DOMINANT MUTATORS IN *ESCHERICHIA COLI*

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Manuscript received August 4, 1981
Revised copy accepted October 15, 1981

ABSTRACT

In this paper we report on the isolation and genetic analysis of a series of strong mutators mapping at five minutes on the *E. coli* chromosome. These mutations are dominant and show no evidence of interaction in merodiploids. Cultures grown in broth medium exhibit mutant frequencies five to six orders of magnitude higher than mut$^+$ strains. Cultures propagated in minimal salts media mutate at rates one to three orders higher than wild-type. Three-factor crosses have been used to order these mutators relative to *metD*, *proA*, and a Tn*10* insertion near five minutes.

THE *mutD* mutator gene of *Escherichia coli* is a powerful mutator that increases mutation rates for all tested classes of transitions, transversions and frameshifts (FOWLER, DEGNEN and COX 1974; COX 1976). This gene has a conditional phenotype. Cultures grown in minimal salts medium mutate at a relatively low rate while cultures grown in L-broth or minimal medium supplemented with thymidine mutate at rates approximately 10^{-6} to 10^{-4} per locus per generation (DEGNEN and COX 1974; FOWLER, DEGNEN and COX 1974). To be active as a mutational effector thymidine must be phosphorylated (ERLICH and COX 1980).

In this paper we report on the isolation of additional mutators with the *mutD* phenotype. These new isolates have been mapped and tested for complementation with *mutD5*, the original isolate. All but one map near *mutD5* are dominant and are characterized by mutant frequencies low in minimal medium and high in L-broth.

MATERIALS AND METHODS

Bacterial strains: Bacterial strains used in this study are listed in Table 1. They are all derivatives of *E. coli* K12. The construction of stocks containing Tn*5* and Tn*10* was by the methods outlined in SHAW and BERG (1979) and KLECKNER, ROTH and BOTSTEIN (1977).

Media: Minimal medium is the minimal salts medium of VOGEL and BONNER (1956). Minimal plates contain minimal medium solidified with 1.5% agar and supplemented with 0.2% glucose and 1 μg/ml thiamine and, when required, 20 μg/ml of the appropriate amino acid. L-broth is 1% Bacto tryptone, 0.5% Bacto yeast extract and 0.5% NaCl. Tryptone plates consist of 1% tryptone and 0.5% NaCl solidified with 1.5% agar. Drug resistant mutant frequencies were measured on tryptone plates containing 50 μg/ml nalidixic acid, 100 μg/ml dihydrostreptomycin sulphate, or 100 μg/ml rifampicin. A solution of 0.85% NaCl was used for diluting and

TABLE 1

Bacterial strains

Strain	Relevant genotype#	Origin
AB1157†	F- *proA2 lacY1 argE3*	DEWITT and ADELBERG 1962
AB1369	F- *proA his-4 cysB38 argE3*	E. ADELBERG
AT713	F- *cysC39 argA8 lysA10 rpsL*	TAYLOR and TROTTER 1967
BW113	Hfr *metB1*	LOW 1973
CGSC4288	F- *metD*+ ▽XIII (*proB-lac*)/F′ *metD*+ *proA*+,*B*+ *lac*+	E. SIGNER
EC1018‡	*mut-18*	P1(AB1157*) × KD1079 → Pro+, Mut-
KD1079	F- *proA2*	DEGNEN and COX 1974
KD1087	F- *mutD5*	DEGNEN and COX 1974
KD1088	F- *mut-14*	P1(AB1369*) × KD1079 → Pro+, Mut-
KH1013	F- *zaf-13*::Tn*10*	P1(W3110::Tn*10*) × KD1079 → Pro+, Tet^R
KH1079	F- *proA2 metB1 lacY1*	P1(TJC13) × KD1079 → Arg+, Met-
KH1080	F- *metD1 proA*+ *metB1*	P1(LT1000) × KH1079 → Pro+, MetD-
KH1113	Hfr *zaf-13*::Tn*10 proA metB1*	P1(KH1301) × BW113 → Tet^R, Pro-
KH1213	F- *zaf-13*::Tn*10*	P1(KH1013) × W3110 → Tet^R
KH1301	F- *zaf-13*::Tn*10 proA*	P1(KH1013) × TJC13 → Tet^R, MetD+, Pro-
KH2079	F- *metD1 mutD5*	P1(LT1001) × KD1079 → Pro+ Mut-, MetD-
KH2080	F- *metD1 mut-14*	P1(LT1002) × KD1079 → Pro+, Mut-, MetD-
KH2081	F- *metD1 zaf-13*::Tn*10*, *mutD5*	P1(KH1013) × KH2079 → Tet^R, Mut-
KH2082	F- *metD1 zaf-13*::Tn*10*, *mut-14*	P1(KH1013) × KH2080 → Tet^R, Mut-
KH2101	F- *metD1 zaf-13*::Tn*10*, *mut-101*	P1•(KH1213) × KH1079 → Mut-
KH2129	F- *metD1 zaf-13*::Tn*10*, *mut-129*	P1•(KH1213) × KH1079 → Mut-
KH2130	F- *metD1 zaf-13*::Tn*10*, *mut-130*	P1•(KH1213) × KH1079 → Mut-
KH2131	F- *metD1 zaf-13*::Tn*10*, *mut-131*	P1*(KH1213) × KH1079 → Mut-
KH3100	F- ▽XIII (*proB-lac*)	Acridine orange cured CGSC4288
KH3103	F- *mutD5* ▽XIII (*proB-lac*)	1. P1(KH2081) × KH3100 → Tet^R, Mut- 2. Fusaric acid cured → Tet^S
KH3104	F- *mut-14* ▽XIII (*proB-lac*)	1. P1(KH2082) × KH3100 → Tet^R, Mut- 2. Fusaric acid cured → Tet^S
KH3105§	F- ▽XIII (*proB-lac*)/F′ *zaf-13*::Tn*10*, *mutD5*	P1(KH2081) × CGSC4288 → Tet^R, Mut-

TABLE 1—Continued

Strain	Relevant genotype#	Origin
KH3106	F⁻ ▽XIII (*proB-lac*)/F′ *zaf-13*::Tn*10*, *mut-14*	P1(KH2082) × CGSC4288 → Tet^R, Mut⁻
KH3107	F⁻ *mutD5* ▽XIII (*proB-lac*)/F′ *mut*⁺ *proA,B*⁺ *lac*⁺	see MATERIALS AND METHODS
KH3108	F⁻ *mut-14* ▽XIII (*proB-lac*)/F′ *mut*⁺ *proA,B*⁺ *lac*⁺	see MATERIALS AND METHODS
KH3109	F⁻ *mutD5* ▽XIII (*proB-lac*)/F′ *zaf-13*::Tn*10*, *mutD5*	see MATERIALS AND METHODS
KH3110	F⁻ *mut-14* ▽XIII (*proB-lac*)/F′ *zaf-13*::Tn*10*, *mut-14*	see MATERIALS AND METHODS
KH3111	F⁻ *mut-14* ▽XIII (*proB-lac*)F′ *zaf-13*::Tn*10*, *mutD5*	see MATERIALS AND METHODS
KH3112	F⁻ *mutD5* ▽XIII (*proB-lac*)/F′ *zaf-13*::Tn*10*, *mut-14*	see MATERIALS AND METHODS
KH3401	F⁻ *zaf-13*::Tn*10 mut-101* ▽XIII (*proB-lac*) *zij-1*::Tn*5*/F′ *zaf-13*::Tn*10*, *mutD5*	see MATERIALS AND METHODS
KH3429	F⁻ *zaf-13*::Tn*10 mut-129* ▽XIII (*proB-lac*) *zij-1*::Tn*5*/F′ *zaf-13*::Tn*10*, *mutD5*	see MATERIALS AND METHODS
KH3430	F⁻ *zaf-13*::Tn*10 mut-130* ▽XIII (*proB-lac*) *zij-1*::Tn*5*/F′ *zaf-13*::Tn*10*, *mutD5*	see MATERIALS AND METHODS
KH3431	F⁻ *zaf-13*::Tn*10 mut-131* ▽XIII (*proB-lac*) *zij-1*::Tn*5*/F′ *zaf-13*::Tn*10*, *mutD5*	see MATERIALS AND METHODS
KH4101	F⁻ *metD1 zaf-13*::Tn*10*, *mut-101*	P1(KH2101) × KH1079 → Mut⁻
KH4129	F⁻ *metD1 zaf-13*::Tn*10*, *mut-129*	P1(KH2129) × KH1079 → Mut⁻
KH4130	F⁻ *metD1 zaf-13*::Tn*10*, *mut-130*	P1(KH2130) × KH1079 → Mut⁻
KH4131	F⁻ *metD1 zaf-13*::Tn*10*, *mut-131*	P1(KH2131) × KH1079 → Mut⁻
KH5101	F⁻ *zaf-13*::Tn*10*, *mut-101* ▽XIII (*proB-lac*)/F′ *mut*⁺	see MATERIALS AND METHODS
KH5129	F⁻ *zaf-13*::Tn*10*, *mut-129* ▽XIII (*proB-lac*)/F′ *mut*⁺	see MATERIALS AND METHODS
KH5130	F⁻ *zaf-13*::Tn*10*, *mut-130* ▽XIII (*proB-lac*)/F′ *mut*⁺	see MATERIALS AND METHODS
KH5131	F⁻ *zaf-13*::Tn*10*, *mut-131* ▽XIII (*proB-lac*)/F′ *mut*⁺	see MATERIALS AND METHODS
LT1000	F⁻ *metD1*	P1(KD1087) × TJC13 → Pro⁺, Mut⁺
LT1001	F⁻ *metD1 mutD5*	P1(KD1087) × TJC12 → Pro⁺, Mut⁻
LT1002	F⁻ *metD1 mut-14*	P1(KD1088) × KD1079 → Pro⁺, Mut⁻
TJC13	F⁻ *metD1 proA* ▽ (*lac-172*) *his metB1 argI*	W. EPSTEIN
W3110	F⁻ prototroph	Cox and YANOFSKY 1969

Symbols follow the conventions of DEMEREC, ADELBERG, CLARK, and HARTMAN (1966) and BACHMANN and Low (1980). Transposon insertion sites are designated according to HONG and AMES (1971).

† For simplicity, only the genotypes relevant to the experiments reported in this paper are described for AB1157 and its derivatives, KD1079 *et seq.* The full KD1079 genotype is: *thr-1 ara-14 leu-6 proA2 lacY1 tsx-33 galK2* ▽ (*tonB trpAB*) *his-4 spcA12* (*rpsE12*) *mtl-1 xyl-5 argE3 thi-1 sup-37*.

‡ Stock construction: the notation used here, *e.g.*, P1(KD1088) × KD1079 → Pro⁺, Mut⁻, means that a P1 lysate propagated on KD1088 was used to transduce KD1079 to Pro⁺, followed by a screen for mutator activity. When this notation grows unwieldy, the reader is referred to MATERIALS AND METHODS for details. A mutagenized P1 or bacterial stock is designated P1* or AB1157*, respectively.

§ KH3105 and its derivatives could be *metD1*, since it arose from a cross in which TJC13 was the donor.

washing cells. Fusaric acid plates, used to cure strains of tetracycline resistance (Tet^R) carried by Tn*10*, were 1% tryptone, 0.5% yeast extract, 1% NaCl, 0.2% glucose, 24 μg/ml Chlorotetracycline·HCl (Sigma), 1% $NaH_2PO_4 \cdot H_2O$, 12 μg/ml fusaric acid and 0.1 mM $ZnCl_2$. All but the fusaric acid and $ZnCl_2$ were sterilized together by autoclaving (20 min, 15 lb pressure). Sterile solutions of fusaric acid and $ZnCl_2$ were added when the autoclaved media had cooled to 50° (B. BOCKNER and B. AMES, personal communication). Tetrazolium lactose plates were prepared according to MILLER (1972). Tet^R transductants were selected on minimal plates containing 15 μg/ml Tetracycline (Sigma) or on L-broth plates containing 20 μg/ml.

Isolating new mutD *alleles*: Two methods were used. In the first (*mut-14*, *mut-18*) the appropriate strain was mutagenized in 0.01 M maleate buffer, pH 6.5 containing 100 μg/ml N-methyl-N′-nitro-N-nitrosoguanidine at 37° for 20 min. Following two washes in saline, cells were diluted and plated on minimal plates in 0.5% top layer minimal agar, approximately 2,000 colonies per plate. When the colony size was approximately 0.5 mm each plate was stained for constituitive β-galactosidase and alkaline phosphatase synthesis, essentially by the methods described in HOMBRECHER and VIELMETTER (1979), using 6-bromo-2-naphthyl-β,D-galactoside and naphthol-AS-MX-phosphate coupled with Fast Blue RR base. Colonies sectored for both β-galactosidase and alkaline phosphatase were purified and checked for mutator activity. In the second method (*mut-101*, *129*, *130*, *131*) a *zaf-13*::Tn*10* P1vir lysate was concentrated and mutagenized with hydroxylamine (HONG and AMES 1971; HONG, personal communication). Following transduction and selection for Tet^R on minimal-tetracycline plates at 30°, colonies were replica printed onto a second plate and incubated at 45°, at which temperature *mutD* alleles are slightly temperature sensitive. Isolates using both methods were crossed out of the parent strain and back-crossed at least once before they were used for further studies (see, for example, the pedigree of KH4101, Table 1).

Mutant frequencies: Five tubes containing the appropriate media were inoculated with a mutant-free inoculum containing a few thousand cells and grown overnight with aeration at 37°. Samples from each tube were plated on appropriate selective plates and simultaneously diluted in saline for total cell number on tryptone plates. Mutant frequencies are reported as the average number of mutants divided by the average number of cells. For mutation rates as high as those studied here the variance among independent cultures started from a single revertant-free colony is low, as would be expected (LEA and COULSON 1949; Table 5).

Acridine orange curing: Merodiploids for the *mutD* region were converted to haploids by culturing cells in L-broth containing 60 μg/ml acridine orange (MILLER 1972) followed by plating on tetrazolium-lactose plates.

Transduction: Mapping and stock construction was accomplished by standard methods with P1vir (FOWLER, DEGNEN and COX 1974). Mutator linkage was determined by spotting 25 μl of a saturated L-broth culture, started from a purified colony, on two antibiotic plates, one containing nalidixic acid, the other rifampicin (DEGNEN and COX 1974). This procedure allows one to score Mut^+ and Mut^- colonies with a high degree of confidence.

Episome transfer: Donor and recipient cells were mated by standard techniques (MILLER 1972). With strains carrying *zaf-13*::Tn*10* on the episome it was possible to use Tet^R for selection since tetracycline resistance is dominant. This method was used in a two-step procedure to select for *mutD* on the F′*lac* episome carried by CGSC4288 (for example, KH3105, Table 1). A P1 lysate grown on an F^- *zaf-13*::Tn*10 mutD* haploid was used as donor and CGSC4288 as recipient. Greater than 95% of the Tet^R transductants in this cross integrate in the chromosome (data not shown). The transduction mixture was therefore grown to saturation, diluted several fold, and mated with the appropriate recipient. Tet^R was used to select for the merodiploid and against the haploid recipient. CGSC4288 was chosen for these studies because in preliminary crosses with TJC13 we showed that the CGSC4288 episome spanned the *metD-lac* interval. Kan^R, carried by Tn*5* integrated near *argE*, was used in several crosses to select for the recipient and against the donor (KH3401 *et seq.*).

RESULTS

Isolation of zaf-13::*Tn*10: The *mutD* region of the chromosome is poorly marked. To overcome this difficulty we isolated a series of Tn*10* insertions in the

metD-proA-lac region using standard methods (KLECKNER, ROTH and BOTSTEIN 1977; KLECKNER, personal communication). One such isolate, *zaf-13*::Tn*10*, is tightly linked to *mutD5*, as the results in Table 2 show. This insertion confers no detectable phenotype on the cell other than Tet^R and was used extensively in this study.

TABLE 2

mutD *linkage analysis: selection for* proA+

	P1 donor*:	*metD1*	*mutD*	*zaf-13*::Tn*10*		+	
	Recipient†:	+	+	(—)		*proA2*	
	Selection:	Pro+					
Recombinant phenotype			Donor allele				
Met	Mut	Tet	*mutD5*	*mut-14*	*mut-101*	*mut-129*	*mut-130*	*mut-131*
+	+	S	84	89	91	63	71	72
—	+	S	1	0	0	2	0	0
+	—	S	0	0	1	0	0	0
—	—	S	1	0	0	0	0	0
+	+	R	5	4	6	21	14	5
—	+	R	0	0	0	8	1	1
+	—	R	6	5	2	2	8	10
—	—	R	3	2	0	0	5	7

* P1 lysates were grown on KH2081, KH2082, KH4101, KH4129, KH4130, and KH4131 containing the mutators *mutD5*, *mut-14*, *mut-101*, *mut-129*, *mut-130*, and *mut-131*, respectively.
† The recipient was KH1079.

New mutators: *cytochemical screening*: *mutD5* was originally isolated by testing mutagenized colonies for high mutation rates to streptomycin and nalidixic acid resistance (Str^R and Nal^R, respectively). Additional mutators with the *mutD* phenotype (a high mutation rate dependent on L-broth) were detected by examining several hundred thousand colonies stained with cytochemical dyes chosen to detect *lacI phoR* sectors in the same colony. These were invariably due to mutator action. Two new isolates linked to *proA* by P1 transduction, EC1018 (*mut-18*) and KD1088 (*mut-14*), were crossed out of the mutagenized background (AB1369 and AB1157, respectively) into KD1079 for further study.

New mutators: *linked mutagenesis*: HONG and AMES (1971) showed that temperature sensitive mutations linked to a known marker can be isolated following mutagenesis of a P22 transducing lysate. We have used this method to isolate additional mutators linked to *zaf-13* by mutagenizing with hydroxylamine a P1 lysate propagated on W3110 *zaf-13*::Tn*10 mut*+. By selecting first for Tet^R and then screening by replica printing for temperature sensitivity at 45° we have isolated four additional mutators in the *zaf-13* region initially designated *mut-101*, *mut-129*, *mut-130* and *mut-131* (KH2101-KH2131, Table 1). These mutants were isolated by screening 8700 Tet^R transductants. This screen takes advantage of the fact that *mutD* colonies are slighlty growth sensitive on minimal medium at 45°, although mutant frequency is not (data not shown).

Mapping mut-14, 18, 101, 129, 130, 131: Our mapping strategy was to cross newly isolated mutators out of the original background into KH1079, selecting for Pro+ and screening for Mut-. Both KD1079 *mut-14* and KD1079 *mut-18* were

subsequently transduced to Tet^R with a P1 lysate propagated on KH1013 (*zaf-13*::Tn*10*). Then P1 lysates made on each strain carrying the mutator and Tn*10* were used as donors with various recipients.

TABLE 3

mutD *linkage analysis: selection for* zaf-13::*Tn*10

P1 donor*:			*metD1*	*mutD*	*zaf-13*::Tn*10*		+	
Recipient†:			+	+	(—)		*proA2*	
Selection:			Tet^R					
Recombinant phenotype			Donor allele					
Met	Mut	Pro	*mutD5*	*mut-14*	*mut-101*	*mut-129*	*mut-130*	*mut-131*
+	+	+	0	0	1	1	0	0
—	+	+	0	0	0	2	0	3
+	—	+	8	15	26	16	24	24
—	—	+	2	1	10	10	14	6
+	+	—	1	3	0	1	1	0
—	+	—	0	0	0	4	0	2
+	—	—	15	12	5	10	4	6
—	—	—	24	19	6	4	5	6

* P1 lysates were grown on KH2081, KH2082, KH4101, KH4129, KH4130, and KH4131 containing the mutator alleles *mutD5*, *mut-14*, *mut-101*, *mut-129*, *mut-130*, and *mut-131*, respectively.

† The recipient was KH1079.

The linkage and gene order of *mutD5* and *mut-14* depends on both the donor and recipient, apparently because various *E. coli* K12 strains are polymorphic for inversions in this region of the chromosome (Cox and Horner, in preparation). However, a consistent and stable linkage and gene order for *mutD5*, *mut-14*, *101*, *129*, *130* and *131* can be established by choosing the appropriate recombinants from the appropriate crosses. These results are presented in Tables 2 and 3 and summarized in Figure 1. All six mutators appear to map to the same region of the

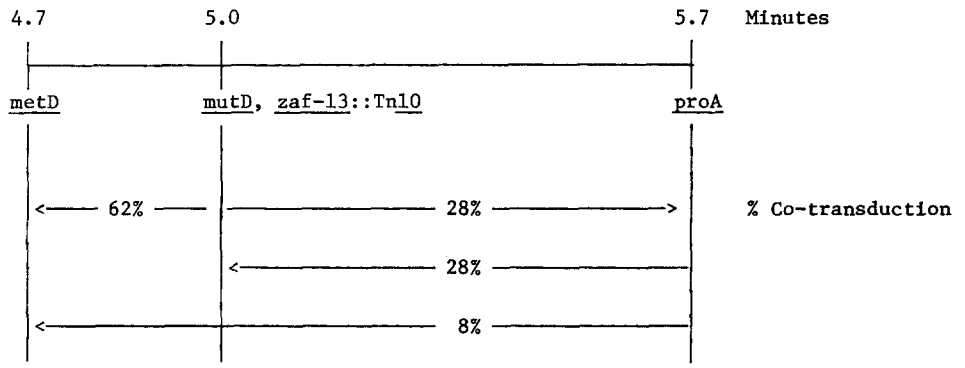

Figure 1.—Map of the *metD mutD proA* region. This map is based on average cotransduction frequencies taken from Tables 2 and 3, using the mapping function of Wu (1966) and assuming that *proA* is correctly placed close to six minutes (Bachmann and Low 1980). The *mutD-3af-13*::Tn*10* order is based on the preponderance of Pro+ Tet^R Mut+ over Pro+ Tet^S Mut- recombinants. The arrows originate at the selected marker.

chromosome, and they exhibit the same antibiotic resistance spectrum at roughly the same rate and in response to L-broth (Table 4).

The initial *mut-18* isolate was backcrossed into KD1079. One Pro$^+$ transductant was mutator, responded to L-broth and added thymidine (Table 4), showed the same spectrum of mutant frequencies to antibiotic resistance, and mutated at about the same rates at various loci as *mutD5*. However, in subsequent P1 transduction experiments using a KD1079 *mut-18* donor, *mut-18* proved not to be linked to *proA* (0/100 Pro$^+$ transductants). Nor could *mut 18* be crossed out of the KD1079 *mut-18* background in a P1 (KH1013) × KD1079 *mut-18* cross with selection for Tet^R (0/200 transductants).

Three features of the data stand out. First, the gene order, as deduced from the relative frequency of single and double crossovers, is *mutD zaf-13 proA* in these crosses. Second, although the variance within the five samples used to determine mutant frequency is low (for typical values in L-broth see the caption to Table 5), there is nonetheless considerable variation between replicas determined in both minimal medium and L-broth for the same strain, and between various mutator isolates in minimal, TdR supplemented minimal, and L-broth. This high variance in the data obtained in different experiments started from different single colonies appears to be attributable to the unavoidable accumulation of modifiers of both the mutation rate and the expression of mutants at various loci. It could mask intrinsic differences between the various isolates studied here but it is not, in our experience, avoidable. Third, *mut-101* and *mut-129* do not respond to added TdR, although the maximum mutation rate in L-broth does not differ significantly from other *mutD* isolates (Table 4).

Complementation studies: *mutD5* and *mut-14* have been tested in *trans* with *mut*$^+$ and with each other. These results are summarized in Table 5, where Rif^R, Nal^R and Str^R mutant frequencies are reported for growth in L-broth and minimal medium (minimal medium supplemented with thymidine does not increase the mutant frequency in CGSC4288 and so could not be tested).

TABLE 4

Haploid mutant frequencies

Strain	Mutator	Mutant frequencies × 10^{9}* Nal^R LB	Nal^R TdR	Nal^R Min	Str^R LB	Str^R TdR	Str^R Min
KD1079	*mut*+	1.0	1.8	0.5	<0.4	<0.4	<0.4
EC1018	*mut-18*	5,900	3,500	720	17,000	3,700	110
KH2081	*mutD5*	44,000	76,000	11,000	21,000	11,000	570
KH2082	*mut-14*	56,000	13,000	3,200	32,000	13,000	60
KH4101	*mut-101*	110,000	410	550	40,000	75	8
KH4129	*mut-129*	43,000	540	320	23,000	<5	<1
KH4130	*mut-130*	47,000	49,000	5,900	11,000	13,000	3,700
KH4131	*mut-131*	51,000	36,000	2,000	6,000	4,700	400

* Mutant frequencies were determined as described in MATERIALS AND METHODS in L-broth (LB), minimal medium supplemented with 2×10^{-6} M thymidine (TdR), and minimal medium (Min).

It is clear that *mutD* is dominant. In general there is no evidence for complementation between *mutD5* and *mut-14* and no significant differences between the measured mutant frequencies for any of the *mut*+, *mutD5* and *mut-14* combinations shown in Table 5. Those differences that we see do not suggest systematic differences between *mutD5* and *mut-14*. For example, even though the Rif^R and Nal^R frequencies are roughly four-fold higher in KH3105 than KH3106, there is no measurable difference in Str^R for these two strains and the apparent differences between *mutD5* and *mut-14* depend on the particular stock, are not reproducible, and appear to be attributable to background differences that rapidly accumulate.

Because these results show that *mutD* is dominant it was important to show unambiguously that the CGSC4288 episome was not deleted for *mutD*, thus allowing the expression of a recessive allele on the chromosome. This was done in two ways. For those partial diploids with *mutD5* and *mut-14* on the *episome* (KH3105, KH3106) we showed that the Mut⁻ phenotype could be transferred into TJC13 and that acridine orange curing of the parent merodiploid produced Mut+ Lac⁻ Pro⁻ segregants at high frequency. For those stains carrying *mutD* on both the endogenote and exogenote (KH3109-KH3112) we demonstrated that the Mut⁻ phenotype could be transferred with high frequency into TJC13. It should also be noted that the method used to construct KH3105 and KH3106 is in itself good evidence that the CGSC4288 episome carried the *mutD* region (see Table 1).

In general, the merodiploids used in this study are stable, exhibiting little tendency to form homogenotes. In preliminary studies using an exogenote derived in this laboratory from BW113 and a *recA* derivative of KD1079, heterogenotes proved to be unstable with a tendency to form homogenotes with high frequency. These results have not been pursued further.

TABLE 5

mutD5 *and* mut-14 *complementation results*

Strain	Mutator genotype†	Mutant frequencies × 10⁹* Rif^R LB	Rif^R Min	Nal^R LB	Nal^R Min	Str^R LB	Str^R Min
KH3100	*mut*+	27	19	0.44	0.39	0.1	<0.4
KH3103	*mutD5*	192,000	7,290	53,000	54	3,630	33
KH3104	*mut-14*	63,400	1,500	14,300	26	2,880	26
CGSC4288	*mut*+/*mut*+	0.85	<1.0	1.02	1.3	<0.1	<0.1
KH3107	*mutD5*/*mut*+	131,000	2,030	54,700	39	6,760	22
KH3108	*mut-14*/*mut*+	120,000	750	55,600	5.0	8,010	10
KH3105	*mut*+/*mutD5*	197,000	3,520	72,300	26	7,650	0.5
KH3106	*mut*+/*mut-14*	53,400	1,790	22,700	28	5,690	34
KH3109	*mutD5*/*mutD5*	183,000	767	54,500	9	4,550	<0.4
KH3110	*mut-14*/*mut-14*	86,200	1,840	7,940	28	3,430	11
KH3111	*mut-14*/*mutD5*	124,000	1,520	86,300	84	8,260	71
KH3112	*mutD5*/*mut-14*	55,300	320	10,600	<1	2,680	<0.6

* Drug resistant mutant frequencies were determined as described in MATERIALS AND METHODS in L-broth (LB) and minimal (Min) medium. Some typical results for KH3103 and KH3104 Nal^R frequencies in L-broth (± SD) are: 5.30 (± 0.61) × 10^{-5} and 1.43 (± 0.29) × 10^{-5}; for KH3110 and KH3111, 7.9 (± 1.63) × 10^{-6} and 8.62 (± 0.61) × 10^{-5}, respectively.

† For diploids the endogenote genotype is followed by the exogenote.

mut-101, 129, 130 *and* 131 *are dominant*: Table 6 summarizes data showing that these isolates are dominant. The procedure used to construct these strains (see Table 1), plus the observations that the CGSC4288 episome covers the *metD-proA* region (MATERIALS AND METHODS) and can transfer both *zaf-13*::Tn*10* and *mutD*, provide the evidence that the genotypes assigned are correct. The differences between the measured mutant frequences in the strains used in Table 6 are not reproducible, probably for the reasons mentioned above.

Complementation studies: We have tested *mut-101*, *129*, *130* and *131* with *mutD5* in *trans* (Table 7). It is clear that these mutators do not complement *mutD5* and that mutant frequencies in minimal medium and L-broth are very similar if not identical to haploid mutant frequencies.

DISCUSSION

Mutator phenotypes: The new isolates described here, including *mut-18*, which could not be located following the first back-cross into KD1079, are alike in that the mutant frequency is low in minimal salts medium and high in L-broth. Both *mut-101* and *mut-129*, however, do not respond to added TdR with an increase in mutation rate (Table 4). The failure to respond is not attributable to a requirement for a higher concentration of TdR in the growth medium, since both mutators have been tested at 10^{-6} M (Table 4) and 10^{-4} M (data not shown). The optimum for *mutD5* is approximately 10^{-6} M (DEGNEN and Cox 1974). Increased concentrations beyond 10^{-6} M neither increase nor decrease the mutation rate in *mutD5* strains (ERLICH and Cox 1980).

TABLE 6

mut-101, 129, 130, 131 *dominance tests*

Strain	Mutator genotype	Mutant frequencies × 10^{9}* Rif^R LB	Rif^R Min	Nal^R LB	Nal^R Min	Str^R LB	Str^R Min
KH5101	*mut-101/mut*+	40,600	3,880	9,500	73	7,190	21
KH5129	*mut-129/mut*+	58,700	1,890	19,000	896	14,900	34
KH5130	*mut-130/mut*+	75,000	873	93,300	1,550	56,500	6
KH5131	*mut-131/mut*+	133,000	930	12,900	<46	4,040	<46

* Mutant frequencies were determined as described in MATERIALS AND METHODS.

TABLE 7

Complementation studies with mut-101, 129, 130, 131 *and* mutD5

Strain	Mutator alleles†	Mutant frequencies × 10^{9}* Rif^R LB	Rif^R Min	Nal^R LB	Nal^R Min
KH3401	*mut-101/mutD5*	25,000	7,250	91,800	313
KH3429	*mut-129/mutD5*	58,900	7,600	31,500	299
KH3430	*mut-130/mutD5*	124,000	6,380	37,600	795
KH3431	*mut-131/mutD5*	37,800	795	18,900	540

* Mutant frequencies were determined as described in MATERIALS AND METHODS.
† The endogenote is followed by the exogenote.

It should now be possible to ask if this failure to respond to TdR is dominant or recessive, since *mut-101* can be studied in merodiploids with a responder, such as *mutD5*, in *trans*. Unfortunately, this test cannot be carried out with the stocks used here, since CGSC4288 strains containing *mutD5* do not respond to added TdR (data not shown).

Are the mutation rates caused by the various mutators reported here the same? As noted earlier, and as may be seen in Tables 4 to 7, there is considerable variability between repeated measurements carried out on the same strain, and thus we can only say that mutation rates induced by the mutators described here fall within the same range. However, it is worth noting that during the isolation of *mut-14* many other mutators with mutation rates 10 to 100-fold lower than these, but mapping elsewhere, were also isolated (data not shown). This result argues, to a first approximation, that most mutants with the *mutD* phenotype are likely to have similar high mutation rates.

The mutD *region:* We have found (Cox and HORNER, in preparation) that the *mutD*, *zaf-13*::Tn*10-proA* cotransduction frequencies vary depending on the background, and that the *mutD*, *zaf-13*::Tn*10* order is also variable. Our interpretation of these results is that *E. coli* K12 strains are polymorphic for an inversion in this region, and that the two parental strains from which *mutD5* and *mut-14* were derived, AB1157 and AT713, respectively, contain a *zaf-13-mutD* inversion relative to KH1113. Nonetheless, once a recombinant within the inversion has been used as a donor in a backcross, a stable linkage and gene order is established for a given recipient, and all but *mut-18* map to the same position (Figure 1).

The *metD-proA* region of the chromosome has been mapped in detail in two other laboratories. Our result for the *metD-proA* cotransduction frequency (8%) agrees well with those of LATHE (1977), who found cotransduction frequencies of 7% in a detailed study of the *leu-proA* interval, and HORIUCHI, MAKI and SEKIGUCHI (1978), who found 6.8% cotransduction for these two genes.

Other mutators have been mapped to this general region of the chromosome. One, named *dnaQ49*, shows 16% linkage to *proA* and exhibits mutation rates to drug resistance at 40° comparable to those reported here (HORIUCHI, MAKI and SEKIGUCHI 1978). The *dnaQ49* isolate mutates at a rate similar to *mutD5* in minimal medium when it is cultured at 30°. DNA replication is slowed at 44.5°. The difference in cotransduction frequencies between *dnaQ49*, *mutD5* and *proA* may not be significant, for, as we have mentioned, cotransduction frequencies in the *proA-metD* region are exceptionally strain dependent (Cox and HORNER in preparation). Complementation studies with *dnaQ49* have not been published and so further comparison with *mutD5* cannot be made.

HOMBRECHER and VIELMETTER (1979) have isolated several different mutators that also map in the *proA-metD* region. These mutators mutate at a higher rate in broth cultures than in unsupplemented minimal medium. However, the mutation rates to Nal^R in broth are roughly two orders of magnitude lower than those reported here and one isolate, *mut-8*, is recessive and the mutator phenotype is *recA*-dependent. Thus *mut-8* is probably not an allele of *mutD* since *mutD5*

activity is *recA*-independent (DEGNEN and Cox 1974) as are the other mutators described here (data not shown).

Complementation tests: All of the mutator isolates studied are dominant and none shows interaction with another. *mutD5* and *mut-14* have been tested in all permutations, both with wild-type and each other. It is important to recognize that changes in mutation rate in diploids resulting from complementation would have to be roughly 5-fold to be studied reliably. Thus there could be substantial interaction between these various isolates which we would miss at these high mutation rates. At the same time, it is worth recalling that recessive mutators such as *mutT*, *mutL*, *mutR*, *mutS* and *uvrE* exhibit wild-type mutation rates in mut^+/mut^- merogenotes, and hence recessivity itself is not difficult to detect in mutator strains (Cox 1976).

There are several plausible interpretations for the dominance results reported here.

First, it is possible that the *zaf-13* region of the chromosome codes for several genes whose mutant products act as dominant mutators. This possibility cannot be formally eliminated at this time, although we do not favor such an explanation.

Second, all but *mut-18* could be allelic and dominant because they are mutant in regulator genes that code for some step in proof-reading. Such mutations would be analogous to i^d and O^c mutants in the *lac* operon. They can be distinguished from each other by the appropriate *cis/trans* tests (SMITH and SADLER 1971). At the moment, however, we have no mutator product that can be so assayed.

Third, and assuming that *mutD5*, *14*, *101*, *129*, *130* and *131* are allelic, the mutator product could participate in a multimeric structure involved in DNA replication or editing which in an altered conformation causes high mutation rates. In such a situation those multimers which were wild-type, even though the probability of a wild-type multimer might be low in a mut^+/mut^- diploid, would not contribute to the number of mutants in the culture and would not measurably decrease the number of mutants caused by the mutator product. A model of this kind predicts that dominance is a function of genomic copy number. For a multicopy episome or plasmid carrying the mut^+ product over a *mutD* chromosome the mutator phenotype should be recessive if competition between mut^+ and *mutD* subunits for a small number of multimeric structures is used to explain the data. Such an explanation is favored by the results of DEGNEN (1974; DEGNEN and Cox, in preparation). This model would also apply to any multimeric structure used in small numbers, be it a regulatory or catalytic multimer (reviewed for the *lacI* gene by MILLER 1980).

The results described here and discussed above do not allow one to distinguish between these several quite different hypotheses. Neither do they address the question of the role played by TdR and other factors in L-broth in turning on the high mutating state (ERLICH and Cox 1980). Presumably, TdR acts directly or indirectly either to regulate the synthesis of the *mutD* product (model two) or modulate the activity of a multimeric structure (model three).

This work was supported by Public Health Service grant GM28923. We thank many colleagues for supplying strains, but especially N. KLECKNER for the detailed methods and stocks used to construct Tn*10* derivatives.

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Corresponding editor: J. W. DRAKE