreover, *strA*+ recombinants were successfully recovered from some of these *strA* strains in the second series of crosses described below. In addition it

has been shown that *strA*$^+$/*strA1*, *strA*$^+$/*strA2*, *strA*$^+$/*strA40*, and *strA*$^+$/*strA60* merodiploids are all phenotypically arginine leaky and sensitive to Sm (BRECKENRIDGE and GORINI 1969).

Recombination was then examined between representative alleles of Groups I and II. Two alleles with each of the four phenotypes were chosen at random; these eight alleles were then crossed in all possible combinations. As indicated in Table 9, both Group II alleles recombined with all Group I alleles, and moreover, alleles with the same phenotype recombined with practically identical frequencies. The *strA5* allele, which confers dependence on Sm, was then mapped relative to the resistant alleles. The data in Table 10 indicate that *strA5* defines a third site (termed Group III) in the *strA* gene. These data, however, are less certain than those in Table 9, since carryover of Sm to the selective plates made detection of recombinants more difficult. (In all cases, homologous phage controls, i.e., crosses between donor and recipient carrying the same *strA* allele, were negative (<0.003% recombination).)

The average recombination frequencies between pairs of alleles with the same phenotypes are presented in Table 11. Comparable results were obtained when the Arg$^\pm$ Aro$^+$ Sm^s frequencies were expressed relative to the transduction frequencies of an unlinked marker, *his*$^+$ (Table 11), although the absolute numbers were different, since *his*$^+$ is transduced about 30% as frequently as *aro*$^+$. In both cases, small but reproducible differences are evident between Group Ia, Ib, and Ic alleles either as donors or recipients, although these alleles are inseparable by recombination. Whether this reflects some effect of the *strA* alleles on the

TABLE 10

Recombination frequency between dependent and resistant strA *alleles*

Cross			Transductants Arg$^\pm$ Aro$^+$ Sm^s		
	strA alleles				Recombination
Groups crossed	Donor	Recipient	Number	Frequency ($\times 10^{-8}$)	frequency
Ia × III	T2	A5	18	.94	.028
	A5	T2	42	8.2	.068
Ib × III	E26	A5	36	2.8	.11
	A5	E26	131	13.	.11
Ic × III	H14	A5	55	6.2	.20
	A5	H14	74	10.	.061
II × III	E11	A5	9	.73	.029
	A5	E11	41	2.1	.016
	T8	A5	4	.30	.0072
	A5	T8	26	2.7	.024

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.

The *strA* allele T2 represents Group Ia (*strA60* type), E26 Group Ib (*strA2* type), H14 Group Ic (*strA1* type), E11 and T8 represent Group II (*strA40* type), and A5 represents Group III (*strA5* type).

TABLE 11

Average recombination frequencies between strA *alleles*

Group		Arg± Aro+ Sms / Aro+ Smr		Arg± Aro+ Sms / His+	
Donor	Recipient	Average	Combined average	Average	Combined average
Ia	II	.23		.67	
Ib	II	.28	.28	.86	.84
Ic	II	.34		1.0	
II	Ia	.15		.41	
II	Ib	.073	.089	.16	.23
II	Ic	.045		.097	
Ia	III	.028		.066	
Ib	III	.11	.11	.28	.35
Ic	III	.20		.69	
III	Ia	.068		.19	
III	Ib	.11	.08	.25	.19
III	Ic	.061		.14	
II	III	.018		.031	
III	II	.020		.041	

The average recombination frequencies for the 34 reciprocal crosses listed in Tables 9 and 10 are expressed (in percent) as the ratio of the frequency of Arg± Aro+ Sms transductants over the frequency of Aro+ Smr cotransductants or the frequency of Arg± Aro+ Sms transductants over the frequency of His+ transductants.

physiology of the donor and recipient cells or whether it reflects some effect of the base sequences of the mutated *strA* gene could not be determined in these experiments.

While the reciprocal crosses summarized in Table 11 do not permit an unequivocal ordering of the three sites, they do suggest that a) Group I lies further from *aroE* than Group II, since recombinants were three times as frequent in the Group I × Group II crosses as in the reciprocal Group II × Groups I crosses and b) that Group III lies closer to Group II than to Group I, since the Group III × Group II recombinants were less frequent than the Group III × Group I recombinants. A tentative map, consistent with these data, is presented in Figure 1, together with the map distances expressed as the percent recombination observed in the more favorable crosses. Correlations between map position and phenotype do exist, although their significance is not known at the present time. Site I includes the three classes of resistant alleles responding least well to Sm suppression. Site II consists of the resistant alleles responding best to Sm suppression, and close to site II lies a dependent mutation which also responds dramatically to Sm suppression.

DISCUSSION

In the course of this investigation, a total of four classes of *strA* alleles have been distinguished in *E. coli* B on the basis of the patterns of phenotypic sup-

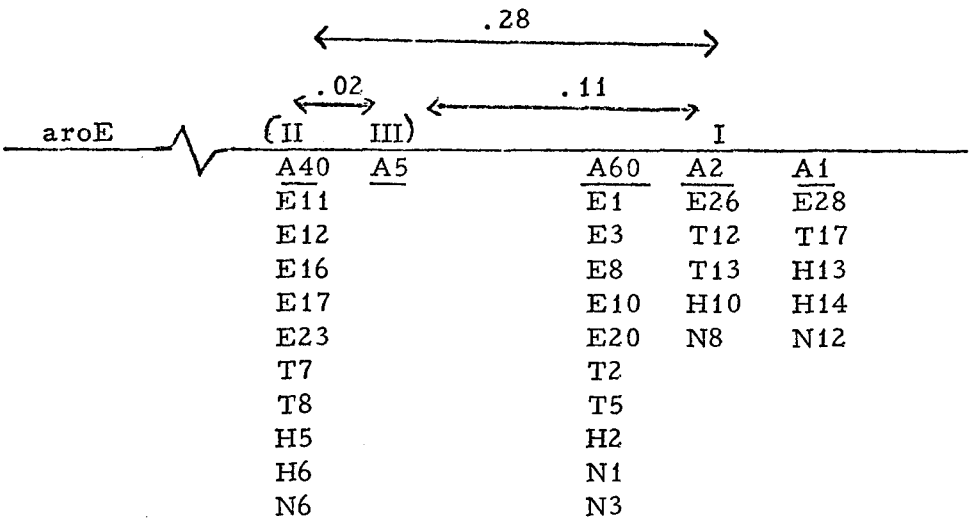

FIGURE 1.—The map distances represent the average percent recombination between each pair of groups of *strA* alleles.

The relative position of groups II and III is not yet established.

pression by Sm and Bm they permit in a strain that carries two nonsense mutations. Compared to the wild-type *strA*$^+$ allele, these four classes can be ordered with respect to increasing incompetence for phenotypic suppression as follows: *strA60*, *strA40*, *strA2*, and *strA1*. These four classes of alleles similarly restrict to an increasing degree the efficiency of genetic suppression of nonsense codons (Table 3) as well as that of missense codons (BISWAS and GORINI, unpublished; GORINI 1969). It thus appears that the *strA* mutations impose a generalized restriction on translation, possibly at the level of tRNA-ribosome binding (STRIGINI and GORINI 1970; GORINI 1969).

While only four classes of *strA*-resistant alleles are distinguishable on the basis of different levels of competence for phenotypic suppression, it might be supposed that more types of alleles could be distinguished by quantitatively screening *strA* alleles for restriction of genetic suppression of a phage or bacterial function. So far this has not proven the case. GARTNER and ORIAS (1966), for example, detected four classes of *strA* alleles in *E. coli* K on the basis of the degree with which they restricted suppression of amber and ochre T4 *rII* mutants by an ochre suppressor. These four classes closely paralleled the *E. coli* B classes both in growth rate (the growth rate decreases slightly with increasing incompetence or restriction) and in the streptomycin effect, in that this drug-stimulated suppression with all alleles but particularly with the second class (ORIAS and GARTNER 1966), the class analogous to *strA40*. OTSUJI and AONO (1968) found three classes of *strA* alleles; KUWANO, ISHIZAWA and ENDO (1969) reported five classes. In this laboratory a

search for *strA* alleles which permitted unusual patterns of suppression of phage S13 nonsense mutations similarly failed to reveal any new types of alleles (TESSMAN and WALD, unpublished). It should be pointed out that in no case have the differently restricted *strA* alleles been selected directly for this phenotype. It is thus conceivable that more types of restricted (or even de-restricted) *strA* alleles would be discovered if the search were not limited to the class of *strA* mutants which are resistant to killing by streptomycin, as it currently is.

The mutagenic data indicate that all four classes of resistant mutations are point mutations: the *strA60*- and *strA40*-type mutations appear to originate in transitions since they are induced by *mutS1* as well as by several chemical mutagens, and *strA2*- and *strA1*-type mutations appear to originate in transversions, since they are induced by *mutT1*. *strA2* has been successfully reverted to $strA^+$ (frequency about 10^{-9}), strengthening the idea that it is a point mutation: $strA^+$ revertants have been selected from L44-2 (*argF40 leu-2 strA2*) as simultaneously Arg and Leu "leaky" revertants which are streptomycin sensitive and able to transduce sensitivity but not resistance into appropriate recipients.

All four classes of resistant alleles map at one of only two sites in the *strA* gene: all the CSD *strA40*-type alleles map in one site (termed II), and all the CBD *strA60*-type alleles and incompetent *strA2*- and *strA1*-type alleles map in a second site (termed I). Thus alleles with three distinct phenotypes map at one site; i.e., they are not separable by recombination at the level of 0.002%.

How precisely can these "sites" be defined? If one could estimate the recombinational length of the *strA* gene, one could correlate this with estimated size of the gene, based on the fact that the *strA* gene codes for a protein with a molecular weight of 19,000 or about 200 amino acids (CRAVEN, VOYNOW, HARDY and KURLAND 1969); however, the recombinational length of the gene was not provided by this work. If recombination in the *strA* gene is comparable to recombination in the tryptophan synthetase gene, *trpA*, where 0.01 to 0.02 map units correspond to one amino acid residue (YANOFSKY *et al.* 1964), then the 0.28 map units between sites I and II would correspond to a separation of 15 to 30 amino acids in the protein. Moreover, recombination frequencies of the order of 0.003% should represent recombination between adjacent nucleotides.

Thus the failure to observe recombination between *strA60*, *strA2* and *strA1*-type alleles in these experiments (generally $<0.002\%$ but $<0.0001\%$ in one case) suggests that these mutations lie at the same nucleotide site or at adjacent sites, possibly within the same codon. Alternatively, these mutations may occur at three distinct sites around which recombinational events are inhibited for some reason, analogous to the situation reported by ROTHEIM and RAVIN (1968) in Pneumococcus. If *mutT1* induces AT to CG transversions exclusively, then our mutagenic data imply that *strA2* and *strA1*, both induced by *mutT1*, are not mutations in the same nucleotide base pair. Thus the true distance between the *strA* mutations that are inseparable by recombination remains undetermined by this investigation.

The one dependent mutation studied here, *strA5*, a spontaneous mutation, maps close to the resistant alleles and defines a third site in the *strA* gene. The

low frequency of recombination between dependent and resistant mutations is compatible with the idea that they are alleles of one cistron. Additional evidence for allelism comes from complementation tests in merodiploids in which the *strA5* allele failed to complement with any of four resistant alleles to give the wild-type phenotype: in each case the dependent phenotype was dominant over the resistant phenotype (BRECKENRIDGE and GORINI 1969). In any case, biochemical evidence for allelism has recently been provided by the isolation and characterization of the *strA* protein from a dependent cell (BIRGE and KURLAND 1969). Thus the same ribosomal protein is altered in dependent and resistant mutants. Rather than considering the streptomycin-dependent mutant as a peculiarity to be treated separately, the possibility should be explored that this class comprises a type of *strA* allele severely restricted but easily responsive to the de-restrictive action of streptomycin (GORINI, ROSSET and ZIMMERMANN 1967). As a matter of fact the streptomycin-dependent mutants are usually highly competent for suppression by streptomycin.

Thus mutations in at least two sites in the *strA* gene lead to high level resistance to streptomycin; mutations at another site in the *strA* gene lead to high level dependence on the drug. Mutations at still other sites in the gene may lead to ribosomes which do not show an altered response to streptomycin or to ribosomes which are nonfunctional and therefore lethal to the cell.

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SUMMARY

Four classes of streptomycin-resistant *strA* alleles have been distinguished in *Escherichia coli* on the basis of the patterns of phenotypic suppression by streptomycin and bluenomycin they permit in a strain that carries two nonsense mutations. Several thousand mutants were examined; they arose spontaneously or were induced by various chemical mutagens and then were selected as survivors to high doses of streptomycin. Compared to the wild-type (*strA*$^+$) allele, these four classes can be ordered with respect to increasing incompetence for phenotypic suppression. All classes are point mutations; two appear to originate in transitions while two appear to originate in transversions since they are induced by specific mutators, *mutS1* and *mutT1*, respectively.—A genetic map of the *strA* gene has been constructed, taking advantage of the fact that recombination can be detected in the *strA* locus by the use of a strain carrying a nonsense mutation which is phenotypically leaky in *strA*$^+$-sensitive cells but negative in *strA*-resistant or dependent cells. Thirty-five resistant mutations map at one of two sites and one dependent allele maps at a closely linked third site. Thus mutations in at least two sites in the *strA* gene, the structural gene for a 30S ribosomal protein, lead to high level resistance to streptomycin, while mutations in a third site lead to dependence on the drug.

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