Studies on a mutator gene in Escherichia coli
by Guylyn Rea Warren

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY in Genetics
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Abstract:
The precise location of mut-1, one of three separately isolated mutator genes which have been studied extensively in bacterial systems, has been determined by the use of transduction and Hfr x F- timed mating methods. It has been found to map very close to the locus for sodium azide resistance (azi), between azi and the locus for T1,T5 resistance (ton).

Mut-1 is thus not allelic to either of the other two mutator genes (ast in E. coli, mut-3 in S. typhimurium). The existence of three mutator loci indicates that the mutant DHA polymerase model is inadequate as a general explanation for all instances of mutability.

Some mutagens have been found to produce effects on mapping data, such as "loosening of linkage" by UV irradiation; therefore, the possibility of such an effect produced by mut-1 was investigated. Paired transduction experiments using isogenic mutable and non-mutable phage donor strains showed no measurable effects of mut-1 on linkage distances. Paired conjugations using isogenic mutable and non-mutable derivatives of HfrC as donors suggested a slight increase in numbers of quadruple crossover types caused by mut-1 although their effect on timed mating data used for location of mut-1 was negligible.

Since the effects of mut-1 on linkage data are negligible, the mutator genes mut-1, ast and mut-3 may be ordered as follows with respect to other well-known loci in the region.

met mut-3 purA thi thr leu val azi mut-1 ton ast pro lac

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Genetics

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ABSTRACT

The precise location of *mut-1*, one of three separately isolated mutator genes which have been studied extensively in bacterial systems, has been determined by the use of transduction and Hfr x F^- timed mating methods. It has been found to map very close to the locus for sodium azide resistance (*azi*), between *azi* and the locus for T1,T5 resistance (*ton*).

Mut-1 is thus not allelic to either of the other two mutator genes (*ast* in *E. coli*, *mut-3* in *S. typhimurium*). The existence of three mutator loci indicates that the mutant DNA polymerase model is inadequate as a general explanation for all instances of mutability.

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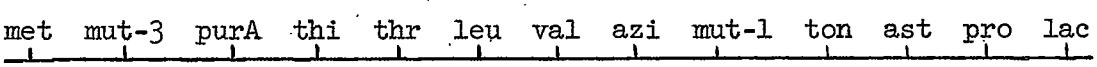


INTRODUCTION

Studies by R. A. Emerson (1914), in which he proposed the cause of mosaicism in plants to be mutation, cleared the way for studies of increased mutation rates at various loci in genetically suitable material. In nearly every type of organism which has been the focus of intensive genetic study, instances of mutational instability have been reported.

Mutable loci at which forward mutations are unstable and revert to wild type or some other state with high frequency have been described in plant, animal, and bacterial systems. Such loci as rose-alpha and lavender-alpha investigated by Demerec (1931), and the color locus p* reported by Dawson (1955) in Delphinium, the dwarf locus in Portulaca (Blakeslee, 1920; Faberge and Beale, 1942), and the c locus controlling anthocyanin pigmentation of the kernel in maize (Schwartz, 1960) have been studied in higher plants. In Drosophila, the miniature-gamma locus (Demerec, 1929a, 1929b) and mt-3a (Zamenhof, 1945) have been investigated. Lederberg (1952), Zamenhof et al. (1958) and Barnett and deSerres (1963) have found such mutants in microorganisms.

"Controlling elements" of mutation, probably episomal, have been studied extensively in maize in such systems as a_1-Dt (dotted) by Rhoades (1938, 1941) and Nuffer (1961), and activator-dissociator by McClintock (1951, 1956). In Drosophila, controlling elements, possibly viral, have been found by Mampell (1943, 1945, 1946), by Levitan (1962), Levitan and Williamson (1965) and Ehrman and Williamson (1965). Systems of episomal control of mutation in bacteria have been described by Gunderson et al. (1961, 1963) in Escherichia coli and by Dawson and Smith-Keary (1963) in

Salmonella typhimurium. A system of viral effect on mutation has been described in E. coli by Taylor (1964) caused by the temperate bacteriophage mu-1, and one in maize caused by an RNA virus, barley stripe mosaic (BSMV), by Sprague et al. (1963). In all of these systems the controlling element is heritable and often infectious, but it cannot be mapped at any specific and unique locus in the genome.

The system of particular interest here is that of a true mutator gene, which has a unique chromosomal locus and is inherited in a Mendelian fashion, and which affects rates of mutation at other loci not closely linked with it. Several such genes have been studied in Drosophila by Demerec (1937), Plough and Holthausen (1937), Goldschmidt (1939, 1945), Neel (1942) and Ives (1943, 1950). Most of these gene affect the yellow locus as well as causing chromosomal aberrations, with the exception of hi, studied by Ives (1950), which affects the forked locus. Demerec (1937) proposed that the presence of mutator genes in wild populations of Drosophila would allow increased rates of evolution by maintaining greater adaptability to changes than that allowed by normal mutation rates.

Speyer (1965) has recently discovered a series of mutator alleles in bacteriophage T4. Gene 43, which has been identified as the structural gene for T4 DNA polymerase (Edgar, 1964; deWaard, 1965), is the locus of these alleles. Tests of the rII system of T4 indicated that temperature sensitive (ts) mutant L-56 caused a 2000-fold increase in the reversion frequency of spontaneous forward mutations in the rII region and also affected the back mutation frequency of some mutations caused by base

analogs, e.g. AP129. The mutagenic ts alleles do not affect reversion of deletions or of frame shift rII mutations. It was proposed that mutant DNA polymerases may permit mistakes in base pairing, thereby increasing mutation frequency.

Four independent mutable strains of bacteria have been described, three of Escherichia coli and one of Salmonella typhimurium. The first was a substrain of E. coli K12, 58-278M*, discovered by Treffers et al. (1954) by virtue of its enhanced (ca. 100-fold) mutation rate from streptomycin sensitivity to resistance. Subsequently, by means of appropriate $F^+ \times F^-$ crosses, Skaar (1956) showed that a localized region of the E. coli linkage map was responsible for the enhanced mutability. He concluded that a single mutator gene was involved. Since more than one of the mutator loci to be discussed have been given the designation mut, for the sake of clarity in this presentation the mutant allele of of 58-278M* will be referred to as mut-1. Skaar (1956) also concluded that the mut-1 locus was very close to the leucine (leu) locus, which marks the Y-10 line (Lederberg, 1947). Further, although the pertinent recombinants were not numerous enough to allow an unambiguous answer, it appeared that the mut-1 locus was to the "right" of leu, between leu and the locus for T1,T5 resistance (ton), rather than between the leu and threonine (thr) loci. Later HfrC x F^- crosses performed by Gunderson et al. (1961) were interpreted by these workers to indicate that the mut-1 locus was not close to leu, but was rather to the "left" of thr, beween that locus and the methionine (met) locus.

There are a number of clues as to the mode of action of mut-1.

(1) The multiplicity of effects of mut-1 observed by Treffers et al. (1954) suggested a generalized effect, and hence a mutagenic gene product. They reported enhanced mutability for such diverse traits as resistance to chloramphenicol, sodium fluoride and bacteriophages T1, T4, T5, and T3h, and fermentation of sugars, but no effect on the mutation rate from T2 sensitivity to resistance. Skaar's demonstration (1956) that the streptomycin locus affected was only remotely linked to mut-1, and that the near-by loci, thr and thi (thiamine), were also affected, strengthened this inference. Auxotrophic mutants are significantly more frequent in mut-1 cultures (Skaar, personal communication) and these constitute the same general array of types observed in non-mutable strains, mapping throughout the genome. Of especial significance is the finding (Pierce, 1966b) that mut-1 is capable of increasing the mutation rate in the virulent bacteriophage T4. This argues that the "mutagen" is not a defective polymerase since this phage makes its own DNA polymerase.

(2) Bullas and Skaar (personal communication), (upon one occasion), isolated appreciable amounts of a fifth UV-absorbing component from the DNA of a mut-1 strain. Several of its properties, including its unique spectral profile and its ability to satisfy growth requirements of a thymine-requiring bacterium, suggest that it was not an artifact, but rather a mut-1 specific substance (possibly the mutagen) produced only under certain conditions of growth.

(3) Treffers et al. (1954) pointed out the marked discrepancy

between the array of streptomycin resistant mutants evoked by mut-1 and those which arise in other K12 strains. In the latter, 25% are streptomycin dependent; whereas, less than 1% of those arising in mut-1 strains are dependent. Since dependence and resistance without dependence were thought to be allelic (Newcombe and Nyhom, 1950) they suggested an allele-specific action of mut-1. Though these types of streptomycin resistance are not allelic (Hashimoto, 1960), this suggestion was supported by the work of Bacon and Treffers (1961a, 1961b), who found that mut-1 selectively raises the rate of one specific mutation type (the S48 type) at the ornithine locus.

(4) Finally, Yanofsky (1966) showed that amino acid replacements, resulting from action by mut-1 on the tryptophane synthetase A protein, are consistent with a base transversion from A-T to C-G in the A cistron.

The second mutable strain was a substrain of MSFB and was designated the Harvard strain (ATCC 11887) of E. coli by Goldstein and Smoot (1955) and is also referred to as DZ-1 by Zamenhof (1966). Populations of this organism grown on nutrient medium normally contain a variety of auxotrophic mutants, at a mean frequency of about 4%. Goldstein and Smoot (1955) found that many of these auxotrophs are unstable and revert to wild type with high frequency. The mutability of this strain was found to be a stable hereditary characteristic and was proposed to be the result of a mutator gene. This gene was recently mapped by Zamenhof (1966) and was given the designation ast, for the Greek astasia (instability, mutability). Ast was found to lie between the locus for T1,T5 resistance (ton) and that

for proline (pro). The percentage of auxotrophic mutants in DZ-1 ranged from 5 to 12, while that of its closest known ancestor was only 0.005, and 50% of these mutants were highly unstable. The mapping of this gene was done with K12 derivatives of DZ-1 which were F-lac since establishment of F in DZ-1 strains was impossible (possibly due to lack of homology with the F genome or to restriction) precluding selection of DZ-1 Hfr. Possible host restriction or modification of DZ-1 DNA by K12 strains was noticed, causing "unlinking" of markers in crosses. It was observed that ast does not appreciably increase the mutation rate to streptomycin resistance in its host strain (DZ-1) but does so in K12 strains, suggesting a difference between strains in the mechanism of this resistance. Further, ast causes an increase in rate of mutation from T2 sensitivity to resistance in K12 strains. However ast affects loci for amino acid, purine and pyrimidine requirements, phage resistance other than T2, resistance to antibiotics and sugar fermentation as does mut-1. Also like mut-1, ast has recently been found to be capable of affecting virulent phage (T4) mutation rates (Zamenhof, 1967).

The third mutable strain was isolated and studied extensively by Miyake and Demerec (1959) and Miyake (1960) from S. typhimurium and has been designated LT-7. The mutability of this strain was shown to be caused by a single mutator gene, which will be referred to hereafter as mut-3 in order that it may be distinguished from the first isolated mutator gene. This gene exhibits allele specific activity and affects the rate of mutation at the streptomycin locus, as well as at all others

tested, with 87.7% of the auxotrophs produced exhibiting high reversion frequencies. This gene was mapped by Hfr x F^- crosses and was reported to be located to the "left" of thr, near the purine A (purA) locus (Demerec et al, 1965; Miyake, 1960) as inferred from timed matings, although extensive data are not available. Mut-3 also has the peculiar property of increasing the efficiency of its host as a receptor for transduction by the transducing phage PLT-22 H1 (Miyake and Demerec, 1959). Kirchner is said to have found an unusual base present in mut-3 strains, but he has not been able to introduce this substance into cells to prove that it is mutagenic (in Pierce, 1966a), in agreement with his earlier mutagen hypothesis (Kirchner, 1960). He also has evidence of other possible mutator genes and other mutagenic bases in Salmonella.

The fourth mutable strain was isolated by Zamenhof (1958a, 1958b) by selection of a 5 bromouracil (5 BU) resistant mutant of E. coli I (ATC 11117) which requires thymine. The strain was designated I/Br and yields pinpoint colonies at high rates on nutrient medium as does the Harvard strain, DZ-1, which harbors ast. This mutability must be a property of resistance to 5 BU since all resistant mutants were unstable and back mutants to sensitivity were stable. This type of mutant cannot be isolated without the presence of 5 BU but the presence of 5 BU was not required for the expression of mutability of the resistant strains. 5 BU resistant mutants of other strains did not exhibit this mutability.

The three mutator genes which have been mapped are located within a segment constituting only about 10% of the E. coli genome. The map of Salmonella (Demerec et al., 1965) indicates close correlation of the

area containing mut-3 with the same area of the E. coli map (Taylor and Thoman, 1964). Despite the fact that each of the mutator genes seems to have its unique pattern of action, the possibility that they are allelic requires re-examination. On the basis of published positionings, ast and mut-3 are the loci furtherst apart. Hence, they are least likely to be misplaced in the genome. The mut-1 locus, however, is between the others (according to Skaar) and the allelism of mut-1 with both mut-3 (Demerec, 1959) and ast (Zamenhof, 1966) has been suggested. In the present investigation, the precise position of mut-1 is examined, using the more powerful tools of transduction and timed Hfr matings.

The precise positioning of mut-1, in addition to answering questions about the multigenic basis of mutability, is a necessary prerequisite to any further study of a possible gradient of mutability due to proximity to the gene.

Certain mutagens (e.g. ultraviolet light) affect recombination frequencies (Jacob and Wollman, 1955, 1959) and therefore representation of linkage distances in mapping. The possibility of a similar effect of mut-1 was investigated. This possibility is interesting for its own sake, and is also a necessary part of an investigation attempting to position the gene.

MATERIALS AND METHODS

Bacterial and bacteriophage strains

The bacterial strains used were derivatives of Escherichia coli K12.

CS19 (Skaar, 1956) requires phenylalanine and is mutable and is therefore 58-278M* (Treffers et al., 1954) renamed after loss of its biotin requirement. GW1 is a derivative of CS19 which is resistant to sodium azide and to valine.

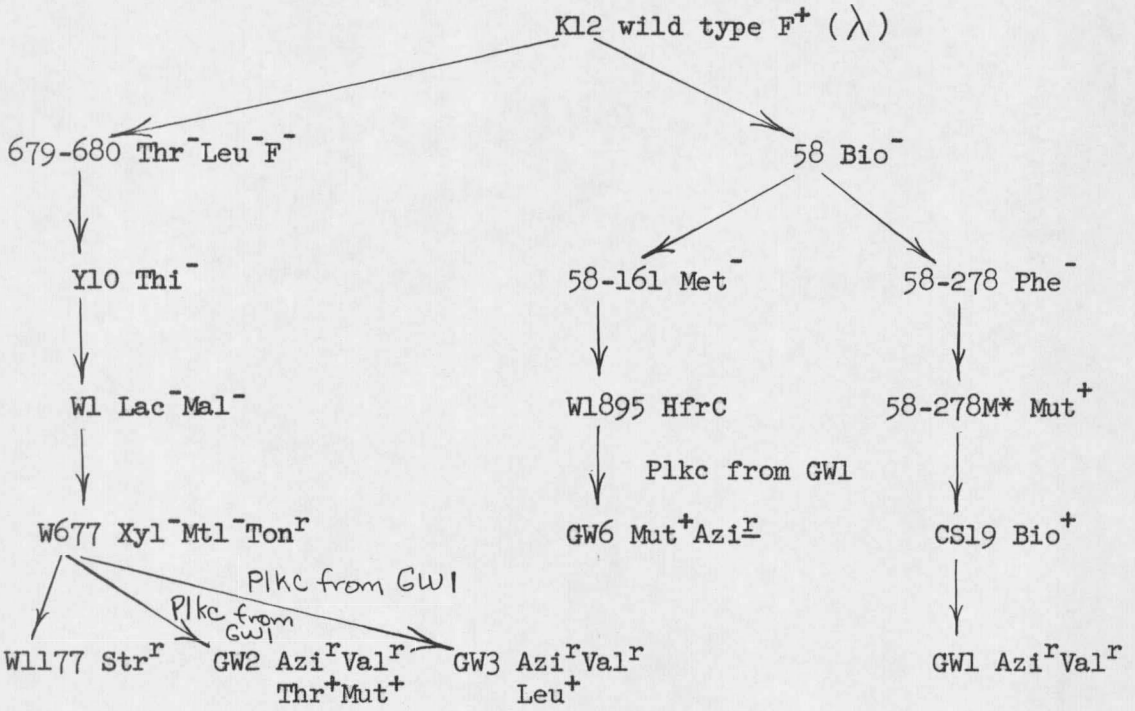
W677 is a multiply marked derivative of Y10 (Lederberg, 1947) which requires threonine, leucine and thiamine, cannot ferment lactose, maltose, mannitol or xylose, and is resistant to coliphages T1 and T5. W1177 is a streptomycin resistant derivative of W677.

W1895 is a derivative of 58-161 which is HfrC, the Cavalli strain (Cavalli, 1950) and requires methionine.

GW6 was constructed by transduction of W1895 by P1kc grown on GW1 and is HfrC, requires methionine, is mutable, and is resistant to sodium azide. GW6 was used as the donor in timed mating experiments.

GW2 was constructed by transduction of W677 with P1kc grown on GW1 and requires threonine and thiamine, does not ferment lactose, maltose, mannitol or xylose, is resistant to coliphages T1 and T5, is mutable and resistant to sodium azide and valine. GW3 is isogenic with GW2 with the exception that it is not mutable. These strains were used as isogenic donors in paired transduction experiments.

The phage strains used were P1kc, a mutant of P1 with an increased efficiency of plating on K12 strains, which was used as the generalized transducing agent; and T5, a virulent coliphage used to test the resistance



Only new characteristics are indicated, at all other loci the strains are identical with their immediate predecessors.

Figure 1. Pedigree of K12 strains used.

of transductants and recombinants to T1,T5.

Media and supplements

Final concentrations are given in grams/liter of distilled water unless otherwise indicated. Media were sterilized in an autoclave at 18 pounds pressure for 20-25 minutes.

Nutrient Agar (NA): Difco Nutrient Broth 8, NaCl 5, agar 15,

L Broth (LB): Bactotryptone 10, Bacto-Yeast Extract 5, NaCl 5, Bacto-glucose 1.

L Agar (LA): LB plus 10 grams agar, for phage add 1 ml/l 2.5 M $CaCl_2$ (Lennox, 1955).

Eosin Methylene Blue Agar (EMB): Bacto-Casitone 8.0, Bacto-Yeast Extract 1, NaCl 5, K_2HPO_4 2.0, eosin Y 0.4, methylene blue 0.065, sugar 10.0, agar 15.

Pennassay Broth (PB): Bacto-Beef Extract 1.5, Bacto-Yeast Extract 1.5, Bacto Peptone 5.0, Bacto-Dextrose 1.0, NaCl 3.5, K_2HPO_4 3.6, KH_2PO_4 1.3.

Davis Minimal Agar (DMA): KH_2PO_4 2.0, K_2HPO_4 7.0, crystalline sodium citrate 0.5, crystalline $MgSO_4$ 0.1, crystalline $(NH_4)_2SO_4$ 1.0, Bacto-Dextrose 1.0, agar 15. The salts were added to 500 ml of water and sterilized. The glucose and agar were added to 500 ml water and mixed with the salt solution after sterilization.

Tris Glucose Minimal Agar (TGA): NaCl 5.4, KCl 3.0, NH_4Cl 1.1, $CaCl_2$ 0.011, $MgCl_2$ 0.095, $FeCl_3$ 0.00162, KH_2PO_4 0.0872, Na_2SO_4 0.0227. Tris (hydroxymethyl) aminomethane 12.1. sugar 2.0, agar 10.0. The salts were added to 500 ml water and sterilized, the sugar and agar were added to 500 ml water and mixed with the salts after sterilization just prior to pouring into petri plates. In some cases lactose was substituted for glucose as the sugar and was added by diluting in 10 mls sterile water and adding to the agar after sterilization.

Phage Dilution Fluid (PDF): NaCl 3, Bacto-Peptone 1, $MgSO_4$ 2.46.

Top Layer Agar (TL): Bacto-Nutrient Broth 8.0, NaCl 5.0, agar 6.5.

Saline: NaCl 8.6.

Streptomycin: (dihydrostreptomycin sulphate) 250 mg/ml.

Sodium Azide: 5 ml/l of 0.4 M solution - used in LA.

Valine: 5 ml/l of 20 mg/ml solution - used in TGA.

Growth Factors: The following were added to TGA or DMA when required: methionine 0.02, threonine 0.04, leucine 0.02, thiamine 0.0001, phenylalanine 0.02.

Storage of bacterial and bacteriophage strains and preparation of working cultures and lysates.

Bacterial strains. Bacterial strains were stored on nutrient agar slants at 4°C and transferred every 4-6 months. Transfers always included a single colony isolation and tests for relevant growth requirements. All inoculations were made directly from the stock slants and stocks in prolonged use were reisolated and tested periodically.

All cultures were grown in an incubator or water bath at 37°C. Pennassay and L Broth cultures were grown overnight (12-16 hours) and log phase cultures in these media were obtained by diluting 1/10 from an overnight culture and incubating 90-120 minutes, depending upon the medium.

Bacteriophage techniques. Phage lysates were obtained by the confluent lysis plate technique described by Adams (1959). After collection, phage suspensions were spun in a Servall angle head centrifuge to remove cells and the supernate decanted and treated with chloroform. The resulting lysates were stored at 4°C. A concentration of 2.5×10^{-3} M $CaCl_2$ was added to both L Agar and top layer agar when working with phage P1kc.

Lysates were assayed by the agar-layer method at appropriate

dilutions (in PDF) on L agar plus calcium. Lysates were aerated 20 minutes at 37°C to remove chloroform prior to transduction procedures.

Transduction procedures

High-titer lysates (at least 10^9 phage/ml) of P1kc were harvested on appropriate donor bacteria as above. Incubation of a 1/10 dilution of an overnight culture of W677, the recipient, for 150 minutes in LB produced a log phase culture of approximately 10^8 cells/ml. Five mls of this culture were centrifuged for five minutes and resuspended in 0.5 ml LB plus calcium, which allowed concentration to 10^9 cells/ml. 0.5 ml of the phage suspension, with chloroform removed, was added allowing multiplicities of infection of approximately 1:1. This phage-cell mixture was incubated 30 minutes to allow maximum adsorption and was then diluted, a step found to be critical for frequency of transduction, by a factor of five in saline and allowed to stand at room temperature for 15 minutes. The cells were then washed twice by centrifugation in saline, resuspended in 1 ml saline, and 0.1 ml samples of this suspension spread on appropriate selective media. For example, if leucine were the selective requirement, the medium was supplemented with threonine and thiamine. The freshly spread plates were incubated at room temperature for two hours as suggested by Luria et al. (1960), and then incubated 48 hours at 37°C. Control plates were spread with the recipient strain alone to check for spontaneous revertants and the phage lysate was tested for sterility in each experiment.

Tests of transductants for marker alleles. Recombinant clones were

picked and streaked on nutrient agar, single colony isolations were made from these streaks and L broth cultures (5 ml) inoculated from each. These cultures were incubated 12-22 hours and tested for unselected markers and checked for the selected marker as well. Growth requirements such as those for threonine and leucine were tested by patching on appropriately supplemented TGA and noting absence (-) or presence (+) of growth after 48 hours of incubation; resistance to valine by patching on TGA plus valine and all necessary supplements and noting absence (s) or presence (r) of growth after 24 hours; resistance to sodium azide by patching on L agar plus sodium azide and reading at 24 hours; and resistance to T5 by cross brushing the cultures with T5 on nutrient agar and scoring presence (s) or absence (r) of plaques or lysis of the streaks. Fermentation markers were tested on EMB plus the appropriate sugar and noting color reaction. Tests for mutability were performed by spreading 0.1 ml of each culture on nutrient agar plus streptomycin as described by Skaar (1956). Any clones not giving a positive test were diluted, re-grown, and tested again. After scoring each broth culture was stored at $4^{\circ}C$ for future reference.

Mating procedures

Matings following the kinetics of transfer of the mutator gene (mut-1) and other pertinent loci were performed by the method developed by deHaan and Gross (1962) as modified by Wendt (1965). Log phase Hfr and F^- cultures were mixed to concentrations of 1-2 x 10^7 Hfr/2-4 x 10^8 F^- per ml., usually by diluting the Hfr 1/10 into the F^- when grown in pennassay

broth. Mating mixtures were incubated for five minutes to allow pairing and then diluted into 10^{-2} PB (PB diluted in saline) to halt further pairing. 0.1 ml. samples were removed from the diluted mixture at either 2.5 or 5 minute intervals and spread on appropriately supplemented TGA (usually with lactose) or on EMB-lactose to assay recombinants. In all cases resistance to streptomycin was the contra-selective marker, and streptomycin was added to all media to kill the donor cells. Each recombinant was picked and purified on the selective medium and tested for markers as in the transductions, with the exception that a new test for mutability was devised, since the recombinants are streptomycin resistant. The mutable allele causes a 100-fold increase in mutation rate from mannitol minus to mannitol formentation (Skaar, personal communication), therefore, the cultures were patched on EMB-mannitol and the presence or absence of mutability scored by observing increase in pappillation.

Gene terminology

As nearly as possible the suggestions of Demerec et al. (1966) were followed as regards genetic terminology. When the genotype of the strains is discussed small italicized letters are used, and when phenotype is discussed the first letter of the designation is capitalized, not italicized and such characteristics are resistance, sensitivity etc., as conferred by alleles of the same gene may be discussed. The abbreviations most commonly used are as follows:

locus	genotype	allelic phenotypes
threonine	thr	Thr^- or Thr^+
leucine	leu	Leu^- Leu^+
valine	val	Val^s Val^r
sodium azide	azi	Azi^s Azi^r
mutability	mut-1	Mut^- Mut^+
T1,T5 receptors	ton	Ton^s Ton^r

The plus sign used in phenotypic designations normally indicates the wild type ability to perform a function and does so here with the exception of its use with Mut^+ where it is the designation for mutability while Mut^- is the wild type.

EXPERIMENTAL RESULTS

The position of the mut-1 gene as inferred from transductions

Linkage of mut-1 to leu. Due to the disparity of the conjugal mapping data of Skaar (1956) and that of Gunderson et al. (1961), it was first necessary to investigate the relative proximity of mut-1 to leu and thr. This problem was approached by transductional techniques, which allow detection of linkage only between loci that are situated close enough together on the chromosome to be included in one fragment carried by an infecting phage particle. The generalized transducing phage P1kc, a strain of the largest of the temperate phages, which is competent on K12 strains of E. coli and known to co-transduce leu and thr (Lennox, 1955), was chosen as the agent of transfer. Transduction experiments were performed as described in Materials and Methods. Since mut-1 cannot be selected directly, selection for another locus (leu or thr) was imposed and the frequency of co-transduction of unselected markers was scored. Proximity of mut-1 to leu was examined by imposing selection of those transductants manifesting the donor (GW1) phenotype for leucine (Leu^+) and scoring their respective phenotypes for the val, thr, mut-1 and ton (T1,T5 resistance) loci. A common recipient strain, W677, was used in all transduction experiments, thus minimizing the variability of transducing ability from strain to strain shown by P1 (Lennox, 1955). The results of this experiment are reported in Table I, a. As is to be expected among P1kc transductants, the donor thr allele was found among very few (less than 1%) and the donor ton allele was never found (Lennox, 1955). Among these transductants 82.5% carried the donor val allele and

TABLE I. PERCENT CO-TRANSDUCTION OF UNSELECTED MARKERS WHEN LEU^+ OR THR^+ WERE SELECTED.

Donor	Recipient	Selected marker	No.	Unselected marker inheritance (%) Thr^+	Leu^+	Val^r	Azi^r	Mut^+	Ton^s
a. GW1	W677	Leu^+	200	1	-	82.5	*	20.5	0
b. GW1	W677	Leu^+	458	0.6	-	76.4	19.4	14.6	0.5
c. GW1	W677	Leu^+	192	0	-	83.9	19.3	15.6	0
d. GW2	W677	Leu^+	111	0.9	-	72.0	23.4	20.7	0
e. GW2	W677	Leu^+	207	0	-	76.5	24.2	23.2	0
f. GW3	W677	Leu^+	159	0	-	71.7	24.6	**	0
g. GW3	W677	Leu^+	111	0	-	79.3	20.7	**	0
h. GW1	W677	Thr^+	96	-	4.2	1.0	0	0	0

Each transductant clone was picked, streaked, and purified by single colony isolation on NA (nutrient agar). Transductions a., b. and h. were selected on DMA (Davis Minimal) plus appropriate supplements, while the rest were selected on TGA (Tris Glucose Minimal).

* No test of these transductants was made for azide resistance.
** No test for mutability was possible, since the donors were Mut^-.

20.5% carried the donor mut-1 allele. This indication of the closeness of mut-1 to leu was further tested by the performance of a transduction identical to the first but imposing selection for donor phenotype at the thr locus. The results of this experiment are reported in Table I, h. Among 96 transductants tested, 4% carried the donor leu allele and 1% carried the donor val allele. None of them carried the donor mut-1 allele; in other words, mut-1 was not observably co-transduced with thr.

The results of these experiments indicate a much closer linkage of mut-1 to leu than to thr, but that mut-1 is not so close to leu as is val. On control platings, spontaneous revertants of the recipient strain to either Leu^+ or Thr^+ were never present in concentrations greater than $1/10^8$ cells; corrections for background mutation were therefore unnecessary.

Positions of val and mut-1 in relation to azi and leu. Unambiguous positioning of mut-1 or val to the "left" or "right" of leu cannot be inferred from the data of the first two experiments due to the small number of Thr^+ transductants tested, although location to the "right" is suggested. A locus controlling resistance or sensitivity to sodium azide (azi) has been positioned by transduction (Lennox, 1955) and timed mating (Maccacaro and Hayes, 1961) techniques to the "right" of leu and between it and ton. Since this is the only azide locus which has been described in E. coli and it is co-transducible with leu, azi was assumed as the "right" reference locus for the following transduction experiments. This assumption will be examined later. In order to establish the positions of val and mut-1 unambiguously in relation to leu and azi, two separate transductions were performed imposing selection for Leu^+ and a total of 650 transductants were purified and scored for unselected markers. The results are reported in Table I, b. and c. Among 650 Leu^+ transductants, approximately 80% were Val^r, 19% were Azi^r and 15% were Mut^+ (or mutable). Since frequency of transfer is directly proportional to distance from the selected marker (Lennox, 1955), these data indicate that leu is