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Ordering of Mutant Sites in the Isoleucine-Valine Genes of *Escherichia coli* by Use of Merogenotes Derived from F^{14}: a New Procedure for Fine-Structure Mapping[1]

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F-merogenotes derived from F^{14} by transductional shortening have previously been found to consist of the sex factor plus one or more of the *ilv* genes. It is shown here that they carry one or more *ilv* genes and a variable portion of the adjacent proximal *ilv* gene. This observation was used to develop a method, analogous to deletion mapping, for ordering mutant sites within the *ilv* genes. This method requires the use of a series of merogenotes each carrying an increasingly longer segment of the gene being mapped. A simplified method of fine-structure mapping is also described which requires only one or two F′ donor strains to map any one gene. This method is based on the large differences observed in recombination frequencies for mutant sites at various distances from the origin of the incomplete merogenote gene. The sequence of 25 mutant sites within three of the *ilv* genes was determined by use of the simplified procedure.

P1 lysates prepared on AB 1206, an F′ strain of *Escherichia coli* K-12, which is haploid for the region of the chromosome carried on the F^{14} merogenote (Fig. 1 and 2), were used by Pittard (11; Bacteriol. Proc., p. 138, 1963) to transduce the isoleucine-valine (ilv^+) merogenote genes into ilv^- recipients. A small percentage of the Ilv^+ transductants could act as donors in conjugation experiments. Most of these could transfer the *ilv*, *metB*, and *argH* genes, which had all been carried on the original F^{14} in the P1 donor strain. Pittard also found among the ilv^+ transductants some strains that transferred only the *ilv* genes in mating experiments. Among these latter, some transferred either one gene, two genes, three genes, four genes, or five genes (Fig. 3). This observation was used by Ramakrishnan and Adelberg (14) to determine the order of genes in the *ilv* cluster. They found the order shown in Fig. 4. The sex factor of the F^{14} (and merogenotes derived from F^{14} by transductional shortening) was distal to *ilvE* but is probably very close (15).

Our preliminary studies suggested that the transductionally shortened merogenotes carried one or more complete *ilv* genes plus a portion of the adjacent proximal *ilv* gene. This was confirmed by more detailed study as outlined here. The data obtained in this study led to the development of two methods for fine-structure mapping of *ilv* mutations within a gene. The order of 25 mutant sites within three *ilv* genes was determined by use of one of these methods.

MATERIALS AND METHODS

Media. Haploid F^{14} strains and all F^- strains were grown in L broth (10). Z broth was L broth with 0.25×10^{-3} M $CaCl_2$. Merogenote-containing strains were grown in a synthetic medium lacking isoleucine-valine. The synthetic medium was described by Adelberg and Burns (1). L agar and synthetic agar contained 2% agar. Z agar contained 1% agar.

Bacterial strains. The bacterial strains used are described in Table 1. The haploid F^{14} strains, AB 1206 and AB 1013, were used as the donor strains for making transductionally shortened merogenotes. AB 1013 differs from AB 1206 only in that it carries the mutation *ilvO113*, conferring resistance to 10^{-2} M valine (Val-r), concomitant with derepression of the three-gene *ilv* operon A (13).

Each shortened merogenote derived from F^{14} by transduction was originally obtained in rec^+ recipients, either strain AB 2070 or AB 1450. The shortened merogenotes were transferred by conjugation to

[1] Florida Agricultural Experiment Station Journal Series 4070.

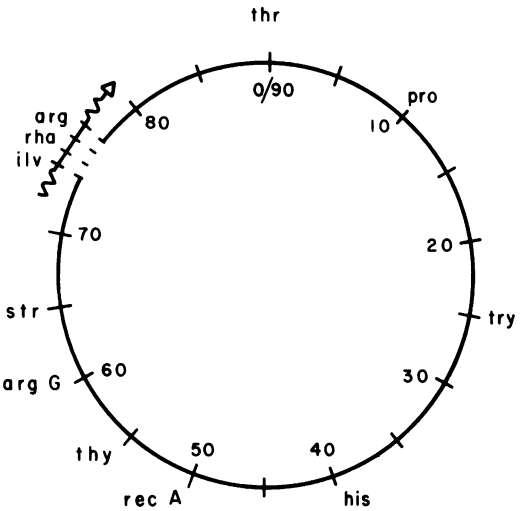

FIG. 1. *E. coli K-12 chromosome showing the segment of the chromosome carried by the merogenote in the haploid F'[14] strain AB 1206.*

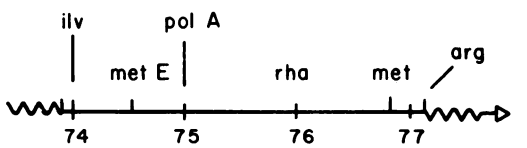

FIG. 2. *Some of the genes known to be carried on F'[14].*

the *recA* recipient strain AB 2987 (Table 1). These F' *recA* strains were then used as donors in the matings to be described.

Most of the *ilv* mutations were obtained from the collection of E. A. Adelberg, and a few were obtained from K. Kiritani (University of Tokyo). Each *ilv* mutation was transduced into a common F^- strain, AB 2200 (Table 1). These resultant strains were used as recipients in the matings to be described.

Bacteriophage. Two stocks of generalized transducing phage were used. One was a single-plaque isolate of P1kc of Lennox (10) labeled P_{38}, which was obtained from Roy Curtiss III (Oak Ridge National Laboratory). The other was the virulent strain, P1vir, of Ikeda and Tomizawa (8) obtained from Herbert Boyer. These phages will be referred to as P1 or P1vir.

Making transductionally shortened merogenotes. P1 lysates of haploid F'[14] strains (AB 1206 or AB 1013) were used in these transductions. The procedures of Lennox (10) were generally followed, and a multiplicity of infection (MOI) of about 1 was used. The recipient F^- strains carried an *ilv* mutation, and selection was for the merogenote gene *ilv*$^+$. Ilv$^+$ transductants were then tested for their ability to transfer merogenote markers by cross-streaking appropriately marked F^- strains. After characterization, the shortened merogenotes were transferred by conjugation into the *recA ilvE* strain (AB2987) with selection for Ilv$^+$.

Production of P1 lysates. Two methods were used, each for different purposes. (i) Lysates used to transfer *ilv* mutations from the primary strains into AB 2200 were prepared by infecting early-log-phase cultures grown in Z broth with P1vir (8) at an MOI of 0.01. The cultures were harvested after lysis by exposing them to 0.25 ml of chloroform for up to 5 min in ice, and then centrifuging them. The supernatant fluid was used for transduction at an MOI of 0.1 to 1. (ii) P1 lysates of haploid F'[14] cells were made by use of the agar-layer method of Swanstrom and Adams (16). Mid-log-phase cells grown in Z broth were mixed with sufficient P1 to produce about 5×10^5 plaques per plate of Z agar. After 15 min of preadsorption, the infected cells were added to soft agar, poured onto fresh wet Z agar, and incubated overnight (9 to 12 hr) at 37 C. The soft-agar layers from 5 to 10 plates were collected in a 50-ml centrifuge tube and homogenized with about 0.5 ml of chloroform by use of a Virtis tissue homogenizer at very low speed. The tissue homogenizer was equipped with special blades, obtained from The Virtis Co., Inc., Gardner, N.Y., which did not whip air bubbles into the homogenized soft agar. After incubation at room temperature for 20 min, the homogenized soft agar was centrifuged at about 5,000 $\times$ *g* for 20 min. The resulting supernatant layer (about 15 ml from 10 plates) contained high titers of P1 phage [10^{10} to 5×10^{10} plaque-forming units (PFU) per ml from P1 lysates of haploid F'[14] strains, 2×10^{11} to 5×10^{11} PFU/ml from most F^-, F^+, or Hfr strains]. These lysates could be stored at refrigerator temperatures for long periods of time without significant loss of titer. After 6 to 12 months, titers may drop about 50%.

Cross-streak test for complementation. Approximately 0.1-ml quantities of the appropriate recipient strains [grown in L broth and resuspended in 56/2 buffer (1)] were distributed in a straight line across a selective synthetic agar plate. The donor cultures were usually grown overnight in presterilized Microtiter plates containing 0.1 ml of the synthetic medium per well. The synthetic medium was designed to maintain diploid strains in the diploid state by omitting the amino acids isoleucine and valine. Sterile toothpicks were dipped into synthetic medium cultures of the donor strains to be tested and streaked across the line of recipient culture described above. The mating plates were incubated for 2 days and observed for the presence of colonies along the streak. Complementation was indicated by heavy confluent growth of large colonies. Recombination was indicated by a smaller number of isolated colonies. Cross-feeding between *ilv* mutants was recognized as light creamy or smooth growth with no evidence of large colonies at the periphery of growth. The three types of growth were easily distinguishable.

Mating procedure for ordering mutant sites. Overnight cultures of the recipient strains were transferred into fresh L broth and grown until the recipient reached a turbidity of 250 Klett units (red filter; Klett Manufacturing Inc., New York, N.Y.). Males were similarly grown in synthetic medium to 50 Klett units and mixed immediately in equal volumes with the female. The mating was terminated

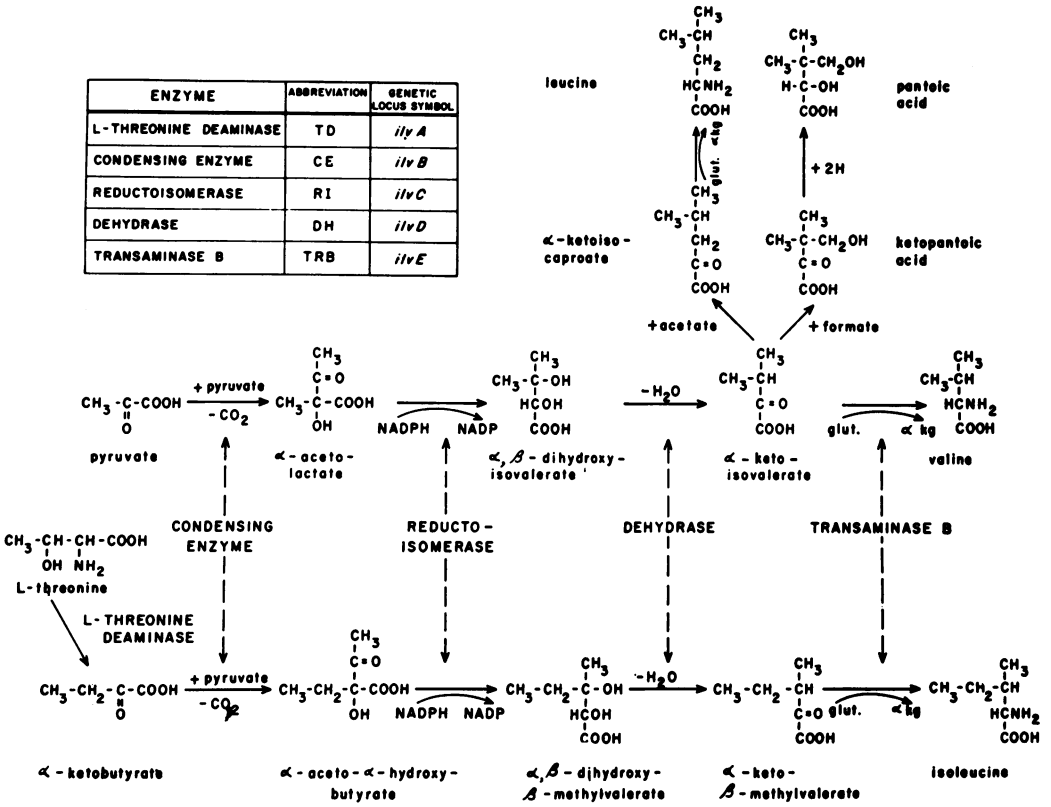

FIG. 3. *Isoleucine-valine biosynthetic pathway.*

FIG. 4. *Order of ilv genes and enzyme reaction sequence.*

by vigorous swirling on a Vortex mixer (Scientific Products, Evanston, Ill.) after 60 min at 37 C, and dilutions were plated on appropriate selective media. After 2 days of incubation at 37 C, colonies were counted and recombination frequencies were calculated on the basis of the numbers of participating donor cells.

Enzyme assays. Dehydrase and threonine deaminase were assayed by the methods described by Wechsler and Adelberg (18), except that cysteine was used rather than NH_4Cl in the threonine deaminase assays. Reductoisomerase was assayed as described by Ramakrishnan and Adelberg (13), except that phosphate buffer (*p*H 7.5, 0.02 mmole per ml) was used in place of tris(hydroxymethyl)aminomethane (Tris) buffer. (Tris buffer is inhibitory in the reductoisomerase assay.) Transaminase B was assayed by a modification (*in preparation*) of the procedure used by Ramakrishnan and Adelberg (13). The important modification in this method was in the indirect method of keto acid determination of Friedmann and Haugen (6). The hydrazone of the monocarboxy keto acid was extracted into toluene as it was being formed.

RESULTS

Characterization of shortened merogenotes. When P1 lysates prepared on a haploid F^{14} donor were used, about 4% of the Ilv^+ transductants were able to transfer, during conjugation, several of the F^{14} genes (*argH, metB, rha, metE,* and *ilv*) at a high frequency. These same transductants were not able to transfer the chromosomal genes of the transductional donor or of the transductional recipient strains at high frequency. Among the Ilv^+ transductants were found some F′ strains that would transfer only *ilv* genes. Cross-streak complementation tests showed that some transductants would form large numbers of Ilv^+ colonies only with the *ilvE12* test recipient. (The mutant genes of all test recipients had previously been characterized by enzyme assay.)

TABLE 1. *Escherichia coli K-12 strains*[a]

Strain	Sex	*ilv*	*met*	*arg*	*pro*	*trp*	*his*	*thy*	*str*	*thi*	*rec*	Remarks
AB 1450	F^-	D16	B1	H1	+	+	1	+	R	−	+	
AB 2070	F^-	E12	E46	+	+	3	4	+	R	−	+	
AB 2930	F^-	E12	+	G12	+	+	42	26	S	+	+	
AB 2987	F^-	E12	+	G12	+	+	42	+	S	+	A1	MA 1079 × AB 2930 for *thy*$^+$, *recA*
AB 2200	F^-	+	E46	+	+	3	4	+	R	−	+	
MA 1079[b]	Hfr	+	+	+	+	+	+	+	S	−	A1	From B. Low
AB 1206	F^{14}	+	+	+	2	+	4	+	R	−	+	Haploid for the F^{14} region
AB 1013	F^{14}	O113	+	+	2	+	4	+	R	−	+	Derived from AB 1206, Val-r phenotype

[a] Allele numbers are those assigned by the laboratory where the mutations were isolated.
[b] MA 1079 is also *serA* and injects in the order ←*thy-rec-his-trp-ilv*.

TABLE 1A. *F' strains constructed by conjugal transfer into AB 2987*[a]

Strain	F-merogenote[b]	Source
AB 2988	F^{310}(F *ilvEDAC1*)	This laboratory
AB 2989	F^{16} (F *ilvEDAC2*)	AB 1528 (Pittard)
AB 2990	F^{216}(F *ilvEDA1*)	AB 1526
AB 2991	F^{311}(F *ilvEDA2*)	This laboratory
AB 2992	F^{312}(F *ilvEDA3*)	This laboratory
AB 2993	F^{313}(F *ilvEDA4*)	This laboratory
AB 2994	F^{314}(F *ilvEDA5*)	This laboratory
AB 2995	F^{25} (F *ilvED1*)	AB 1545 (Pittard)
AB 2996	F^{315}(F *ilvE1*)	This laboratory
AB 2997	F^{316}(F *ilvE2*)	This laboratory

[a] These strains differ only in the F-merogenote carried. All are *ilv*$^+$/*ilvE12*. Allele numbers are those assigned by the laboratory where the mutations were isolated.
[b] The complete *ilv* genes transferred by these merogenotes are given in parentheses.

Others would form large numbers of Ilv^+ colonies only with the *ilvE* recipient and an *ilvD16* test recipient. Others transferred the *ilvD* and *ilvE* genes and formed many colonies with an *ilvA201* test recipient but not with the *ilvC* strain. Still others transferred the complete *ilvE*, *ilvD*, *ilvA*, and *ilvC* genes. None has been found to transfer the proximal gene, *argH*, of the parental F^{14}. It seems likely to us that these merogenotes may differ in length. They carry sufficient sex factor deoxyribonucleic acid (DNA) to replicate, to transfer, and to absorb male-specific phage.

To facilitate presentation and comprehension of concepts, we will refer to these merogenotes by the complete *ilv* genes which they transfer, F *ilvE*, F *ilvED*, F *ilvEDA*, and F *ilvEDAC*, rather than by their assigned merogenote numbers.

Identification of nonfunctional genes in the ilv^- recipients. The above methods roughly characterized the transductionally shortened merogenotes with respect to the number of complete *ilv* genes which they carried. By use of four selected merogenotes, one of each type (F *ilvE*, F *ilvED*, F *ilvEDA*, and F *ilvEDAC*), it is possible to characterize each of the unknown *ilv* mutants by cross-streak complementation tests. For example an *ilvE* mutant strain would be complemented and become Ilv^+ upon receiving any of the above four merogenotes. An *ilvD* would be complemented by the presence of any of the merogenotes except F *ilvE*. An *ilvC* mutant would not become Ilv^+ after mating with donors carrying F *ilvE* or F *ilvED*, but would be complemented after receiving either of the other two merogenotes. In this manner, each of 25 *ilv* mutants was classified as being altered in the *ilvE*, *ilvD*, *ilvA*, or *ilvC* genes. No *ilvB* mutants were used, as none suitable for these studies was known to us.

Ordering of mutant sites. In cross-streak complementation tests, the merogenote-recipient pairs formed large numbers of Ilv^+ diploid colonies if the merogenote complemented the *ilv* lesion in the recipient, but no colonies if the merogenote did not carry the gene corresponding to the mutated gene in the recipient. Results were obtained with some transductionally shortened merogenotes and some ilv^- recipients which did not fit either of the above patterns. In these cross-streak tests, only a few ilv^+ colonies appeared along the cross-streak after the donor had crossed the recipient. It seemed likely that these results could be explained if the merogenote carried less than the complete gene corresponding to the mutant gene in the recipient. Under these conditions, complementation could not occur, but recombination could occur between the endogenote gene and the merogenote gene fragment, if the exogenote carried the wild-type codon corresponding to the mutant codon in the endo-

TABLE 1B. *F⁻ strains constructed by cotransduction into AB 2200*[a]

Strain	*ilv* allele	Source
AB 3515	*ilvE12*	*ilv* allele from AB 1514 (Adelberg)
AB 3516	*ilvE316*	*ilv* amber antipolar mutation from this laboratory
AB 2950	*ilvD109*	*ilv* allele from Kiritani
AB 2970	*ilvD21*	*ilv* allele from AB 1285 (Adelberg)
AB 2971	*ilvD52*	*ilv* allele from AB 1424 (Adelberg)
AB 2972	*ilvD22*	*ilv* allele from AB 1286 (Adelberg)
AB 2973	*ilvD46*	*ilv* allele from AB 1415 (Adelberg)
AB 2974	*ilvD23*	*ilv* allele from AB 1287 (Adelberg)
AB 2975	*ilvD62*	*ilv* allele from AB 1436 (Adelberg)
AB 2976	*ilvD45*	*ilv* allele from AB 1414 (Adelberg)
AB 2977	*ilvD29*	*ilv* allele from AB 1293 (Adelberg)
AB 2927	*ilvD16*	*ilv* allele from AB 1450 (Adelberg)
AB 2978	*ilvD211*	*ilv* allele from AB 1460 (Adelberg)
AB 2979	*ilvD60*	*ilv* allele from AB 1433 (Adelberg)
AB 2980	*ilvD43*	*ilv* allele from AB 1411 (Adelberg)
AB 2981	*ilvD61*	*ilv* allele from AB 1435 (Adelberg)
AB 2949	*ilvD106*	*ilv* allele from Kiritani
AB 2982	*ilvD39*	*ilv* allele from AB 1406 (Adelberg)
AB 2983	*ilvA24*	*ilv* allele from AB 1288 (Adelberg)
AB 2928	*ilvA201*	*ilv* allele from AB 1255 (Adelberg)
AB 2984	*ilvA44*	*ilv* allele from AB 1412 (Adelberg)
AB 2944	*ilvC285*	*ilv* amber mutation from this laboratory
AB 2947	*ilvC288*	*ilv* amber mutation from this laboratory
AB 2985	*ilvC101*	*ilv* allele from Kiritani
AB 2945	*ilvC286*	*ilv* amber mutant from this laboratory
AB 2986	*ilvC49*	*ilv* allele from AB 1419 (Adelberg)
AB 2929	*ilvC7*	*ilv* allele from AB 1203 (Adelberg)
AB 3590	*ilvDAC115*	*ilv* deletion from Kiritani

[a] These strains differ only in their *ilv* allele. Allele members are those assigned by the laboratory where the mutations were isolated.

genote. The recombination frequency would be low enough to account for the numbers of colonies observed in the cross-streak test.

Such observations and explanations led us to develop a method of ordering mutant sites within a gene. The initial concept was to construct, by transductional shortening, a series of merogenotes each carrying a different length of the gene to be studied. In mating these merogenote strains with a series of *ilv* mutants of that gene, one would obtain *ilv*⁺ recombinants, depending on whether the merogenote carried the wild-type sequence of bases corresponding to the mutated bases in the endogenote. A diagrammatic representation of the concept is shown in Fig. 5. Merogenote C could form recombinants with all mutant sites within the gene except site 4. Merogenote B could form recombinants with mutants 1 and 2, but not 3 or 4. Merogenote A could form recombinants only with mutant site 1.

If several merogenotes, each carrying an increasingly longer segment of the gene, were crossed with several recipients, each carrying an independent mutation of that gene, one should be able to order the mutant sites on the basis of the presence or absence of recombinants arising from the various matings.

Such matings were carried out with five donor strains, each carrying a merogenote consisting of the sex factor, the *ilvE*, *ilvD*, and *ilvA* genes, plus variable segments of the *ilvC* gene. These merogenote strains were crossed with seven recipients, each carrying a different mutation in the *ilvC* gene. The results of these matings are shown in Table 2. The mutant sites are arranged in the order deduced from the data, and the merogenotes are listed in order of size.

Variable ilv gene segments on shortened merogenotes. The merogenote F *ilvEDA1* formed recombinants with all seven of the *ilvC* mutations. The merogenote F *ilvEDA2* formed recombinants with five of the mutants. When crossed with the mutants *ilvC49* and *ilvC7*, no

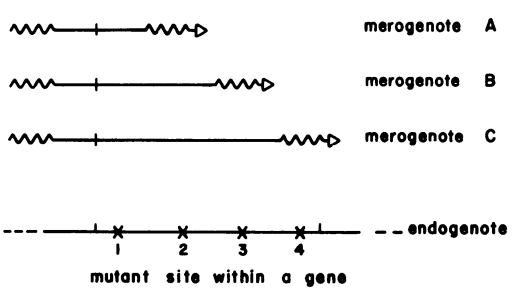

FIG. 5. *Diagrammatic representation of the principle behind a method for ordering mutant sites within a gene by the use of a series of merogenotes of differing lengths. The recipient is represented as if the several mutations existed on a single chromosome.*

TABLE 2. *Recombination frequencies of shortened merogenotes with recipients carrying a series of ilvC mutations*[a]

Merogenote	*ilv* alleles							
	A201	C44	C285	C288	C101	C294	C49	C7
F *EDA1*	1.2×10^{-2}	8.4×10^{-3}	3.4×10^{-3}	4.4×10^{-4}	4.8×10^{-5}	4.5×10^{-5}	3.6×10^{-6}	6.4×10^{-7}
	1.1×10^{-2}	1.3×10^{-2}	2.0×10^{-3}	4.5×10^{-4}	1.9×10^{-5}	1.9×10^{-5}	4.0×10^{-6}	5.0×10^{-7}
Avg	1.2×10^{-2}	1.1×10^{-2}	2.7×10^{-3}	4.5×10^{-4}	3.3×10^{-5}	3.2×10^{-5}	3.8×10^{-6}	5.7×10^{-7}
F *EDA2*	1.4×10^{-2}	2.6×10^{-2}	5.4×10^{-3}	2.7×10^{-4}	9.5×10^{-5}	2.6×10^{-5}		
	1.7×10^{-2}	1.4×10^{-2}	4.6×10^{-3}	2.3×10^{-4}	5.1×10^{-5}	7.1×10^{-5}	$<1 \times 10^{-8}$	—
Avg	1.5×10^{-2}	2.0×10^{-2}	5.0×10^{-3}	2.5×10^{-4}	7.3×10^{-5}	4.9×10^{-5}		
F *EDA4*	1.2×10^{-2}	1.7×10^{-2}	8.4×10^{-7}	3.5×10^{-7}	2.0×10^{-7}			
	1.1×10^{-2}	1.7×10^{-2}	1.8×10^{-6}	8.2×10^{-8}	8.7×10^{-8}	$<1 \times 10^{-8}$	—	—
Avg	1.2×10^{-2}	1.7×10^{-2}	1.3×10^{-6}	2.1×1.0^{-7}	1.4×10^{-7}			
F *EDA5*	1.3×10^{-2}	2.2×10^{-2}	3.4×10^{-6}					
	1.3×10^{-2}	8.1×10^{-3}	6.7×10^{-7}	$<1 \times 10^{-8}$	—	—	—	—
Avg	1.3×10^{-2}	1.0×10^{-2}	2.0×10^{-6}					
F *EDA3*	1.4×10^{-2}	2.5×10^{-3}						
	1.2×10^{-2}	8.7×10^{-4}	$<1 \times 10^{-8}$	—	—	—	—	—
Avg	1.3×10^{-2}	1.7×10^{-3}						

[a] Recombination frequencies are given as the number of recombinants arising per donor cell in the mating mixture. The dashes indicate $<10^{-8}$ recombinants.

colonies were detected at the lowest dilution from that mating mixture, and hence the result is listed as a recombination frequency of less than 10^{-8}. Similarly, F *ilvEDA4* formed *ilv*$^+$ recombinants with *ilvC101* but not *ilvC294*, F *ilvEDA5* recombined with *ilvC285* but not *ilvC288*, and F *ilvEDA3* could recombine with *ilvC44* but not *ilvC285*. Complementation/recombination frequencies with *ilvA201* are discussed below.

We have interpreted the data for the F *ilvEDA2* matings to indicate that the *ilvC* gene segment on this merogenote terminates between the site of *ilvC294* and that of *ilvC49*. Similarly, the merogenote F *ilvEDA4* carries an *ilvC* gene segment that terminates between the site of the *ilvC101* mutation and that of the *ilvC294* mutation. The F *ilvEDA4* terminates between *ilvC285* and *ilvC288*, and F *ilvEDA3* terminates between *ilvC285* and *ilvC44*.

Ordering mutant sites within the ilvC gene. The confirmation of the proposed method of ordering mutant sites is evident. The mutant site *ilvC7* is proximal (with respect to the origin of the merogenote) to the other six because it forms recombinants only with the longest merogenote. Similarly, *ilvC49* is proximal to *ilvC249* since *ilvC49* forms recombinants with the longest merogenote but not with F *ilvEDA2*. Similar reasoning was used to establish the order of each of the remaining *ilvC* mutants as shown in Table 2.

Transfer frequencies of these five merogenotes into a strain carrying a mutation in the *ilvA* gene, which is distal to the *ilvD* gene, are included to show Ilv$^+$ colony frequencies when a merogenote gene complements the recipient mutation. We have no data yet to indicate what fraction of these Ilv$^+$ colonies are recombinants and what fraction are diploids. This will be determined by comparing frequencies of Ilv$^+$ colonies obtained by mating with *rec*$^+$ and *rec* recipients and by testing the Ilv$^+$ colonies among the *rec*$^+$ recipients for ability to act as donors of the merogenote *ilv* gene. The latter will be complicated because the Ilv$^+$ colony may well contain F$^-$, F′, and Hfr types.

The possibility that the F *ilvEDA1* carries some of the *ilvB* gene could not be tested directly because of the dearth of mutants in that gene. If it did carry any *ilvB* DNA, it would carry all of the *ilvC* gene and would have complemented all mutants in the *ilvC* gene. This would have resulted in Ilv$^+$ colony frequency (diploids and recombinants) of 10^{-2} per participating donor, rather than the range observed.

The *ilvC44* mutant gave recombination frequencies similar to complementation/recombination frequencies of the *ilvA201* mutant. The obvious question then is whether this mutation is in the *ilvC* gene or in the *ilvA* gene. Two pieces of evidence suggest that it is not in the A gene: (i) the shortest merogenote, F *ilvEDA3*, yielded 10-fold differences in *ilv*$^+$ colony frequencies when crossed with recipients carrying *ilvA201* versus *ilvC44* (Table 2); and (ii) strains carrying *ilvC44* do not grow in the presence of isoleucine alone as do other known *ilvA* mutants. The recombination frequencies obtained

with the two merogenotes of differing length with *ilvC44* have significance in the simplified mapping procedure discussed below.

Simplified method for ordering mutant sites. When the results obtained with the F *ilvEDA1* were examined closely, it was observed that the recombination frequencies obtained with the various *ilvC* mutants increased (to a surprisingly large degree) with their distance from the origin of the merogenote. The same was true of the recombination frequencies of the *ilvC* mutants with the other merogenotes.

A simplified explanation for this gradation of recombination is shown in Fig. 6. It is not immediately obvious, however, why there should be such large differences in recombination frequencies across a gene. A double crossover would be necessary to generate, by recombination, wild-type progeny from an exogenote gene segment and a mutant endogenote gene. One crossover would be to the left and the other to the right of the mutant site. (In the case of an F merogenote, or any exogenote capable of circularizing, a single reciprocal crossover to the right of the mutant site would generate a wild-type gene, in this case making an Hfr strain. The following explanation for differing crossover frequencies would still hold for this special case.) If crossover frequency is constant per unit of DNA length, then under limiting conditions the frequency of crossovers in recombinational events would be related to the extent of available homologous DNA in the participating strands on both sides of the mutant site. In the limiting conditions used in Fig. 6, there is considerable homologous DNA to the left of mutant site 3, but very little homologous merogenote gene DNA to the right of site 3, that is, between mutant site 3 and the proximal end of the exogenote gene. Pairing and crossover become more likely if the mutant site is more distal to the origin of the merogenote. The vertical dotted lines represent some of the possible first crossover possibilities.

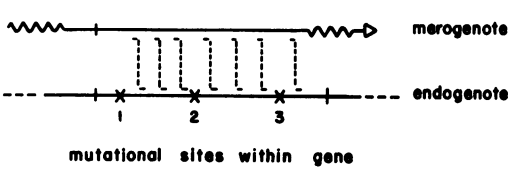

FIG. 6. *Diagrammatic representation of an explanation for the gradation of recombination frequencies observed when a single merogenote carrying a segment of a gene is crossed with recipients which are mutant at different sites within that gene. The recipient is represented as if the several mutations existed on a single chromosome. The vertical dashed lines represent some of the possible first crossovers.*

It would seem possible, then, to order mutant sites within a gene by use of only one or two merogenotes. A merogenote carrying almost all of the gene being mapped would be used to determine the sequence of the majority of the mutant sites within the gene, but in order to resolve mutants at the distal end of the gene a shorter merogenote would be necessary, as indicated above for the *ilvC44* mutation. The use of only one or two merogenotes would simplify the fine-structure mapping procedure by reducing the number of matings required. This simplified procedure was used to order the mutant sites within the *ilvD* and *ilvA* genes.

Ordering mutant sites in the ilvD gene. Six selected mutants in the *ilvD* gene were crossed with two independently isolated F *ilvE* merogenotes. The *ilvD* mutants were ordered according to their increasing recombination frequencies with the two merogenotes, as shown in Table 3. Increasing recombination frequencies were taken as an indication of distance of the mutant site from the origin of the merogenote. No conflicts are evident in ordering these mutants as determined with the two merogenotes used. Complementation frequencies are also shown for the reference mutation *ilvE12* and the *ilvE316* which was located in this gene by Wechsler and Adelberg (18).

The methods that have been used to isolate *ilv* mutants seemed to have favored mutants in the *ilvD* gene, since 16 of the 28 mutants were deficient in *ilvD* function. Recombination frequencies of these 16 *ilvD* mutants with the two F *ilvE* merogenotes are listed in Table 4. All of these recombination frequencies, with two exceptions, lead to the same gene order. The two conflicts are *ilvD52* and *ilvD46*. These are arbitrarily ordered as determined by the results obtained with F *ilvE2*. Recombination frequencies of these mutants with the F *ilvE1* merogenote suggest that each of the mutant sites should be exchanged with one of the mutant sites listed below it in the column. Since the mutant sites in this area of the gene seem to be very close, as indicated by very similar recombination frequencies, it may be that these exceptions indicate that many replicates of the matings may be necessary to resolve the order of such close mutant sites. We are currently developing more sensitive methods allowing a finer resolution. Until this

TABLE 3. *Recombination frequencies of shortened merogenotes with recipients carrying selected ilvD mutants*[a]

Merogenote	*ilv* alleles							
	E12	E316	D109	D62	D16	D60	D106	A201
F *E2*	1.6×10^{-2}	2.3×10^{-2}	1.1×10^{-4}	4.1×10^{-5}	6.9×10^{-6}	1.6×10^{-6}	1.7×10^{-8}	$<1 \times 10^{-8}$
	1.9×10^{-2}	2.0×10^{-2}	1.6×10^{-4}	6.0×10^{-5}	9.0×10^{-7}	7.4×10^{-7}	8.0×10^{-8}	$<1 \times 10^{-8}$
Avg	1.8×10^{-2}	2.2×10^{-2}	1.4×10^{-4}	5.1×10^{-5}	3.9×10^{-6}	1.2×10^{-6}	5.0×10^{-8}	$<1 \times 10^{-8}$
F *E1*	2.0×10^{-2}	3.5×10^{-2}	4.3×10^{-4}	3.1×10^{-5}	3.1×10^{-6}	1.9×10^{-6}	8.0×10^{-8}	$<1 \times 10^{-8}$
	1.7×10^{-2}	3.1×10^{-2}	3.7×10^{-4}	$3.0 \quad 10^{-5}$	6.9×10^{-6}	3.9×10^{-6}	8.0×10^{-8}	$<1 \times 10^{-8}$
Avg	1.9×10^{-2}	3.3×10^{-2}	4.0×10^{-4}	3.0×10^{-5}	5.0×10^{-6}	2.9×10^{-6}	8.0×10^{-8}	$<1 \times 10^{-8}$

[a] Recombination frequencies are given as the number of recombinants per donor cell in the mating mixture.

TABLE 4. *Recombination frequencies of a series of ilvD mutants with two shortened merogenotes*[a]

Ilv mutant	Merogenotes	
	F *ilvE2*	F *ilvE1*
E12	1.8×10^{-2}	1.9×10^{-2}
E316	2.2×10^{-2}	3.3×10^{-2}
D109	1.4×10^{-4}	4.0×10^{-4}
D21	9.6×10^{-5}	6.2×10^{-5}
D52	8.7×10^{-5}	2.1×10^{-5}
D22	7.3×10^{-5}	4.5×10^{-5}
D46	7.0×10^{-5}	1.9×10^{-5}
D23	6.7×10^{-5}	4.4×10^{-5}
D62	5.1×10^{-5}	3.0×10^{-5}
D45	3.1×10^{-5}	2.8×10^{-5}
D29	2.6×10^{-5}	9.7×10^{-6}
D39	1.2×10^{-5}	7.4×10^{-6}
D16	3.9×10^{-6}	5.0×10^{-6}
D211	3.3×10^{-6}	2.5×10^{-6}
D60	1.2×10^{-6}	2.9×10^{-6}
D43	7.0×10^{-7}	3.3×10^{-8}
D61	7.0×10^{-7}	5.0×10^{-8}
D106	5.0×10^{-8}	8.0×10^{-8}

[a] Recombination frequency is given as the number of recombinants arising per donor cell in the mating mixture.

is done, we have shown many of these mutations as being in a limited region of the *ilvD* gene (Fig. 7).

Ordering mutant sites within the ilvA gene. Only two *ilvA* mutants were among the 24 *ilv* mutants obtained from Dr. Adelberg. These two are ordered with respect to each other and to the *ilvD16* and *ilvC7* reference mutations as shown in Table 5. Several more *ilvA* mutants have now been obtained for use in future mapping studies.

On the basis of the recombination frequencies obtained in the above crosses, we have made a tentative fine-structure map of 25 mutations in three of the genes in the *ilv* cluster (Fig. 7). The order of some of the mutations in the *ilvD* gene may be modified as more detailed information is acquired. The order of the *ilvE* mutations is based on the data of Wechsler and Adelberg (18).

DISCUSSION

As has been previously shown, P1 lysates grown on a strain haploid for the region of the chromosome carried by the F^{14} merogenote are able to transduce merogenote genes into suitably marked recipients. Some of the transductants carry shortened merogenotes consisting of only the sex factor plus one or more *ilv* genes proximal to the sex factor. More specifically, as we have demonstrated here, transductionally shortened merogenotes carry one or more complete *ilv* genes plus variable segments of adjacent proximal genes. Merogenotes carrying increasingly greater segments of a particular gene were shown to be useful in ordering mutant sites within a gene.

This new fine-structure mapping procedure, which makes use of several merogenotes carrying different segments of a gene, is analogous to deletion mapping (2, 3, 5) if one considers the shortened merogenote as being deleted for the region lost in transductional shortening. Thus, a merogenote carrying a fragment of a particular gene will form wild-type recombinants with all mutant sites on the endogenote if the corresponding and homologous nucleotides are carried by the "undeleted" portion of the exogenote. Obviously, no wild-type recombinants can be formed if the mutant site on the endogenote lies in a region that is "deleted" in the exogenote.

After this "deletion" mapping of a series of *ilv* mutations in one gene, it was observed, as expected, that wild-type recombination frequencies increased with distance of any mutation site from the origin of the merogenote. This observation led to a simplified method for ordering mutant sites within a gene, a method that requires only one, or perhaps two, merogenotes for each gene to be mapped. One mer-

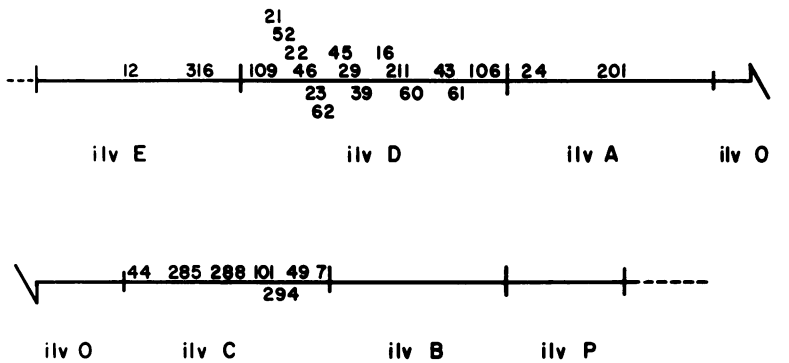

FIG. 7. *Proposed order of 27 mutant sites in four genes in the ilv gene cluster.*

TABLE 5. *Recombination frequencies of a shortened merogenote, F EDI, with two ilv⁻ mutants and two reference mutations*

ilv allele	Recombination frequency[a]
D16	1.2×10^{-2}
	1.4×10^{-2}
Avg	1.3×10^{-2}
A24	1.8×10^{-2}
	2.0×10^{-2}
Avg	1.9×10^{-2}
A201	1.2×10^{-4}
	1.3×10^{-4}
Avg	1.3×10^{-4}
C7	$<1 \times 10^{-8}$
	$<1 \times 10^{-8}$
Avg	$<1 \times 10^{-8}$

[a] Number of recombinants arising per donor cell in the mating mixture.

ogenote should carry all but the very proximal (with respect to the origin of the merogenote) nucleotides of the gene being studied. That merogenote gene segment should be neither functional nor capable of complementing endogenote mutants of that gene. This longer merogenote would be used to order the majority of the mutant sites along the endogenate gene, by their recombination frequencies. The mutants with the lower recombination frequencies are those located near the proximal end of the gene; those with higher frequencies are distal from the origin of the exogenote. It was observed, however, that in matings with longer merogenotes the wild-type recombination frequencies of one mutant located most distal on that gene were quite high. In fact, recombination frequencies were indistinguishable from diploid/recombination frequencies resulting from complementation or recombination, or both, of a mutation in the adjacent distal gene. However, recombination frequencies obtained in matings between the most distal *ilvC* mutant and merogenotes carrying the shortest segment of that gene were different by an order of magnitude from the diploid/recombination frequencies found with the mutant in the more distal *ilvA* gene. Therefore, we conclude that a merogenote carrying a short segment of the gene being studied would be necessary to resolve mutant sites near the distal end of the gene.

It seems likely that recombination frequencies with strains carrying small deletions would be lower than that of point mutations and would therefore lead to erroneous mapping of deletions with respect to other mutation sites. We have no data to estimate this effect. Each of the mutants used in this study was capable of reversion to wild type and so was not likely to be a deletion. No small deletion mutants of the *ilvD*, *ilvA*, or *ilvC* genes are known to us. The *ilvE12* [originally carried in strain 11A16 (10)] is said to be a deletion (H. E. Umbarger, *personal communication*), but we have as yet no merogenotes carrying only a segment of the *ilvE* gene with which we could evaluate the effect of this deletion on recombination frequencies. It may be possible to isolate this type of merogenote in the near future, as described below.

The only other deletion mutation known to us in the *ilv* gene cluster is one isolated and partially characterized by Kiritani et al. (9) as being unable to form recombinants with any *ilvD* or *ilvC* mutants he tested. It seems appropriate to say something about this deletion here, as its existence is not generally known. We have found by enzyme assay that strains carrying this deletion (e.g., AB3590) make transaminase B, but not dehydrase, threonine

deaminase, or the reductoisomerase enzyme. It does make the condensing enzyme (aceto-hydroxy acid synthetase). It seems, then, to be deleted in a portion of the *ilvD* gene, presumably all of the *ilvA*, and a considerable portion of the *ilvC* gene. An estimation of the extent of the *ilvC* segment deleted is based on the absence of wild-type recombinant formation in matings between this deletion and the F *ilvEDA1* merogenote which carries the longest segment of the *ilvC* gene of those tested. A merogenote carrying all of the *ilvC* gene (an F *ilvEDAC* merogenote) will restore Ilv$^+$ phenotype to a strain carrying this deletion.

Kiritani (9) has carried out two-point transductional mapping on a number of mutants that he had isolated in the *ilvD* and *ilvC* genes. He found that *ilvD109* was the most distal from *ilvC* and that *ilvD106* was most proximal to *ilvC*. Our data agree with his mapping, and this observation further supports the validity of the mapping procedure. Kiritani found that *ilvC101* was the most distal to *ilvD*. We found three mutants that map more distally than his *ilvC101*. Kiritani found that his *ilvC102* mutation mapped most proximally to the *ilvD* gene. Despite repeated attempts, we could not transduce the *ilvC102* into AB 2200. This may very well be because this mutation is a nonsense mutation of some type. The AB 2200 strain was originally described as being free from amber and ochre suppressors since it was derived from AB 2550 (4). It carries three ochre mutations (*lac*, *trp*, and *his*), and we have been able to cotransduce into it seven different amber mutations. However, we have been unable to transduce one particular amber mutation (*ilvD283*) into it (Duggan and Adelberg, *unpublished data*). Further, Gudmunder Eggertsson has found that strain AB 2200 is permissive for the T4 phage amber mutant N67 (*personal communication*).

We have isolated several shortened merogenotes carrying four *ilv* genes. These merogenotes complement a recipient carrying the *ilvC7* mutation. Presumably they carry variable segments of the *ilvB* gene and could be of value in ordering mutation sites within the *ilvB* gene. As mentioned above, we are not aware of recessive mutants in this gene suitable for mapping by this method. The *ilvB196* of Ramakrishnan and Adelberg (13) was described as growing slowly in the absence of isoleucine-valine. At any rate, one could hardly "order" one mutant site without another site for comparison.

The shortened merogenotes were isolated by selection for Ilv$^+$ transductants in an *ilvE* recipient because the *ilvE* gene is the closest known site to the sex factor. By selecting transductants in this manner, one can find shortened merogenotes of greater variety than by selecting in recipients carrying *ilv* mutations more distant from the sex factor. This selection method, however, eliminates the finding of shortened merogenotes carrying the segments of the *ilvE* gene necessary to order mutants in this gene. A recipient strain with a mutant gene located between *ilvE* and the site corresponding to the 0-13 sex factor is needed to isolate merogenotes carrying segments of the *ilvE* gene. Recently Wu and Wu (19) reported that the *glmS* gene is located between *ilvE* and *phos*, and is carried on F$'^{14}$. We are currently determining whether this mutation is suitable for selecting such merogenotes.

One of the observations made in this study was that recombination frequencies for mutations located closer to the proximal end of the partial gene carried by the exogenote are lower than for mutations located more distally. This is reminiscent of the observations of Pittard et al. (12, 17). Their time of entry data were initially interpreted to indicate an alteration in the order of gene sequence on the F$'^{14}$ compared with its parent strain. Glansdorff (7) showed that the gene sequence was unaltered, but the most proximal gene (*argH*) appeared at a lower frequency in mating experiments than did more distal genes. In our experiments, the recombination frequency seems to have reached a maximum level within one gene distance (Table 2). We cannot explain the reduced recombination frequency of the *argH* gene of the F$'^{14}$ on the basis of there being only a segment of the *argH* gene on the most proximal end of the F$'^{14}$, because the primary haploid strain, AB 1206, is *arg*$^+$. Further, in the diploid state, the F$'^{14}$ complements the *argH* mutation in AB 1450. It is tempting to speculate, however, that the reduced recombination frequency obtained for very early genes on large merogenotes and on Hfr chromosomes may be the result of limited possibilities for crossovers to occur in the proximal region of the first gene. This should be the case only if the recipient carried a mutation in the proximal region of that gene.

The mechanism of formation of these shortened merogenotes is unknown at this time. We are currently trying to determine whether the merogenotes are shortened at the time of packaging in the donor host or after injection into the recipient.

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