MAP POSITIONS AND SPECIFICITIES OF SUPPRESSOR MUTATIONS IN *ESCHERICHIA COLI* K-12[1,2]

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A suppressor mutation ("suppressor") can be defined as a mutation which reverses the phenotypic effects of a second ("primary") mutation at a different position in the genome. Suppressors may occur either inside or outside the locus containing the suppressed mutation; these are termed intragenic and external suppressors, respectively. Most external suppressors are capable of suppressing particular alleles of different loci. Several external suppressors have been shown to alter the primary structure of proteins by causing amino acid substitutions at the mutant site (BRODY and YANOFSKY 1963; NOTANI, ENGELHARDT, KONIGSBERG and ZINDER 1965; WEIGERT and GAREN 1965).

The units of the genetic code, presumably base-pair triplets, have been referred to as codons, each codon determining one amino acid in a polypeptide chain. Codons which code for the normal, or wild-type, amino acids are called "sense"; a mutant codon which causes the wrong amino acid to be inserted in the polypeptide during translation is called "missense"; and a mutant codon for which the translation machinery provides no corresponding amino acid is called "nonsense" (CRICK 1963). Both missense and nonsense mutations are found among primary mutations which are genetically suppressible (BRODY and YANOFSKY 1963; BENZER and CHAMPE 1962; GAREN and SIDDIQI 1962). When a missense or a nonsense mutation is suppressed, an amino acid is substituted in the polypeptide at the position affected by the primary mutation. The substituted amino acid may be the same as that found in the wild-type polypeptide or may be a different one which nevertheless restores function. It is generally believed that suppression takes place during the "translation" of the nucleotide sequence in messenger RNA into the amino acid sequence of the polypeptide, and not during the "transcription" of the nucleotide sequence of DNA into that of RNA. Translation of a missense codon may be ambiguous in a suppressed strain; i.e., the cell makes two forms of the polypeptide, the original mutant form and the form containing the new amino acid at the mutant site (BRODY and YANOFSKY 1963).

Assuming that suppression does occur at the level of translation, it is still not clear which component of the translating (protein synthesizing) system is affected by such suppressors. YANOFSKY and ST. LAWRENCE (1960) proposed that suppression may be caused by specificity alterations in transfer RNA (T-RNA)

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or amino acid activating enzymes. More recently a role of the ribosomes in determining coding specificity has become evident (GORINI and KATAJA 1964a,b; DAVIES, GILBERT and GORINI 1964), suggesting the possibility that suppressor mutations may affect the genetic code by causing alterations in ribosome structure. Further possible mechanisms of suppressor action have been considered by BRENNER and STRETTON (1964).

As a step towards elucidating the mechanisms of action of external suppressors in *Escherichia coli*, it was decided to investigate the genetic behaviour of such suppressors. The main purposes of our study were: (1) To determine the map positions of suppressor loci; (are they distributed randomly over the genetic map or do they tend to occur in clusters?) (2) To determine the number of suppressor loci for given suppressible mutations. (3) To study the allele specificity of suppressors.

Mutations of the structural loci governing the biosynthesis of isoleucine and valine, the *ilv* loci, were employed as "primary" mutations in obtaining external suppressors, i.e., suppressors were obtained as "reversions." The five *ilv* loci in *E. coli* are known to be located in a single cluster on the chromosome (PITTARD, LOUTIT and ADELBERG 1963), consisting of three operons, one of which comprises three of the five loci (RAMAKRISHNAN and ADELBERG 1964, 1965a,b). The relationship of the *ilv* loci to the isoleucine-valine biosynthetic enzymes is summarized in Figure 1.

In the following sections we describe the isolation, properties, and genetic mapping of a large number of suppressors obtained in *ilv* mutants.

MATERIALS AND METHODS

Bacterial strains: A primary list of bacterial strains which were used is provided in Table 1. They are all derivatives of *Escherichia coli* K-12. Derivatives of these strains are described in Tables 2 and 3. Chromosome markers, and Points of Origin of the Hfr strains, are shown in Figure 2. Throughout this paper, male strains will be classified according to the Points of Origin shown in Figure 2; e.g., a strain exhibiting Point of Origin O-1 during chromosome transfer will be referred to as an "O-1 male," etc.

Bacteriophages: The following were used: P1kc, λ, T6, and the phage T4 amber mutants N130 and N135. The T4 mutants were kindly provided by DR. A. GAREN.

Media and culture methods: Unless otherwise mentioned, those previously described by ADELBERG and BURNS (1960) were used.

Bacterial mating: The conditions described by ADELBERG and BURNS (1960) were employed

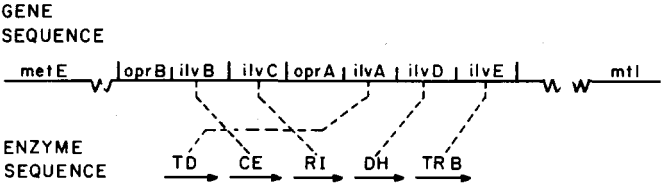

FIGURE 1.—Sequence of genes controlling the formation of enzymes in the isoleucine-valine biosynthetic pathway. TD, threonine deaminase; CE, condensing enzyme; RI, reductoisomerase; DH, dehydrase; TRB, transaminase B; *oprA*, *oprB*, operator regions; *metE* and *mtl*, see Figure 2.

TABLE 1

Primary list of bacterial strains

Strain No.	Sex*	Suppression of amber mutation N130	Genotype†												
			ilv	*met*	*arg*	*try*	*his*	*thi*	*gal*	*lac*	*mal*	*mtl*	*str*	*T6r*	other mutant alleles
AB1940	♀	?	+	*27*	+	*3*	*4*	*1*	*2*	*1* or *4*	*1*	*1*	*8* or *9*	*3*	*pro-2, ara-9*
AB1976	♀	yes	+	*E46*	+	*3*	*4*	*1*	*2*	*1* or *4*	*1*	*1*	*8* or *9*	*3*	*pro-2, ara-9*
AB2070	♀	yes	*E12*	*E46*	+	*3*	*4*	*1*	*2*	*1* or *4*	*1*	*1*	*8* or *9*	*3*	*pro-2, ara-9*
AB2527	♀	yes	*D132*	+	*G6*	+	*1*	?	*10*	*1* or *4*	+	+	+	*59*	*thy-3, purC1*
AB2531	♀	yes	*188*	+	*G6*	+	*1*	?	*10*	*1* or *4*	+	+	+	*59*	*thy-3, purC1*
AB2533	♀	yes	*188*	+	+	+	+	?	*10*	*1* or *4*	+	+	+	*59*	*thy-3, purC1*
AB2550	♀	?	*188*	*E46*	+	*3*	*4*	*1*	+	*Z13*	*1*	*1*	*8* or *9*	*3*	.....
AB2584	♀	no	+	*E46*	*F1*	+	*45*	?	+	+	+	+	*54*	+	.....
AB2596	♀	no	*188*	+	*F1*	*23*	*45*	?	+	+	+	+	*54*	+	.....
AB663	Hfr O–12	yes	+	+	*F1*	+	+	?	+	+	+	+	+	+	*xyl-4, purC1*
AB2234	Hfr O–11	yes	+	*E46*	*F1*	+	+	?	+	*1* or *4*	*1*	*2*	*8* or *9*	+	*xyl-4*
AB2529	Hfr O–13	yes	+	*E47*	+	+	+	*1*	+	*4*	*1*	+	*8*	+	*leu-6, xyl-4*
AB2545	Hfr O–1	no	+	*E46*	+	+	+	?	+	+	+	+	+	+	.....
AB2572‡	Hfr O–1	?	+	+	+	+	+	?	+	*Z13*	+	+	+	+	.....
AB2597§	Hfr O–2	yes	+	+	+	+	+	?	+	+	+	+	+	+	.....
AB2598§	Hfr O–2	yes	+	+	+	+	+	?	+	+	+	+	+	+	.....
AB2599§	Hfr O–2	yes	+	+	+	+	+	?	+	+	+	+	+	+	.....

* The symbols O–1, O–2 etc. refer to points of origin in transfer.

† Genetic symbols refer to loci concerned with the biosynthesis of isoleucine and valine (*ilv*), methionine (*met*), arginine (*arg*), histidine (*his*), tryptophan (*try*), proline (*pro*), leucine (*leu*), thiamine (*thi*), thymine (*thy*), purine (*pur*), and with the utilization of the carbohydrates arabinose (*ara*), galactose (*gal*), lactose (*lac*), maltose (*mal*), mannitol (*mtl*) and xylose (*xyl*). The symbols *str* and *T6r* denote loci which can mutate to give resistance to streptomycin and phage T6, respectively. Capital letters in locus symbols are used in agreement with TAYLOR and THOMAN (1964), with the exception of symbols for *ilv* loci which are used as indicated in Figure 1. Numbers are allele numbers allotted to mutant alleles in these laboratories. In the case of *str* and *T6r*, the mutant alleles confer resistance to streptomycin and phage T6, respectively. + = wild-type allele; ? = not tested.

‡ Strain AB2572, previously numbered 3106, was obtained from DR. N. SCHWARTZ.

§ Strains AB2597, AB2598, and AB2599, previously numbered H12R8a, S26R1E, and S26R1D, respectively, were obtained from DR. A. GAREN.

TABLE 2

Suppressible ilv *mutations in strains derived from AB663 (male) and AB2070 (female)*

ilv mutation*	Mutagen used for induction	AB663-derived strain carrying the *ilv* mutation†	AB2070-derived strain carrying the *ilv* mutation‡
ilvD75	UV	AB684	AB2257
ilvD87	UV	AB1952	AB2278
ilvD88	UV	AB1953	AB2279
ilvD105	EMS	AB2028	AB2071
ilvD130	UV	AB2077	AB2220
ilvD132	UV	AB2081	AB2221
ilvD138	UV	AB2088	AB2222
ilvD139	UV	AB2089	(not constructed)
ilvD144	UV	AB2094	AB2258
ilvD145	EMS	AB2096	AB2277
ilvD185	EMS	AB2267	AB2290
ilv–188	EMS	AB2270	AB2291

* Locus assignments based on enzyme assays described in RESULTS.
† Strains listed in this column are induced *ilv* mutants of AB663; they are isogenic with AB663 except for *ilv* alleles.
‡ Strains obtained by introducing *ilv* mutations from AB663 mutants into AB2070 by co-transduction with *ilvE*+, as described in MATERIALS AND METHODS. These strains are isogenic with AB2070 except for *ilv* alleles.

with the following modifications and exceptions. In all interrupted-mating experiments separation of mating couples (referred to as "blending") was effected by subjecting samples (1 ml) of the mating mixture to agitation on a Vortex Junior mixer for 1 min. In interrupted mating experiments with O-11, O-12, and O-13 males, the male cells were killed by bacteriophage T6, to which the female cells were resistant; 0.1 ml of a phage-T6 lysate (containing approximately 10^{11} T6 particles per ml) was added to each sample of mating mixture immediately prior to blending. In interrupted-mating experiments with O-1 male strains the mating mixture was, after 5 min of mating, diluted 1:500 in minimal medium (DEHAAN and GROSS 1962). The diluted cells

TABLE 3

A partial list of strains carrying suppressors of ilv *mutations**

Strain No.	Sex	Genotype		
		ilv	*sup*†	other genetic markers
AB2273	Hfr O–12	*145*	*H11*	same as in AB663
AB2275	Hfr O–12	*188*	*O1*	same as in AB663
AB2285	Hfr O–12	*88*	*H12*	same as in AB663
AB2300	Hfr O–12	*188*	*L2*	same as in AB663
AB2536	Hfr O–11	*145*	*H11*	*metE*+; other markers same as in AB2234
AB2542	Hfr O–12	*188*	*L2*	(λ+); other markers as in AB2300
AB2544	♀	*188*	*L2*/+‡	*gal*+/*gal-10*‡; other markers as in AB2527
AB2546	Hfr O–12	*188*	*M20*	same as in AB663
AB2547	Hfr O–12	*188*	*N23*	same as in AB663
AB2551	Hfr O–13	*132*	*T10*	*metE*+; other markers same as in AB2529
AB2561	Hfr O–13	*188*	*N23*	*metE*+; other markers same as in AB2529
AB2562	Hfr O–1	*188*	*O1*	*metE*+; other markers same as in AB2545

* Symbols explained in Table 1 and in section on Nomenclature concluding MATERIALS AND METHODS.
† Refers to suppressors of *ilv* mutations only. Capital letters are locus designations.
‡ Former allele present on a λ prophage, the latter on the chromosome (λ *gal*+ *supL2*/*gal-10 supL*+).

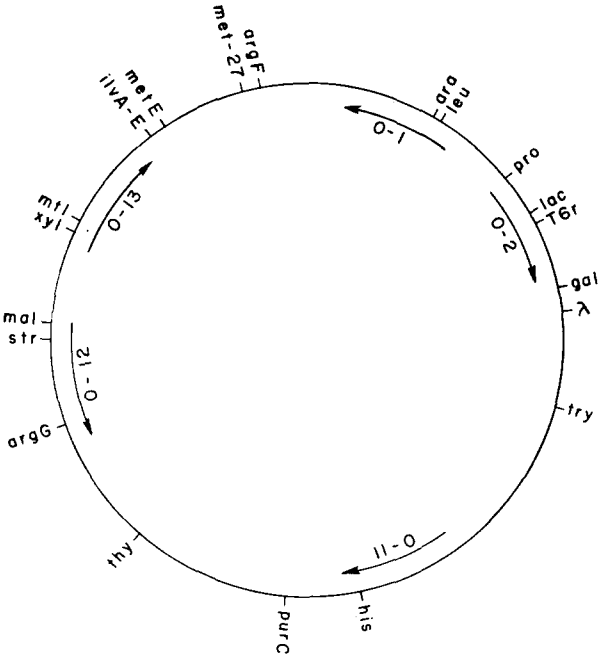

FIGURE 2.—Genetic map of *E. coli* K-12 showing the location of pertinent loci. Arrows indicate Points of Origin of the Hfr strains employed (O-1, O-2, O-11, O-12 and O-13, originally present in strains Hfr H, Hfr CAVALLI, AB311, AB312, and AB313, respectively).

were incubated at 37°C without aeration, and samples (1 ml) taken at intervals for blending; the male cells were in this case killed by plating on selective media containing streptomycin, to which the female cells were resistant.

Transduction: The methods used in experiments with bacteriophage P1kc (referred to as P1 in this paper) were essentially as described by LENNOX (1955). Transduction by bacteriophage λ was carried out using the methods described by ARBER (1958).

Scoring for unselected markers: The colonies to be tested for unselected markers in conjugation or transduction experiments, were transferred to master plates of the selective medium, and after overnight incubation were replica plated to the appropriate media. Data given on unselected marker frequencies in transduction experiments are based on at least 100 transductants scored.

Enzyme assays: Assays of enzymes of the isoleucine-valine pathway were performed as described by RAMAKRISHNAN and ADELBERG (1964).

Isolation of strains carrying ilv *mutations:* Mutations in the *ilv* loci which were to be tested for suppressibility by external suppressors were obtained in strain AB663. They were induced either by irradiation with ultraviolet light (UV), followed by penicillin selection on agar plates (ADELBERG and MYERS 1953), or by treatment with ethylmethanesulfonate (EMS) as described by STRAUSS (1962), followed by penicillin selection in liquid media. A total of 102 *ilv* mutants were obtained, 48 UV-induced and 54 EMS-induced. Twelve *ilv* mutations which were shown to be suppressible by external suppressors (see RESULTS) were, for the purpose of suppressor-mapping and suppressor-specificity studies, introduced by P1 transduction into the female strain AB2070. Strain AB2070 is deficient in transaminase B *(ilvE12)* and therefore cannot utilize α-keto-β-methylvalerate, the keto-acid precursor of isoleucine (KI). This compound can, however, be utilized by each of the 12 suppressible *ilv* mutants, which are blocked earlier in the pathway. Using AB2070 as recipient, and donor lysates prepared on the suppressible *ilv* mutants, selection was made for $ilvE^+$ transductants on a medium supplemented with KI and valine. In each case, transductants were obtained which had received the unselected mutant *ilv* allele of

the donor by co-transduction with *ilvE*+. These 12 *ilv* alleles are listed in Table 2, along with the AB663 mutant strains in which they were originally present, and the strains which were obtained by transducing these alleles into AB2070.

Co-transduction of the *ilv* loci with the *metE* locus was observed early in the course of this work and was frequently made use of in constructing strains carrying *ilv* mutations or *metE* mutations. The following observations are relevant to experiments described in this paper. Using recipients which carry *metE46* and an *ilv* mutation (strains carrying mutations in *ilvA*, *ilvD* and *ilvE* were used) and *ilv*+ *metE*+ donors, the total number of *ilv*+ transductants obtained was approximately four times that of *metE*+ transductants; about 16% of the *ilv*+ transductants carried the unselected donor marker *metE*+, whereas about 65% of the *metE*+ transductants carried the unselected donor marker *ilv*+. In case the recipient was *ilv*+ and the donor was an *ilv* mutant, the frequency of the unselected *ilv* marker of the donor among the *metE*+ transductants was approximately 50%.

Isolation of strains containing external suppressors: The external suppressors employed in this work were, unless otherwise mentioned, obtained in *ilv* mutants of strain AB663 as mutations causing "reversion" to the isoleucine-valine independent (Isoval+) phenotype. Isoval+ revertants either were obtained as spontaneous revertants or were induced by the mutagens diethylsulfate (DES) or N-methyl-N′-nitro-N-nitrosoguanidine (NTG). DES-induced and NTG-induced mutants were obtained by adding 0.025 ml of the mutagen (liquid DES or a 2 mg/ml aqueous solution of NTG) to small filter paper discs placed in the center of plates containing selective media which had been spread with about 2×10^8 cells. The cells to be spread were suspended in minimal medium supplemented with nutrient broth at a final concentration of 10%. The induced mutants form colonies which appear in a circular zone surrounding the paper disc at a distance determined by the diffusion rate of the mutagen and the concentration required for mutagenesis (IYER and SZYBALSKI 1958).

The following test, referred to as the *external suppressor test*, was employed in order to test Isoval+ revertants of AB663-derived *ilv* mutants for the presence of external suppressors. A P1 lysate of the revertant was used to transduce the *metE*+ marker into strain AB1976 which carries *ilv*+ and *metE46*. Selection was made for *metE*+ on a medium supplemented with isoleucine and valine. A sample (usually 100) of the transductants obtained was scored for the isoleucine-valine dependent (Isoval−) phenotype. In case the reversion to Isoval+ is due to an external suppressor not co-transducible with *metE*+, approximately 50% of the *metE*+ transductants are expected to be Isoval−, having received the mutant *ilv* marker of the donor by co-transduction with *metE*+. If, on the other hand, the reversion to Isoval+ is due to a mutational event inside the locus containing the original *ilv* mutation, not more than a few percent of the *metE*+ transductants are expected to be of the Isoval− phenotype. (It should be noted, however, that a failure to recover Isoval− transductants in such tests is also expected if the recipient contains a suppressor capable of suppressing the *ilv* mutation that is introduced by co-transduction with *metE*+).

Transfer of suppressors between strains containing homologous *ilv* mutations was frequently accomplished by P1 transduction; suppressor-carrying transductants were obtained directly by selection for the Isoval+ phenotype.

Strains carrying suppressors of *ilv* mutations are described in Table 3.

Testing of suppressor specificity: Suppressibility of different *ilv* mutations was tested by the following method, referred to as the *allele specificity test*. The suppressor mutation to be employed was either introduced by P1 transduction into an AB2070 derived strain (Table 2), donor and recipient carrying identical *ilv* mutations, or, in certain cases, induced in such a strain. Suppressor-carrying derivatives of AB2070 thus obtained were used as recipients in P1 transduction tests with different *ilv* mutants of AB663 as donors. Transductants were selected for *metE*+ on a medium supplemented with isoleucine and valine, and a sample of the transductants was scored for the Isoval phenotype. In case the *ilv* mutation of the donor is not suppressible by the suppressor present in the recipient, approximately 50% of the *metE*+ transductants (i.e., those containing the *ilv* allele of the donor) are expected to be Isoval−. If, on the other hand, the *ilv* mutation of the donor is suppressible by the suppressor present in the recipient, all the *metE*+

transductants are expected to be Isoval+. It should be noted that all the *ilv* mutations employed in these tests had previously been shown to be jointly transducible with *metE*+. Tests in which donor and recipient carried homologous mutant *ilv* alleles served as controls on reversion of the suppressor.

Genetic mapping of suppressors: Mapping of suppressors was carried out by P1 transduction and/or by conjugation. As a first step, a P1 lysate of the suppressor-carrying strain (an *ilv* mutant of AB663) was used to transduce the suppressor into the AB2070-derived strain carrying the same *ilv* mutation as the donor. Selection was made for recombinants carrying the mutant *sup* allele (i.e., having the Isoval+ phenotype). The selective medium was supplemented with adenine and arginine (both required by the donor) together with the growth factors required by the recipient. A sample of the *sup* transductants (usually 100) was then scored for the following donor markers: *argF1*, *purC1*, *str*+, *metE*+, *pro*+, *try*+, *his*+, *mal*+, *xyl-4*, *mtl*+, *ara*+, *lac*+, *gal*+. In case none of the markers scored for was found to be co-transducible with the suppressor, the suppressor-carrying O-12 male strain was crossed to the AB2070-derived strain carrying the homologous *ilv* allele. After 90 min of mating, selection was made for *metE*+, *pro*+, *try*+, *his*+ and *sup* (separately), and the recombinants were scored for unselected markers. Such tests usually allowed a determination of the approximate map position of the suppressor.

In order to ensure (in interrupted-mating experiments) that mapping by time of entry could be carried out for suppressors located at any point of the chromosome, *metE* mutant male strains with Points of Origin O-1, O-11, and O-13 were constructed (strains AB2545, AB2234 and AB2529, respectively). The appropriate suppressible *ilv* mutations were introduced into these strains by joint transduction with *metE*+; suppressors of these mutations were subsequently introduced by P1 transduction.

Nomenclature: Genotypic symbols are used to designate loci and/or mutational sites. They consist of three italicized letters, usually lower-case, followed by a hyphen and a serial isolation number. When it is known in which of several loci concerned with the same general phenotypic property (e.g., biosynthesis of methionine) the mutation has occurred, the hyphen is replaced by a capital letter (e.g., *metE46*). The full symbol, including the locus designation letter and the isolation number, defines a mutant allele. The wild-type allele of any locus is indicated by a plus sign in place of an isolation number (e.g., *metE*+). Minus signs are not used in genotypic symbols.

Suppressor mutation sites will be designated by the symbol *sup*, followed by a serial isolation number. When a particular suppressor locus has been defined by mapping experiments, it is indicated by a capital letter (e.g., *supT*). To be consistent with the general practice stated above, the wild-type (nonsuppressing) allele of any suppressor locus is designated by a + sign (e.g., *supT*+); mutant (suppressive) alleles are indicated only by the inclusion of an isolation number (e.g., *supT3*). (Note: some authors have used the plus sign to mean "suppressive", and a minus sign to mean "nonsuppressive." This practice is not recommended, as it confuses symbols for phenotypic activity with symbols for genetic alleles).

Phenotypic symbols are used to designate strain properties. They are never italicized, and usually consist of abbreviations that cannot be confused with locus or allele designations. Thus, Isoval+ and Isoval- symbolize, respectively, the ability and inability to synthesize isoleucine and valine.

RESULTS

Isolation of suppressed revertants of ilv *mutants:* For the purpose of obtaining external suppressors the 102 *ilv* mutations induced in strain AB663 (see MATERIALS AND METHODS) were tested for reversion by DES. Observations were made on relative growth rates of revertants, since suppressed revertants were expected to grow more slowly than wild type on media without isoleucine and valine (unless otherwise mentioned, statements concerning growth rates of suppressor-carrying strains refer to relative colony size on such media). Forty-four

of 48 UV-induced mutations and 50 of 54 EMS-induced mutations were found to be reverted by DES. Slow growing revertants were obtained in the case of approximately one third of the mutants tested; each mutant giving such revertants also gave revertants having growth rates similar to wild type, but slow growing revertants were frequently the more numerous.

Slow growing DES-induced revertants of 20 *ilv* mutants (11 UV-induced, 9 EMS-induced) were subjected to the external suppressor test described in MATERIALS AND METHODS; One to three revertants of each mutant were tested. In the case of eight of the *ilv* mutants (3 UV-induced, 5 EMS-induced) all the tests were negative, i.e., none of the *metE*$^+$ transductants tested was Isoval$^-$. However, the "slow growing character" of these mutants was in each case co-transducible with the *metE*$^+$ marker at a frequency expected for the *ilv* loci. The Isoval$^+$ phenotype of these mutants is therefore probably due either to intragenic suppressors or to mutations at the original mutant sites causing partial restoration of protein function. (These results could, however, also be accounted for by the alternative explanation described in MATERIALS AND METHODS).

External-suppressor tests with revertants of the remaining 12 *ilv* mutants (listed in Table 2) were all positive, i.e., in each case approximately 50% of the *metE*$^+$ transductants were phenotypically Isoval$^-$, and the Isoval$^+$ phenotype of these revertants could therefore be attributed to external suppressors.

The suppressors used in this study were obtained in representatives of the 12 strains whose *ilv* mutations had thus been shown to be suppressible by external suppressors, or in strains into which these mutations had been introduced by transduction. Unless otherwise mentioned, they were induced with DES.

Enzyme deficiencies caused by suppressible ilv *mutations:* Assays of the dehydrase enzyme of the isoleucine-valine pathway were carried out by T. RAMAKRISHNAN on extracts of the 12 suppressible *ilv* mutants of AB663 (Table 2). These strains were all found to be deficient in dehydrase. Other enzymes of the isoleucine-valine pathway were not assayed in these strains, which were tentatively classified as *ilvD*. Further assays for enzymes of the isoleucine-valine pathway have, however, been carried out on extracts of strain AB2291 which contains *ilv-188* (Table 2). The results, which are preliminary, suggest that this strain is deficient in both dehydrase and threonine deaminase, but contains normal levels of both transaminase and condensing enzyme; (reducto-isomerase was not assayed). It will be recalled that the structural genes for threonine deaminase and dehydrase are located next to each other in an operon which also includes the structural gene for transaminase in the operator-distal position (Figure 1). The dual deficiency caused by *ilv-188* suggests that it may be a polarity mutation (cf. AMES and HARTMAN 1963), but in that case the production of wild-type levels of the operator-distal enzyme, transaminase, is unexpected. This is being investigated further.

Specificity of suppressors: Several suppressors were subjected to allele specificity tests with the results summarized in Table 4. In each case an unambiguous result was obtained, i.e., either approximately 50% of the *metE*$^+$ transductants were Isoval$^-$ or all of them were Isoval$^+$. The suppressors employed could thus

TABLE 4

Results of allele specificity tests with suppressors of ilv *mutations*

Specificity group	Suppressor	Mutations tested for suppression*													Suppressor locus
		ilvD105	*ilvD130*	*ilvD132*	*ilvD144*	*ilvD185*	*ilv-188*	*ilvD145*	*ilvD88*	*ilvD138*	*ilvD139*	*ilvD75*	*ilvD87*	*try-3*	*his-4*	
I	*sup-3*	⊕†	+	+	+	+	—	—	—	—	—	—	—	—	—	*supT*
	sup-4	+	⊕	+	+	+	..	..	..	..	..	..	..	—	—	*supT*
	sup-5	+	+	⊕	+	+	..	..	..	..	..	..	..	—	—	*supT*
	sup-6	+	+	+	⊕	+	..	..	..	..	..	..	..	—	—	*supT*
	sup-7	+	+	+	+	⊕	..	..	..	..	..	..	..	—	—	*supT*
II	*sup-1*	—	—	—	—	—	⊕	—	—	—	—	—	—	+	+	*supO*
	sup-2	—	—	—	—	—	⊕	—	—	—	—	—	—	+	+	*supL*
	sup-20	—	—	—	—	—	⊕	—	—	—	—	—	—	+	+	*supM*
	sup-23	—	—	—	—	—	⊕	—	—	—	—	—	—	+	+	*supN*
III	*sup-11*	—	—	—	—	—	—	⊕	+	—	—	—	—	—	—	*supH*
IV	*sup-9*	—	—	—	—	—	—	—	—	⊕	+	—	—	—	—	not mapped
V	*sup-13*	..	..	..	..	..	..	..	..	..	..	⊕	..	—	—	not mapped
	sup-14	..	..	..	..	..	..	..	..	..	..	..	⊕	..	..	not mapped

* Tests were carried out as described in text. + = suppression; — = no suppression; dots = not tested.
†⊕ indicates the *ilv* mutation of the strain in which the suppressor was obtained.

be divided into five specificity groups (Table 4), with the following characteristics:

Group I: Five of the 12 *ilv* mutations were suppressed. These five were shown by recombination tests to represent at least four different mutational sites. No Isoval⁻ revertants of strains carrying Group I suppressors were observed.

Group II: Only one *ilv* mutation, *ilv-188*, was suppressed, but Group II suppressors also suppress *try-3* and *his-4* which are present in strain AB2070 and its derivatives. Suppression of the *his-4* mutation by *sup-1* and *sup-20* was heat-sensitive: derivatives of AB2291 carrying either of these suppressors grew without histidine supplementation at 30°C or at 37°C, but not at 42°C. Suppression of *try-3* and *ilv-188* by the same suppressors was, however, effective at all three temperatures. The *his-4* mutation was suppressed at 42°C by *sup-2* and *sup-23*.

Suppressors of Group II could be divided into three subgroups or types on the basis of the heat-sensitivity of the *his-4* suppression, and of differences between suppressed strains with respect to growth rates on media with or without isoleucine and valine (Table 5). Among 200 Isoval⁺ revertants of strain AB2291 induced by DES at 37°C, 126 were inferred to carry suppressors of Type 1, and 24 were inferred to carry suppressors of Type 2. (Of the remaining revertants, all of which were tryptophan and histidine dependent, 48 had growth rates similar to wild type, but two were slow growers. In representatives of these two classes of revertants, the Isoval⁺ phenotype was shown to be due to mutations located inside the cluster of *ilv* loci, possibly at the *ilv-188* site.) Three suppres-

TABLE 5

A classification of suppressors of Group II

Suppressor type	Suppressor	Division time of suppressed strains in medium containing isol-val*	Growth rates of suppressed strains on isol-val free media†	Suppression of *his-4* at 42°C	Frequency of Isoval⁻ mutants of suppressed strains
1	*sup-2, sup-23*	60 min (AB2300, AB2547)	+ +	yes	not observed
2	*sup-1*	80 min (AB2275)	+ + +	no	relatively high‡
3	*sup-20*	120 min (AB2546)	+	no	low

* Strain numbers in parentheses refer to strains whose division times were measured. Cultures were grown with aeration in isol-val supplemented minimal medium at 37°C. Division time of strain AB663 in the same medium was about 60 min; isol-val = isoleucine valine.

† Relative colony size on minimal agar plates at 37°C; wild type: + + + + .

‡ Overnight broth cultures started from large inocula ($1–5 \times 10^7$ cells/ml) of cells grown on media free of isoleucine and valine, usually contained 1 to 5% Isoval⁻ colonies.

sors of Type 3 were obtained among 120 slow-growing Isoval⁺ revertants induced at 30°C in strain AB2291. Suppressors of Types 1 and 2 were also obtained at 37°C as a result of spontaneous mutations.

Group III: sup-11 suppressed *ilvD145* and *ilvD88*. Strains carrying *sup-11* revert to the Isoval⁻ phenotype at relatively high frequency.

Group IV: sup-9 suppressed *ilvD138* and *ilvD139*. Isoval⁻ revertants were obtained at a relatively high frequency in strains carrying *sup-9*.

Group V: sup-13 and *sup-14* which suppress *ilvD75* and *ilvD87*, respectively, were not employed in allele specificity tests, but they are known to differ in specificity from Groups I to IV, none of which suppresses *ilvD75* or *ilvD87*. It is not known, however, whether *sup-13* and *sup-14* have identical allele specificities. Strains carrying *sup-13* revert to the Isoval⁻ phenotype at a relatively high frequency.

Mapping of suppressors of Group I: Approximate mapping by conjugation indicated that the suppressors of Group I which are listed in Table 4 are transferred later than *his* by O-12 males. An *ilv* mutation which is suppressible by Group I suppressors, *ilvD132*, was therefore transduced into the O-13 male strain AB2529. A suppressor mutation, *sup-10*, was then induced by DES; (this suppressor was later, by allele specificity tests, shown to have the specificity characteristics of Group I suppressors). The strain thus obtained, AB2551, was used as donor in interrupted mating experiments with the F⁻ strain AB2527, which carried *ilvD132* and also *argG6* and *thy-3*. Selection was made for the donor markers *argG*⁺, *thy*⁺ and *sup-10*. In order to prevent growth of donor cells that might have survived the treatment with bacteriophage T6, leucine, which is required by the donor, was omitted from the selective media. The results of a typical experiment are shown in Figure 3; it is seen that *sup-10* entered the zygotes approximately 10 min after *argG*⁺ and about 1 min before *thy*⁺.

The very close linkage of *sup-10* and *thy*⁺ in interrupted-mating experiments suggested that the two markers might be jointly transducible by phage P1. Transduction was carried out using strain AB2527 as recipient and strain AB2551 as donor. *thy*⁺ and *sup-10* were selected both separately and jointly. Co-transduction

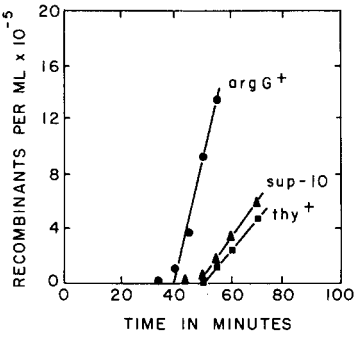

FIGURE 3.—Kinetics of recombinant formation for the *argG*+, *sup-10* and *thy*+ markers in an interrupted-mating experiment. Donor AB2551; recipient AB2527.

of *thy*+ and *sup-10* was demonstrated. The *thy*+ transductants obtained in experiments of this type were consistently about half as frequent as the *sup-10* transductants obtained in the same experiments. This difference was reflected in the frequency of *sup-10* and *thy*+ as unselected markers among *thy*+ and *sup-10* transductants, respectively: 49% of 258 *thy*+ transductants carried the unselected *sup-10* marker, while only 27% of 250 *sup-10* transductants carried the unselected *thy*+ marker.

The five suppressors of Group I which are included in Table 4 were also tested for co-transduction with *thy*. Strain AB2527 was used as recipient in transduction experiments with five *thy*+ donors carrying the *sup-3*, *sup-4*, *sup-5*, *sup-6* and *sup-7* alleles respectively. Selection was made for *thy*+ transductants, of which from 100 to 400 were scored for the presence of the unselected suppressor allele of the donor. The five suppressors were co-transduced with *thy*+ at frequencies of 40 to 53%, comparable to the frequency previously found for *sup-10*. These results suggest that the six Group I suppressors which were employed in these experiments all represent alleles of the same locus, to be designated *supT*. (The presence of a cluster of suppressor loci closely linked to *thy* has, however, not been excluded.)

In an attempt to determine whether any other suppressor locus exists for a given mutation that is suppressible by Group I suppressors, 19 additional slow-growing Isoval+ revertants of an *ilvD132*-carrying strain, AB2081, were obtained (five spontaneous, nine induced by NTG, five induced by DES). These revertants were tested for the presence of suppressors co-transducible with *thy*. The tests were carried out as already described for Group I suppressors *sup-3–sup-7*. All 19 revertants carried suppressors of *ilvD132* that are co-transducible with *thy* at frequencies typical of Group I suppressors. These results, together with the observation that slow growing Isoval+ revertants of the AB2081 strain are very homogeneous with respect to growth rates on media not supplemented with isoleucine and valine, suggest that suppressors of *ilvD132* (and, by inference, of other *ilv* mutations which are suppressible by Group I suppressors) all represent the same suppressor locus, *supT*.

Mapping of suppressors of Group II, Type 1: The loci governing the synthesis of the enzymes of galactose utilization, the *gal* loci, are clustered together in one region of the chromosome. *sup-2*, a Type 1 suppressor, is co-transducible with *gal* by phage P1. Strain AB2300 which carries the *ilv-188*, *sup-2* and *gal*$^+$ alleles was used as donor in transduction experiments with recipient strains AB2291 and AB2531 both of which carry the *ilv-188* mutation and mutant *gal* alleles. Selection was made for suppressed (Isoval$^+$) transductants, a sample of which (about 200 in the case of each recipient) were scored for the unselected *gal*$^+$ allele of the donor. With both recipients, about 64% of the *sup* transductants scored were *gal*$^+$, having received the unselected *gal*$^+$ allele by co-transduction with *sup-2*.

The close linkage of *sup-2* with *gal*, demonstrated in the P1 transduction experiments, suggested that *sup-2* might be transducible by phage λ. In order to test this possibility, strain AB2300 was made lysogenic for λ, and the resulting strain, AB2542, was used as donor in λ-transduction experiments with AB2531 as recipient. Separate selections were made for transductants having the Gal$^+$ and Isoval$^+$ phenotypes. The results of such an experiment are shown in Table 6. It is seen that Gal$^+$ and Isoval$^+$ colonies were obtained in a ratio of approximately 10:1. However, the number of Isoval$^+$ revertants obtained on control plates indicates that the selection for *sup-2* transductants was obscured by a relatively high frequency of Isoval$^+$ revertants. Two out of 400 Gal$^+$ transductants scored had the unselected Isoval$^+$ phenotype, indicating that co-transduction by λ of *gal*$^+$ and *sup-2* occurs at a low frequency. This conclusion was confirmed by the finding that 9 of the 48 Isoval$^+$ colonies obtained after infection with λ in the same experiment carried the unselected *gal*$^+$ marker, while the 32 Isoval$^+$ colonies obtained on control plates (without λ) all carried the mutant *gal* allele of the recipient (Table 6). It is not known whether *sup-2* can be transduced by λ independently of *gal*.

In other experiments with the same donor and recipient strains (AB2542, AB2531), transductants containing both *gal*$^+$ and *sup-2* were obtained by plating λ-infected cells on media jointly selective for both donor markers.

TABLE 6

Transduction of sup-2 *and* gal *by* λ*

Selection	Phenotype	Total number of colonies	Number of transductants per λ p.f.u.	Number of colonies containing unselected marker†	
				gal$^+$	*sup-2*
gal$^+$ transductants	Gal+	475	3.8×10^{-7}	.	2(400)
sup-2 transductants	Isoval+	48	7.2×10^{-9}‡	9(48)	.....
ilv-188 revertants§	Isoval+	32		0(32)	.....

* Recipient AB2531 (*gal-10 sup*$^+$ (λ$^-$)); Donor AB2542 (*gal*$^+$ *sup-2* (λ$^+$)). Multiplicity of infection (i.e., λ plaque forming units per cell): 4 to 5. In each selection, four 0.1 ml aliquots (either of the phage/cell mixture or of the equally concentrated recipient culture without λ) were plated on the appropriate media. Gal$^+$ revertants were not obtained. All the data in this table were obtained in the same experiment.

† Number of colonies scored is shown in parentheses.

‡ i.e., *sup-2 gal*$^+$ transductants.

§ Spontaneous revertants, determined by plating recipient without exposure to transducing phage.

The 11 Gal$^+$ Isoval$^+$ transductants obtained in the experiment described above (Table 6) were all purified and tested for segregation of Gal$^-$ clones by streaking on indicator agar. All of the transductants segregated Gal$^-$ clones at a frequency considerably higher than reported for λ-*gal* heterogenotes (MORSE, LEDERBERG, and LEDERBERG 1956). One of the transductants, strain AB2544, was used for further study. Exponential-phase cultures of this strain which had been grown for 5 or 6 generations in nutrient broth (after initial growth in a minimal medium with galactose as carbon source and unsupplemented with isoleucine and valine) contained Gal$^-$ segregants at a frequency of 20 to 30%. When 200 Gal$^-$ colonies from such a culture were scored for *sup* (i.e., the Isoval phenotype), 97% were *sup*$^+$ (Isoval$^-$). Among 144 Gal$^+$ colonies from the same culture, only 7% were *sup*$^+$. All the Gal$^-$ and Isoval$^-$ segregants were still lysogenic for λ. The parallel loss of *sup-2* and *gal*$^+$ in the majority of the segregants scored indicated that the two markers are, in strain AB2544, carried on a single physical structure (i.e., the λ prophage). These data also demonstrate that the mutant *sup-2* allele is dominant over the wild-type *sup*$^+$ allele in heterozygotes.

In order to test further the nature of the linkage of the *sup-2* and *gal*$^+$ markers in strain AB2544, a high-frequency transducing lysate of this strain was prepared by UV-induction of λ, and used with strain AB2531 as recipient. The AB2544 lysate was used to infect AB2531 at low mlutiplicities (0.01) in the presence of high multiplicities (3 to 5) of a helper phage prepared on a λ-lysogenic derivative of strain AB2531. Isoval$^+$ transductants were selected with or without joint selection for *gal*. In these experiments, the frequency of transductants that had received both donor markers was approximately 10^5 to 10^6 times higher than previously obtained with lysates of strain AB2542. Out of 100 *sup-2* transductants scored, 76 contained the unselected *gal*$^+$ marker. These results confirm the previous conclusion that the two markers, *sup-2* and *gal*$^+$, are both carried on the same λ prophage particle. The suppressor locus, which was thus shown to be co-transducible with *gal* by phages P1 and λ, is designated *supL*. It is not known on which side of *gal supL* is located.

Seven additional suppressors of Group II, Type 1, were tested for co-transduction with *gal*. These were induced in strain AB2291 and introduced by P1 transduction into strain AB2270, which carries *gal*$^+$ and *ilv-188*. Selection was made for the suppressed (Isoval$^+$) phenotype, and samples of the transductants were scored for the donor *gal* allele. Co-transduction with *gal* was found for five of the seven suppressors tested. The observed co-transduction frequencies varied from 43 to 66%; it is tentatively assumed that these five suppressors all represent alleles of the *supL* locus.

Further mapping experiments were conducted with *sup-23*, one of the two Type 1 suppressors which was not co-transducible with *gal*. In conjugation experiments *sup-23* was transferred later than *his* by an O-12 male. An O-13 male, AB2561, carrying *ilv-188* and *sup-23*, was used in interrupted-mating experiments with F$^-$ strain AB2533, which caries *ilv-188*, *thy-3* and *purC1*. Selection was made for the donor markers *thy*$^+$, *purC*$^+$ and *sup-23* (Isoval$^+$). In order to select against donor cells which might have survived treatment with bacteriophage

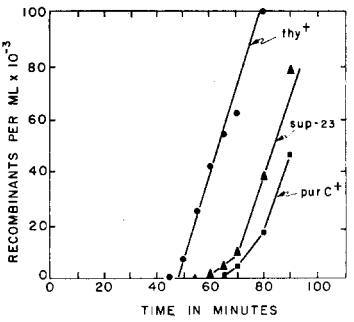

FIGURE 4.—Kinetics of recombinant formation for the *thy*+, *sup-23* and *purC*+ markers in an interrupted-mating experiment. Donor AB2561; recipient AB2533.

T6, leucine, which is required for growth by the donor, was omitted from the selective media. The results of a typical experiment are shown in Figure 4. It is seen that *sup-23* entered the zygotes approximately 10 min later than *thy*+ and approximately 5 min earlier than *purC*+. The suppressor locus which is represented by *sup-23* is designated *supN*.

Mapping of suppressors of Group II, Type 2: The approximate map position of *sup-1* was determined in conjugation experiments using as donor the O-12 male strain AB2275, which carries *sup-1*; the recipient strain was AB2291. Such experiments were complicated by the suppression of *his-4* and *try-3* of the recipient strain by *sup-1* (Table 4). The results, however, indicated that *sup-1* is located near *try*. AB2562, an O-1 male strain carrying *ilv-188* and *sup-1*, was used in interrupted-mating experiments with F⁻ strain AB2596; the latter carries *ilv-188* as well as a *try* mutation (*try-23*) which is not suppressible by *sup-1*. Selection was made for the donor markers *try*+ and *sup-1* (Isoval+). The donor strain was killed by streptomycin present in the selective media. In these experiments the times of entry of the two markers were not significantly different, suggesting very close linkage.

Co-transduction by P1 of *sup-1* with *try* was therefore tested using AB2275 as donor, and AB2596 as recipient. Isoval+ (*sup-1*) transductants were selected and scored for the unselected *try*+ marker of the donor. Among 200 Isoval+ transductants scored, 36% were *try*+.

The suppressor locus which is represented by *sup-1* is designated *supO*. Nine additional suppressors of Group II, Type 2 were mapped. All were found to be co-transducible with *try* at frequencies comparable to that found for *supO1*, and probably all represent the *supO* locus.

Mapping of suppressors of Group II, Type 3: Two suppressors of Group II, Type 3, were mapped: *sup-18* and *sup-20*. Both were obtained in strain AB2291 and introduced into strain AB2270 by P1 transduction. P1 lysates were prepared on transductants carrying *sup-18* and *sup-20*, respectively, and used in co-transduction tests with AB2291 as recipient. Scoring of *sup-18* and *sup-20* transductants for unselected donor markers showed co-transduction of both suppressors

with *argF1*. Among 200 *sup-18* transductants, 45% contained *argF1;* among the same number of *sup-20* transductants, 54% contained *argF1*.

Further information about the map position of *sup-20* was obtained in another P1 transduction experiment. Strain AB2546 which carries *sup-20* and *argF1* was used as donor; AB1940 which carries *met-27* was used as recipient. *met-27* is jointly transducible with *argF* (J. PITTARD, personal communication). Selection was made for the donor markers *met*$^+$ and *sup-20*, both separately and jointly. Strain AB1940 is *ilv*$^+$, but carries *try-3* and *his-4*, both of which are suppressible by *sup-20* (Table 4). Selection for *sup-20* was therefore carried out on media unsupplemented with tryptophan and histidine. All selections were carried out on media containing arginine. Transductants of all selections were scored for the unselected marker *argF1*. In the case of *met*$^+$ and *sup-20* transductants, the unselected *sup-20* and *met*$^+$ markers, respectively, were also scored. The results (Table 7) show that the three donor markers, *met*$^+$, *argF1* and *sup-20*, are all co-transducible; it can be concluded that their order on the chromosome is that given above.

It is assumed that the *sup-18* and *sup-20* mutations have both occurred in the same suppressor locus, which is designated *supM*.

Mapping of suppressors of Group III: The *sup-11* mutation of Group III, which is present in strain AB2273, is co-transducible at a low frequency with *his*. AB2277, which carries *his-4*, was used as recipient; both donor and recipient contain *ilvD145*. Selection was made for the donor markers *his*$^+$ and *sup-11*. Scorings for the unselected donor markers *sup-11* and *his*$^+$, among the *his*$^+$ and *sup-11* transductants, respectively, gave co-transduction frequencies of 4 to 6%.

It will be recalled that *ilvD88* is suppressed by *sup-11* (Table 4). Suppressors of Group III would therefore be expected to be obtainable in strains carrying *ilvD88*. Such a suppressor, *sup-12*, present in strain AB2285, proved to be co-transducible with *his* at a frequency similar to that of *sup-11*. *sup-11* and *sup-12* presumably represent the same locus, which is designated *supH*.

In order to determine more accurately the map position of *sup-11*, an *O-11* male strain, AB2536, carrying this suppressor, was used as donor in interrupted-mating experiments with strain AB2277 as recipient. Selection was made for the

TABLE 7

Map position of sup-20 *determined by P1 transduction**

Selected marker†	Number of transductants per ml	Number of transductants scored for unselected markers	Percent transductants containing unselected marker		
			met$^+$	*argF1*	*sup-20*
met$^+$	7.8×10^3	400	. . .	30.5	18.5‡
sup-20	7.0×10^3	400	12.0	37.2	. . .
met$^+$ *sup-20*	2.0×10^3	280	. . .	96.8	. . .

* Recipient: AB1940 (*met-27*, *argF*$^+$, *sup*$^+$). Donor: AB2546 (*met*$^+$ *argF1 sup-20*). The data given in this table are all obtained in the same experiment.
† *sup-20* was selected for on media lacking histidine and tryptophan.
‡ Sixty-six of 70 transductants of this class carried *argF1*.

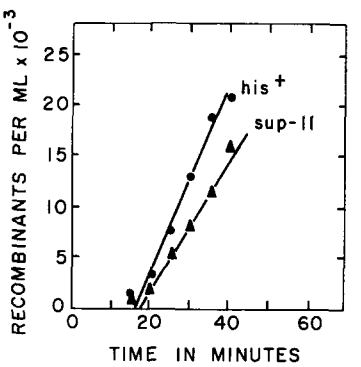

FIGURE 5.—Kinetics of recombinant formation for the *his*+ and *sup-11* markers in an interrupted-mating experiment. Donor AB2536; recipient AB2277.

donor markers *his*+ and *sup-11*. Arginine, which is required for growth by the donor strain, was omitted from the selective media in order to prevent growth of donor cells which might have survived treatment with bacteriophage T6. In such experiments, *sup-11* entered the zygotes approximately 1 minute later than *his*+ (Figure 5).

Mapping of suppressors of Groups IV and V: Approximate mapping by conjugation indicated that both *sup-9* (Group IV) and *sup-13* (Group V) are transferred later than *metE*, but earlier than *pro*, by O-12 males. Both suppressors were introduced into O-1 male strains; neither suppressor was transferred early by such strains. It is therefore tentatively concluded that both suppressors are located in the region containing the *ilv* loci, between the Points of Origin O-12 and O-1, and probably closer to the latter. Presumably two loci are represented by the two suppressors.

Results of suppressor-mapping experiments: a summary: Positions of suppressor loci which have been mapped in this study are shown in Figure 6 (see also Table 4). Of the six suppressor loci mapped, four are represented by Group II, one by Group I, and one by Group III suppressors. The existence of two additional suppressor loci, represented by Groups IV and V, is inferred. Five of the suppressor loci have been mapped by co-transduction with known markers.

Suppression of a lacZ *polarity mutation (a so-called "0°" mutation) by suppressors of Group II:* The pleiotropic effect shown by *ilv-188* suggested that it is a polarity mutation comparable to the 0° mutations found in the *Z* gene of the *lac* operon (JACOB and MONOD 1961; BECKWITH 1964). Suppression by external suppressors of such a mutation, which we will refer to as *lacZ13*, has been demonstrated by BECKWITH (1963a,b) and by SCHWARTZ (1964). External suppressors of the mutation studied by BECKWITH were shown to occur at four distinct chromosomal loci whose approximate map positions were determined. These positions correspond roughly to those determined by us for the four Group II suppressor loci (Figure 6), suggesting that the same suppressor loci have been mapped in both studies. If this suggestion is correct, suppressors of Group II should

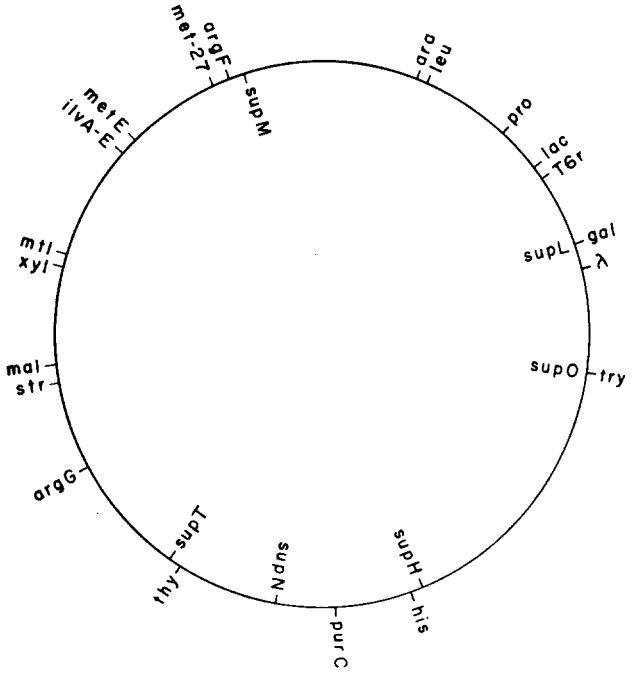

FIGURE 6.—Genetic map of *E. coli* K-12 showing positions of suppressor loci.

be capable of suppressing the *lacZ13* polarity mutation. This possibility was tested as follows: Strain AB2291 was crossed with the O-1 male strain AB2572 (*lacZ13*). Recombinants containing *pro*$^+$ and *gal*$^+$ were selected (the recipient contains *pro-2* and *gal-2*). Streptomycin was added to the selective medium to prevent growth of donor cells. Recombinants were scored for *lacZ13*. A *lacZ13* recombinant, AB2550, which retains the *ilv-188* allele of the recipient strain, was used as recipient in P1 transduction tests with four donor strains carrying *ilv-188* and, respectively, the *supL2*, *supM20*, *supN23*, and *supO1* alleles, representing each of the four Group II suppressor loci. Fifty *sup* (Isoval$^+$) transductants from each experiment were scored for the ability to grow on lactose as sole carbon source. In each experiment, all the transductants tested were capable of utilizing lactose. It is therefore concluded that *lacZ13* is suppressible by each of the four suppressors tested. These results support the inference that *ilv-188* is a polarity mutation.

The ability of *supL*, *supM*, *supN* and *supO* to suppress *lacZ13*, together with the approximate map positions reported by BECKWITH (1963) for his suppressors, suggests that the same four loci have been identified in both his studies and in ours. If so, then his su_A probably corresponds to our *supM*, his su_B to our *supL*, his su_C to our *supO*, and his su_D to our *supN*. Until these correspondences can be verified, however, it seems best to retain the separate designations.

Tests for suppression of amber mutations of phage T4 by suppressors of Group I to V: The amber mutations of phage T4 are considered to represent nonsense

mutations (BENZER and CHAMPE 1962; SARABHAI, STRETTON, BRENNER and BOLLE 1964). The growth of an amber mutant is dependent on the presence in the bacterial host of a suppressor capable of suppressing the amber mutation. Suppression of amber mutations is caused by suppressors of certain mutations in the alkaline phosphatase locus in *E. coli* which are also thought to be nonsense mutations (GAREN and SIDDIQI 1962). Suppressors of nonsense mutations are referred to as nonsense suppressors. Three nonsense-suppressor loci have recently been mapped by GAREN, GAREN and WILHELM (1965).

In preparation for testing whether suppressors of Groups I to V are capable of suppressing nonsense mutations, tests were carried out in order to determine whether nonsense suppressors were already carried by the primary strains in which the suppressible *ilv* mutations and their suppressors had been obtained. An amber mutant of phage T4, N130, was plated on many of the strains listed in Table 1. Most of these strains were found to support growth of the amber mutant, and are therefore inferred to contain nonsense suppressors. The nonsense suppressors carried by these strains have not been mapped, and it is not known whether the same suppressor (or suppressors) is present in all of them. All the suppressible *ilv* mutations used in the present work were induced in strain AB663. Mutations which are suppressible by the nonsense suppressor (or suppressors) that is present in strain AB663 could, therefore, not have been obtained. Neither could suppressors specific for such mutations be among the suppressors of Groups I to V. The possibility remained, however, that some of the latter might be nonsense suppressors whose specificity differed from that of the nonsense suppressor(s) present in strain AB663. In order to test this possibility, suppressors representing Groups I to V were introduced into strain AB2584, which does not contain nonsense suppressors. The suppressible mutations *ilvD132*, *ilv-188*, *ilvD145*, *ilvD138* and *ilvD75* were introduced into this strain by co-transduction with *metE*$^+$. Suppressors representing each of the five sup-

TABLE 8

*Suppressors of Groups I to V, present in derivatives of AB2584, tested for suppression of amber mutations of phage T4**

Suppressor group	Suppressor	*ilv* mutation present in suppressed strain	Effect of suppressor on growth of amber mutants N130 and N135†
I	*supT3*	*ilvD132*	—
II	*supL2*	*ilv–188*	(+)
II	*supM20*	*ilv–188*	+
II	*supN23*	*ilv–188*	(+)
II	*supO1*	*ilv–188*	+
III	*sup–12*	*ilvD145*	—
IV	*sup–9*	*ilvD138*	—
V	*sup–13*	*ilvD75*	—

* *ilv* mutations and suppressors were introduced into strain AB2584 as described in the text. Criterion for suppression: plaque formation in soft agar layers on L-agar.

† −=no plaques formed (no suppression); +=large plaques formed (suppression); (+)=very small plaques formed (attributed to "weak suppression")

pressor groups were subsequently introduced by P1 transduction (selecting for the Isoval$^+$ phenotype) into *metE*$^+$ transductants carrying the appropriate *ilv* mutations. The suppressor-carrying strains thus obtained were tested for ability to suppress amber mutations N130 and N135. The results of these tests are summarized in Table 8. It is seen that only the four suppressors of Group II (representing each of the four Group II suppressor loci) are capable of suppressing these amber mutations. However, two of the four Group II suppressors, *supL2* and *supN23*, showed only very weak suppression of the amber phage mutations.

Suppression of amber mutations by certain suppressors of a polarity mutation (*lacZ13*) has also been reported by BECKWITH (1963b).

Tests for suppression of ilv-188 *by nonsense suppressors:* As suppressors of *ilv-188* were found to be capable of suppressing amber mutations of phage T4, it was of interest to test whether *ilv-188* is suppressible by suppressors representing any of the three nonsense-suppressor loci whose map positions have been determined by GAREN *et al.* (1965). This was done as follows: Strains AB2597, AB2598 and AB2599, (which were kindly provided by DR. A. GAREN), each carries a nonsense suppressor representing a different locus. An *ilv* mutation was induced by NTG in each of these strains, and *metE46* was subsequently introduced by joint transduction with *ilv*$^+$. Finally, the three strains carrying *metE46* thus obtained were used as recipients in transduction experiments with a donor carrying *ilv-188* and *metE*$^+$. Selection was made for *metE*$^+$ transductants, of which 100 from each experiment were scored for the Isoval phenotype. In case *ilv-188* was suppressed by the nonsense suppressor present in the recipient, all the *metE*$^+$ transductants were expected to be phenotypically Isoval$^+$. If, on the other hand, suppression of *ilv-188* by the nonsense suppressor present in the recipient did not occur, approximately 50% of the *metE*$^+$ transductants were expected to be Isoval$^-$. The latter was found with each of the three recipients. It is therefore concluded that the three nonsense suppressors tested are not capable of suppressing *ilv-188*.

DISCUSSION

In the experiments described above, six suppressor loci were mapped, and the existence of two additional suppressor loci was inferred. Our mapping data show no evidence for clustering of suppressor loci. However, one of the nonsense-suppressor loci studied by GAREN *et al.* (1965) is, like the *supO* locus, co-transducible with *try* (D. DUGGAN and A. GAREN, personal communication). The inference that two loci are involved is based only on the difference in specificity between the *try*-linked nonsense suppressors and *supO1*.

Several suppressor loci whose map positions do not appear to coincide with any of those determined in our study have been mapped with varying degrees of accuracy by other workers (BRODY and YANOFSKY 1963; LEDERBERG, CAVALLI-SFORZA and LEDERBERG 1964; GORINI and KATAJA 1964b; GAREN 1964; SCHWARTZ 1964, 1965). On the other hand, the four suppressor loci mapped by BECKWITH (1963a,b) may be identical to our suppressor loci of Group II. The results reported by SCHWARTZ (1965) indicate that certain unstable suppressors

may be associated with episomic elements which can be attached to the chromosome at more than one alternative position.

Our results indicate that the number of suppressor loci for a given suppressible mutation may in certain cases (e.g., *ilvD132*) be only one, in other cases (*ilv-188*, *lacZ13*) at least four, and in the case of certain amber mutations of phage T4 as many as seven (assuming that the three nonsense suppressor loci described by GAREN *et al.* (1965) all are different from our Group II loci). These differences in the number of suppressors per mutation may be related to the number of amino acids whose substitution at the mutant site will restore the function of a given protein. It should be noted, however, that since many point mutations are not reverted by external suppressors, not all theoretically possible functional substitutions can be expected to be effected by such suppressors. The number of theoretically possible functional substitutions may be particularly high in the case of nonsense or polarity mutations. The *ilv* mutations suppressible by suppressors of Groups I, III, IV and V are inferred to be missense mutations, since we did not observe suppression of amber mutations of phage T4 by suppressors of these Groups.

It will be recalled that three types of Group II suppressors could be distinguished phenotypically (Table 5). The difference between suppressors of different types can be explained on the hypothesis that they cause the substitutions of different amino acids. Thus, the heat sensitivity of the suppression of *his-4* by *supO* and *supM* may be due to substitution at the *his-4* site of amino acids which make the mutant enzyme heat sensitive. Since *ilv-188* and *try-3* are suppressed by the same suppressors at 42°C, it seems less likely that the suppression mechanism itself is heat sensitive.

Ambiguous translation caused by a suppressor is expected to affect not only the mutated codon containing the suppressed mutation, but also other codons of the same type, wherever they occur in the genome. If the codon in question occurs relatively frequently, the mistakes in protein synthesis caused by the suppressor would be expected to have deleterious effects on growth, even on fully supplemented media. Growth inhibitory effects of suppressors were observed by BECKWITH (1963a) and by SCHWARTZ (1964). In our study, mutations of both the *supO* and the *supM* loci were found to have such effects (Table 5).

Assuming that a suppressor mutation acts by changing a codon in a structural gene for one of the components of translation (e.g., ribosomes, activating enzymes or t-RNA molecules), it should be dominant in heterozygotes. It is possible, however, that suppressor loci determine enzymes which secondarily modify the components of translation (e.g., by methylation). In that case, suppression could result from either the loss or the gain of enzyme activity. In the former case the suppressor mutation should be recessive; in the latter, it should be dominant. The only suppressor which was tested for dominance, *supL2*, was found to be dominant.

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SUMMARY

Twelve mutations of loci governing the biosynthesis of isoleucine and valine (*ilv* loci) were shown to be suppressible by external suppressors. The suppressors were divided into five groups on the basis of allele specificity tests. Mapping of suppressors was carried out by conjugation and/or by transduction with phage P1. Twenty-five suppressors of Group I were all co-transduced with *thy* and probably represent a single locus, *supT*. On the other hand, suppressors of Group II were mapped at four different loci, *supL*, *supM*, and *supO*, co-transducible with *gal*, *argF*, and *try*, respectively; and *supN*, which is located between *purC* and *thy*. Suppressors of Group III were mapped at a locus, *supH*, which is co-transducible with *his*. Suppressors of Groups IV and V appear to represent two additional loci.

The *supL* locus is transducible by phage λ. A mutation of this locus, *supL2*, is dominant in λ *supL2/supL*$^+$ heterogenotes.

The mutation *ilv-188*, which is suppressed by Group II suppressors, causes deficiency of two enzymes whose structures are controlled by *ilv* loci. Suppressors of Group II were found to suppress a known polarity mutation in the *lac* operon as well as certain amber ("nonsense") mutations of phage T4. *ilv-188* was, however, not suppressed by three different suppressors known to suppress nonsense mutations. Suppressors of Groups I, III, IV, and V did not suppress amber mutations.

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