MUTATOR GENE STUDIES IN *ESCHERICHIA COLI*: THE *mutS* GENE[1]

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Manuscript received May 19, 1972
Revised copy received August 3, 1972

ABSTRACT

We report here on a study of a mutator gene (*mutS*) that causes transition mutations in *Escherichia coli*. We have used the *trpA* system to show that A:T→G:C and G:C→A:T transitions occur. Not all A:T pairs are equally susceptible to *mutS* action however, since the A:T pair at the *trpA223* site reverts at a frequency similar to, if not identical with, the frequency in a *mut*+ background. Presumably this is a consequence of neighboring bases, because other A:T pairs are reverted by *mutS* in the same gene; and an A:T pair in the *lac* operon is reverted at two widely separated points on the chromosome, and in two orientations relative to the *trp* sense strand. In addition, we have shown that the *mutS1* allele is recessive to wild type, and *trans* active.

THE appearance of new mutants in a population is an essential part of the evolution of a species. How new mutants arise is poorly understood, but there are growing experimental data to suggest that many, if not all, spontaneous mutations arise as errors in DNA replication, recombination or repair (SPEYER, KARAM and LENNY 1966; DRAKE *et al.* 1969; COX 1970; WITKIN 1969; GREEN 1970; VON BORSTEL, CAIN and STEINBERG 1971). These studies have shown that the mutation rates in T4, *Escherichia coli* and *Drosophila melanogaster* are under direct genetic control, since mutations in several different genes increase (T4, *E. coli*, Drosophila, yeast) or decrease (T4) the spontaneous mutant frequency. Additional evidence supporting the idea that mutation rates are controlled by the cell comes from studies of *D. melanogaster* populations isolated from various regions (PLOUGH 1941; IVES 1950; WALLACE 1970), where a range of mutation rates between populations has been observed for the first, second and third chromosomes.

The molecular basis for increased or decreased mutation rates is not presently known. The most complete data show that a mutant DNA polymerase directly controls mutation rate in T4: Some alleles act as mutator genes (SPEYER *et al.* 1966; DRAKE *et al.* 1969), others an antimutator genes (DRAKE *et al.* 1969). How the polymerase is involved in control of mutation rate has not been determined, but these studies make it clear that the wild-type rate in T4 is not the realizable minimum, again showing that mutation rate can be adjusted. In *E. coli*

[1] Supported by grants from NIH and NSF.

the data are not as complete, but a mutator gene that increases A:T→C:G transversions (YANOFSKY, Cox and HORN 1966) is active only during DNA replication (Cox 1970). Other systems are more poorly defined, but available evidence suggests that the known properties of the T4 and *E. coli* systems apply to yeast (VON BORSTEL *et al.* 1971) and Drosophila (GREEN 1970), since mutation rates in yeast increase in repair defective strains (VON BORSTEL *et al.* 1971), and there is a *D. melanogaster* mutator stock that is active in females only (GREEN 1970). This latter observation suggests that recombination is one point at which Drosophila can control mutation rate.

In this paper we report on a genetic study of the *E. coli* mutator gene (*mutS1*) isolated by SIEGEL and BRYSON (1964). These authors showed that 2-aminopurine increased the reversion frequency of *mutS1* induced auxotrophs. They inferred from this observation that the *mutS1* allele caused transition mutations, an inference based on the well documented action of 2-aminopurine in *E. coli* and T4 (DRAKE 1970). We have examined this question in more detail with the *trpA* system (YANOFSKY, ITO and HORN 1966) and show here that the *mutS1* allele is specific for transition mutations, in agreement with SIEGEL and BRYSON (1967). However, we have found that not all A:T pairs are equally susceptible, and conclude that nearest neighbors can influence mutant frequency in this strain. In addition, we have mapped the gene more accurately and have shown that it is recessive in merodiploids, and *trans* active.

MATERIALS AND METHODS

Bacteria: The relevant characteristics of all strains are given in Table 1. Unless noted otherwise, all *mut*+ and *mutS1* stocks whose mutation frequencies are compared are Cys+ cotransductants isolated from the same cross.

Media: VB medium is the minimal salts medium of VOGEL and BONNER (1956); M63 is that of PARDEE, JACOB and MONOD (1959). Minimal plates consist of minimal medium solidified with 1.5% agar and supplemented with 0.2% carbon source. TZ plates are tetrazolium indicator plates (OHLSSON, STRIGINI and BECKWITH 1968) supplemented with 0.4% sugar; MC plates are MacConkey's plates prepared according to the manufacturer (Difco) and supplemented with 0.4% sugar. Tryptone broth is 1% "Bacto" tryptone and 0.5% NaCl in distilled water; tryptone plates consist of tryptone broth solidified with 1.2% agar. H broth contains 100 μg/ml acridine orange and is described by HIROTA (1960). All amino acid and vitamin supplemented minimal plates or cultures contained 50 μg/ml of the L-amino acid and 1 μg/ml of the vitamin. Azide, streptomycin,

TABLE 1

Bacterial strains used

Strain	Mut genotype	Relevant phenotypic characteristics* Lys	Arg	Cys	Trp	Prd	Lac	Spc	Sm	Sex	Other characteristics†
KD1001	+	—	—	+	+	—	+	R	S	F⁻	su° for UAG, UAA, UGA A. L. TAYLOR
KD1002	*S1*	—	—	+	+	—	+	R	S	F⁻	
KD1013	+	—	—	—	+	+	+	R	S	F⁻	Grows on 0.2% propanediol (WU, CHUSED and LIN 1967)

TABLE 1—(Continued)

Strain	Mut genotype	Relevant phenotypic characteristics* Lys	Arg	Cys	Trp	Prd	Lac	Spc	Sm	Sex	Other characteristics†
KD1016	*S1*	—	—	+	+	+	+	R	S	F⁻	
KD3001	+	—	—	—	*del*	—	+	R	S	F⁻	ϕ80 *vir/colE* selection on KD1001
KD3002	*S1*	—	—	+	*del*	—	+	R	S	F⁻	
KD3003	+	—	—	+	*del*	—	+	R	S	F⁻	
KD3017	*S1*	—	—	+	+	+	+	R	S	F⁻	
KD4000	+	+	+	+	+	—	*del*/—	S	R	F+	*lac del*/ϕ80d$_{II}$*lacU118* (SIGNER and BECKWITH 1966; NEWTON *et al.* 1965)
KD4002	*S1*	+	+	+	+	—	*del*/—	S	R	F+	*lac del*/ϕ80d$_{II}$*lacU118*
KD4003	+	+	+	+	+	—	*del*/—	S	R	F+	*lac del*/ϕ80d$_{II}$*lacU118*
KD4032	*S1*	+	+	+	+	—	*del*/—	S	R	F+	*lac del*/ϕ80d$_{I}$*lacU118*
KD4033	+	+	+	+	+	—	*del*/—	S	R	F+	*lac del*/ϕ80d$_{I}$*lacU118*
KD5025	*S1*	+	+	+	+	+	*del*/—	S	R	F+	*lac del*/ϕ80d$_{II}$*lacX90*
KD5026	+	+	+	+	+	+	*del*/—	S	R	F+	*lac del*/ϕ80d$_{II}$*lacX90*
KD5032	*S1*	+	+	+	+	+	*del*/—	S	R	F+	*lac del*/ϕ80d$_{I}$*lacX90*
KD5033	+	+	+	+	+	+	*del*/—	S	R	F+	*lac del*/ϕ80d$_{I}$*lacX90*
265,288	+	—	—	+	—	—	*del*/—	S	R	F+	*lac del*/ϕ80d$_{I}$*lacX90*
398,402	*S1*	—	—	+	—	—	*del*/—	S	R	F+	*lac del*/ϕ80d$_{I}$*lacX90*
229,230	+	—	—	+	—	—	*del*/—	S	R	F+	*lac del*/ϕ80d$_{II}$*lacX90*
245,252	*S1*	—	—	+	—	—	*del*/—	S	R	F+	*lac del*/ϕ80d$_{II}$*lacX90*
KD1001/ F143	+/+	—/+	—/+	+/+	+	+/—	+	S	R	F′	
KD1002/ F143	*S1*/+	—/+	—/+	+/+	+	+/—	+	S	R	F′	
KL16-99	+	+	+	+	+	—	+	S	S	Hfr	*recA1*; B. Low
KD3010	+	—	—	+	—	—	*del*/—	R	S	F′	*trpA46*, F′*lacX90*
KD3011	*S1*	—	—	+	—	—	*del*/—	R	S	F′	*trpA46*, F′*lacX90*
KD3012	+	—	—	+	—	—	*del*/—	R	S	F′	*trpA223*, F′*lacX90*
KD3013	*S1*	—	—	+	—	—	*del*/—	R	S	F′	*trpA223*, F′*lacX90*
KD4041	+	—	—	+	—	—	—	R	S	F⁻	*lacX90 trpA223*
KD4042	*S1*	—	—	+	—	—	—	R	S	F⁻	*lacX90 trpA223*
trpA strains											YANOFSKY *et al.* 1966a,b
MX74	+	+	+	+	+	—	*del*	S	R	F⁻	*lac del*
MX90	+	+	+	+	+	—	—	S	R	F⁻	*lacX90*
Mo	+	+	+	+	+	—	+	S	R	F⁻	

* Abbreviations: Lys, lysine; Arg, arginine; Cys, cysteine; Trp, tryptophan; Prd, propanediol; Lac, lactose; Spc, spectinomycin; Sm, streptomycin; +, independence when used with an amino acid, non-mutator when used to describe a mutator phenotype, ability to ferment when used with a sugar; —, dependence when used with an amino acid, mutator when used to describe a mutator phenotype, inability to ferment when used with a sugar; R, resistance to a phage or drug; S, sensitivity to a phage or drug; *del*, a deletion of the character; su°, lack of ability to suppress amber, ochre and opal mutants of T4 *rII*; —/+, mutant allele on the endogenote, wild type on the exogenote; *del*/—, deletion on the endogenote, mutant allele on the exogenote; *S1*/+, mutator allele on the endogenote, wild-type on the exogenote.

† In this column additional pertinent genotypes or phenotypes are recorded, with reference to the strain source if it was not constructed in this laboratory. KD1001 is an acridine orange cured F⁻ derivative of AT713, originally received from A. L. TAYLOR. Other strains, with the exception of the KD5000 series, KL16-99, MX74, MX90 and Mo are descendants of KD1001, prepared by P1 and Hfr crosses or, in the case of KD1013, by nitrosoguanidine mutagenesis. Genotypes and phenotypes are written, where possible, according to the recommendations of DEMEREC *et al.* 1966.

and nalidixic acid resistance was measured on tryptone plates containing 5×10^{-3}M sodium azide, 100 μg/ml dihydrostreptomycin or 50 μg/ml nalidixic acid, respectively.

Transductions: P1kc transductions were carried out as described by YANOFSKY and LENNOX (1959) using UV-irradiated lysates. Each 2 ml P1kc lysate in L-broth (LENNOX 1955) was irradiated for 5 min in a 55 mm Petri dish with a GE germicidal lamp at 30 cm. Experiments with defective φ80 phage and φ80 lysogens were conducted exactly as described by SIGNER and BECKWITH (1966). In all cases their criteria for the site of phage insertion were followed explicitly.

Bacterial crosses: Hfr × F⁻ crosses were performed with freshly isolated stocks as described by Low and WOOD (1965).

Detecting the mutS1 *allele:* An overnight tryptone broth culture of a *mutS1* population contains approximately 100 streptomycin-resistant (Sm^R) cells/ml. This frequency is high enough to detect the mutator gene by replicating onto tryptone-dihydrostreptomycin plates an isolate spotted or streaked on a portion of a tryptone plate. Mutator clones produce many Sm^R papillae within the replica, while Mut+ clones produce none. When a stock is Sm^R to begin with, we assay for azide or T5 resistance on tryptone plates containing 2×10^{-3}M sodium azide, or tryptone plates spread with approximately 10^{10} T5 particles and supplemented with 10^{-3}M $CaCl_2$.

5-methyl tryptophan inhibition tests: This method follows an unpublished procedure of YANOFSKY. Purified single-colony isolates of Trp+ revertants are grown with aeration in VB glucose medium until they reach late log phase. The culture is diluted to 10^6 cells/ml, 0.1 ml is mixed with 2.5 ml VB glucose medium containing 0.65% agar, and the mixture is poured onto a VB glucose plate. When the top layer has solidified, 80 μg of 5-methyl tryptophan (5-MT) in 0.04 ml distilled water is pipetted onto a 13 mm filter paper disc placed on the center of the plate. The plates are then incubated for at least 24 hr at 37°, and the diameter of the zone of 5-MT inhibition is measured. Full revertants grow to within 2 or 3 mm of the disc; partial revertants or suppressed mutants are inhibited to a larger extent by 5-MT and show much larger zones of inhibition. The diameter of the zone is a sensitive and reproducible index of the reversion pattern and distinguishes accurately between full and partial revertants.

Enzyme assays: Enzymes of the *lac* operon were assayed by published procedures (WILLSON *et al.*, 1964; LEIVE and KOLLIN 1967).

RESULTS

Mapping the mutS *gene:* Previous results of SIEGEL and BRYSON showed that the *mutS1* allele was very weakly linked by P1 transduction to *argA* (SIEGEL and BRYSON 1967), as were additional isolates of the *mutS* gene studied by LIBERFARB and BRYSON (1970). In the course of constructing stocks for the work to be presented here we have been able to construct a more detailed map of this region of

TABLE 2

Mapping the mutS1 *allele*

Cross	Selection	Phenotype
(1) P1 (KD1013) × KD1001	Prd+	13/150 Cys⁻
(2) P1 (KD1013) × KD4000	Prd+	10/200 Cys⁻
(3) P1 (KD1016) × KD1001	Cys+	31/64 Mut⁻
P1 (KD1016) × KD1001	Cys+	14/36 Mut⁻
(4) KL16-99 × KD3017	Lys+, Arg+, Spc^R	257/480 Mut⁻ Rec+
		121/480 Mut+ Rec⁻
		8/480 Mut⁻ Rec⁻

Crosses were performed as described in MATERIALS AND METHODS. The *recA1* allele was detected by exposing purified streaks to a Germicidal lamp for a few seconds.

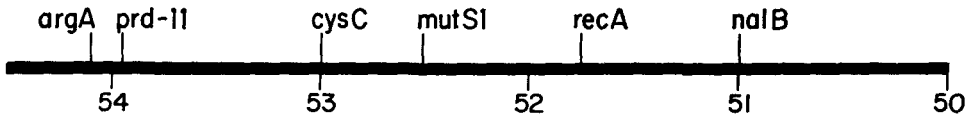

FIGURE 1.—The *mutS* region of the chromosome, drawn to scale. The *prd-11*, *cysC*, *mutS1* and *recA* order and distances are derived from the data of Table 2 and the map of TAYLOR (1970). Co-transduction frequencies were converted to map minutes using the data from Table 9 of TAYLOR and TROTTER (1967).

the chromosome. We have found that a propanediol fermenting mutation (*prd-11*) isolated in this laboratory co-transduces with *cysC*, as shown in Table 2. Cys$^-$ transductants prepared from a *prd-11 cysC* stock can then be transduced with reasonable frequency to *mutS1*. The co-transducation frequencies from such a cross establish the order *prd-11 cysC mutS1*, but do not allow us to orient this group of genes relative to other outside markers. However, the results of cross (4) of Table 2, together with the order *prd-11 cysC mutS1* deduced above, clearly places *mutS1* between *cysC* and *recA*, since the *mutS1 recA* recombinant class is found with a very low frequency. Had the order been *mutS1 cysC recA*, this particular class would be in the majority. A map of this region is shown in Figure 1.

Base-pair specificity of the mutS1 *allele:* SIEGEL and BRYSON showed that many *mutS1*-induced auxotrophs could be reverted by aminopurine (SIEGEL and BRYSON 1967). They inferred from this result that this mutator isolate caused transition mutations. We have extended their analysis by using the *trpA* system developed by YANOFSKY and co-workers (YANOFSKY *et al.* 1966a,b). In our experiments, we distinguished primary site from secondary site mutations by assaying the sensitivity of Trp$^+$ revertants to 5-MT, as outlined in MATERIALS AND METHODS. Figure 2 illustrates some of the data obtained in this way. The first point to notice about these results is that *mutS1*-induced revertants fall into discrete groups when assayed by this method. Revertants of *A223* illustrate this point well, and the same feature is evident for revertants at other sites. We cannot be certain from results of this kind that each discrete class does not contain two or more revertant types, but at least two well studied partial revertants of *A23*, labeled *EMS* and *SPR6* in Figure 2, are clearly separable from full revertants, and from each other. Both of these facts argue that the *mutS1* gene product, or lack of active product, allows a restricted set of base pair interchanges to occur. We can be fairly certain of this conclusion because, as shown in Figure 2, two other mutator genes show a different reversion pattern when examined by this method. One of these, *mutT1*, causes A:T→C:G transversions exclusively (YANOFSKY *et al.* 1966a,b), and as Figure 2 reveals, the A78 reversion pattern in a *mutT1* strain is qualitatively different from the observed *mutS1* pattern. The other mutator, *mutD1*, is a conditional mutator gene that reverts frameshifts as well as point mutants (DEGNEN and COX, in preparation). In this case, the reversion pattern is broad and distinct from either *mutT1* or *mutS1*. Although the final test that *mutS1* causes point mutants of a single transition class must rest on amino acid sequence studies, we feel that this evidence makes it unlikely that

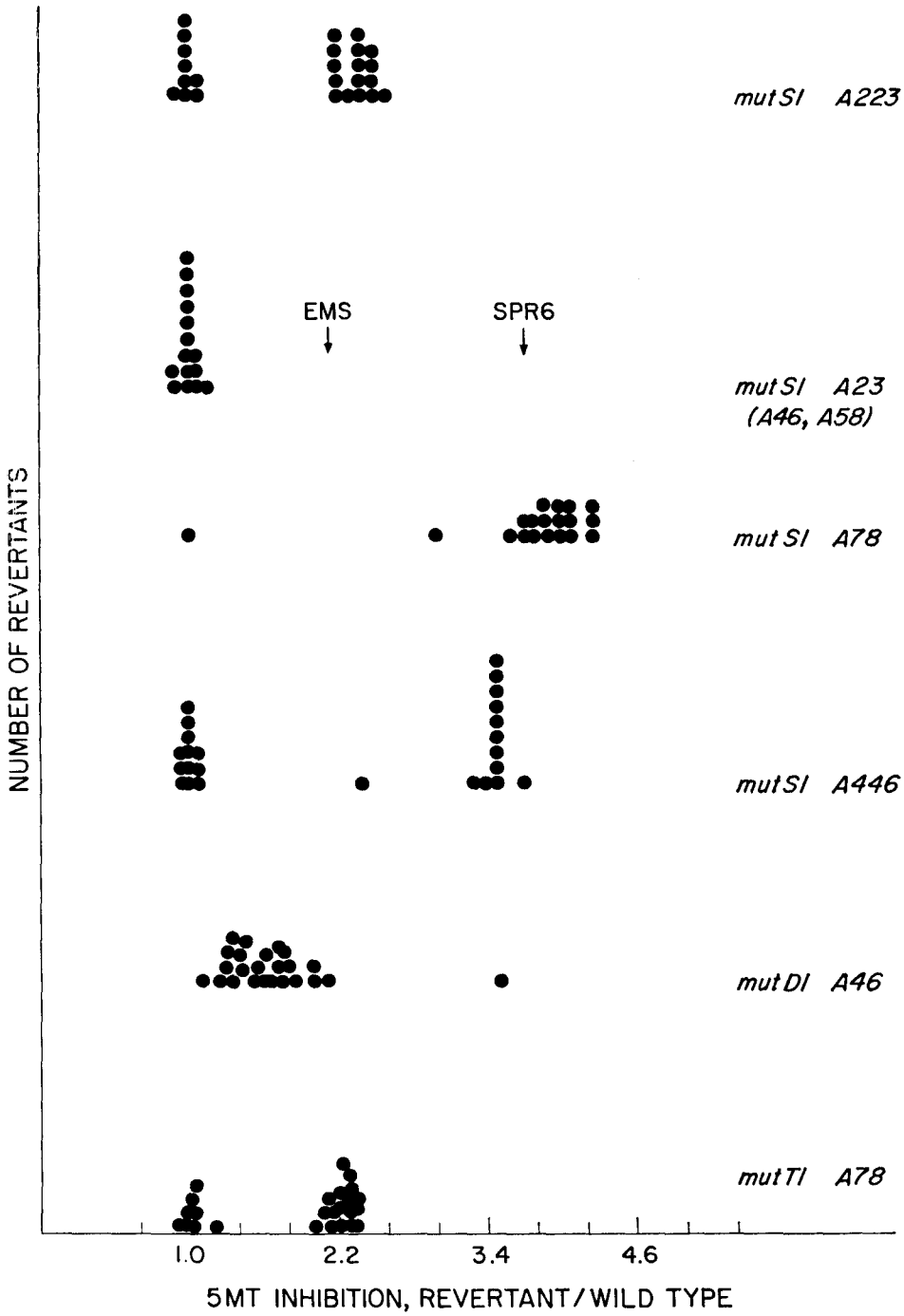
mutSI A223
EMS
SPR6
mutSI A23
(A46, A58)
mutSI A78
mutSI A446
mutDI A46
mutTI A78
NUMBER OF REVERTANTS
1.0
2.2
3.4
4.6
5MT INHIBITION, REVERTANT/WILD TYPE

TABLE 3

Reversion of trpA alleles by mutS1†

trpA allele*	Reversion frequency *mut+* PR	*mut+* FR	*mutS1* PR	*mutS1* FR	*mutS1/mut+* PR	*mutS1/mut+* FR
A23	2×10^{-9}	3×10^{-9}	$<7.5\times10^{-9}$	1.5×10^{-7}	<3.7	50
A46	3×10^{-9}	2×10^{-9}	1×10^{-9}	2×10^{-7}	0.33	100
A58	0.8×10^{-10}	1×10^{-10}	4×10^{-10}	1×10^{-8}	5	100
A446	6.7×10^{-10}	3.5×10^{-10}	2.2×10^{-7}	2.3×10^{-7}	330	660
A78	4.7×10^{-9}	2.0×10^{-9}	2.8×10^{-8}	1.6×10^{-9}	6	0.8
A223	2.3×10^{-9}	7.0×10^{-9}	6.4×10^{-9}	6.5×10^{-9}	2.8	0.93

* Because *trpA446* is a leaky Trp⁻ allele, *A446* reversion analyses were carried out on VB plates containing 0.2 μg/ml 5-methyl tryptophan. This effectively stops plate reversion in the KD1001 and KD1002 genetic background.

† KD3002 *trpA* and KD3003 *trpA* strains were grown in triplicate overnight in L-broth from a revertant-free inoculum. Each culture was washed and plated for total cells and Trp+ cells on supplemented VB glucose plates with and without tryptophan, respectively. The data presented here were averaged for each group of three cultures. Five to ten colonies were chosen at random from each plate, purified, and tested for 5-MT sensitivity, as described in MATERIALS AND METHODS. Partial revertant (PR) and full revertant (FR) frequencies were then calculated from these data and a knowledge of total cell numbers. The data for the *multS1* strains are shown graphically in Figure 2.

A:T→C:G transversions or frameshifts constitute a significant class of events in the *mutS1* background.

The second important result obtained with this method is that although the reversion frequency of the *A223* and *A78* alleles have increased in a *mutS1* background (Table 3), most of this increase can be attributed to an increase in partial revertants, as can be seen by examining Figure 2. In fact, once we separate the full revertants from partial revertants on the basis of 5-MT sensitivity, the full reversion frequency of the *A78* allele is identical in Mut+ and Mut⁻ backgrounds (2.0×10^{-9} and 1.6×10^{-9}, respectively; Table 3). As we shall show later, this effectively rules out *mutS1*-induced A:T→C:G changes, at least at this site. The *A223* case is not as clear. The data from Figure 2, if used to calculate the frequency of full revertants (A:T→G:C transitions), give an answer of 6.5×10^{-9}, a value almost precisely the same as the full reversion frequency obtained in a Mut+ co-isogenic background, 7.0×10^{-9}. A more extensive analysis of the *A223* allele was therefore carried out with a large number of independently arising

FIGURE 2.—Reversion patterns of *trpA* alleles. Each revertant is recorded separately as a function of how strongly it was inhibited by 5-MT. Rather than report the 5-MT zone of inhibition in mm, we have normalized the results by dividing the diameter of the revertant zone by the diameter of the zone obtained with a coisogenic Trp+ strain included as an internal control. The arrow labeled *EMS* corresponds to the zone of inhibition of a known *A23* revertant that arises by a G:C→C:G transversion (YANOFSKY *et al.* 1966); SPR6 is a spontaneous partial revertant, a consequence of a GC→TA transversion (YANOFSKY *et al.* 1966). The *A46* and *A58* reversion patterns were identical to *A23*, and consequently have not been included here. The details of these experiments are described in MATERIALS AND METHODS, and in Table 3, where the partial and full reversion frequencies calculated from these data are recorded and compared to similar data obtained in *mut+* stocks.

TABLE 4

Trp A223 *reversion analysis*

Parental strain	Clone	Cells/ml $\times 10^{-10}$	Trp+/ml	Reversion frequency $\times 10^{8}$
I. KD3003	1	1.9	390	2.1
(*mut*+)	2	2.7	290	1.7
	3	2.1	270	1.3
	4	2.7	60	0.2
	5	2.1	350	1.7
KD3002	1	1.6	1440	9.2
(*mutS1*)	2	2.1	1760	8.5
	3	2.2	1430	6.3
	4	1.9	1380	7.1
	5	0.9	690	7.3
		Full revertants	Partial revertants	Full reversion frequency
II. KD3003		27	12	1×10^{-8}
KD3002		41	32	4.0×10^{-8}

In part I of this Table individual colonies were grown overnight in tryptone broth at 37°. Total cell counts and Trp+ revertants were assayed on VB plates with and without tryptophan, respectively. Only some of the data are shown. In part II, a single revertant colony from each plate of part I was then purified and tested on a 5MT plate. The division into partial and full revertant phenotype was made on the basis of 5MT sensitivity (see Figure 2), and each determination (39 for *mut*+ and 73 for *mutS1*) represents an independent revertant.

revertants in *mut*+ and *mutS1* pairs. One of these experiments is summarized in Table 4, where it may be seen that the full reversion frequency increased approximately four-fold in a mutator background. To rule out the possibility that this was due to selection for full revertants in the *mutS1* strain, four independently arising full revertant colonies were competed individually against four partial revertant colonies by growing mixed cultures of full and partial revertants under the conditions used in the original reversion experiment. Single colony isolates recovered from minimal tryptophan plates showed that the partial to full revertant ratio had not changed during the experiment. Although this control is only an approximation of the actual conditions under which mutants arise, it does rule out simple competition effects and suggests that the increased full revertant frequency, although small, nonetheless reflects an increase in reversion *rate*.

The results summarized in Table 3 show that the *mutS1* allele increases the reversion frequency of *A23*, *A46*, *A58* and *A446* 50 to 500 times over the frequency observed in a Mut+ strain. In contrast, the full reversion frequency of the *trpA78* allele does not increase at all, and the increase of *A223* is marginal.

MutS1-*induced Lac*+ *revertants:* As we will show in the discussion of these results, the only consistent interpretation of the *trpA* reversion data is that the *mutS1* allele causes bidirectional A:T↔G:C transitions. We should therefore observe U↔C changes within a codon, of which *trpA223* is an example (YANOFSKY *et al.* 1966a,b). The data presented here however show that the *A223* allele reverts feebly, at best, in a *mutS1* stock. One explanation for this result is that the *mutS1* allele shows an absolute preference for one strand of the DNA, causing

A↔G errors only. In this case, if the *mutS1*-specific strand should code for the *A223* codon (AT^{T}_{C}), we would not expect to find mutator induced full revertants at the *A223* site (AC^{T}_{C}). A second explanation for these results would be that the *mutS1*-controlled transition rate is influenced by neighboring base pairs, as KOCH (1971) has shown to be the case for 2-aminopurine induced revertants in T4. In this instance, not all A:T pairs would be equally susceptible to mutator action.

We have examined both of these possibilities using ochre codons in the *Z* gene of the *lac* operon, for here a given codon can be examined in two orientations relative to the *trp* operon. In $\phi 80d_{I}lac$ lysogens the *lac* sense strand is parallel to the *trp* sense strand; in $\phi 80d_{II}lac$ lysogens it is antiparallel (SIGNER and BECKWITH 1966). The ochre codon, UAA, can revert by a transition mutation to CAA, UGA or UAG. The two latter codons are chain terminating, and cannot give rise to Lac$^+$ revertants in the *su*^{o} strains used in these studies (results not shown). CAA codes for glutamine which, according to our first hypothesis, should appear as a *mutS1* induced revertant only when the *lac* sense strand is antiparallel to the *trp* sense strand.

Table 5 summarizes our results with *mutS1* $\phi 80d_{I}lac$ and $\phi 80d_{II}lac$ lysogens. In one pair of strains, KD4032 and KD4002, there is a repeatable 10- to 30-fold difference between the reversion frequencies in the two *mutS1* lysogens, but the increase occurs when the *lac* and *trp* sense strands are *parallel* to one another; moreover, the codon is reverted in both orientations. A second *lac* ochre mutation in the *Z* gene, *X90*, does not show this difference; and, when the experiment is conducted in a different genetic background, the reversion frequency of *X90* is identical in both orientations (strains 398 and 402, 252 and 245). Thus it seems unlikely that this allele of the *mutS* gene shows strand specificity, a conclusion that is confirmed by the results in Table 6. Here we have examined the *X90*

TABLE 5

MutS1-*induced reversion of* $\phi 80d_{I}$lac *and* $\phi 80d_{II}$lac *ochre codons*

Strain	Genotype	Lac$^+$ reversion frequency	*mutS1/mut*$^+$
KD4033	*mut*+ ($\phi 80d_{I}lacU118$)	1.6×10^{-9}, 1.7×10^{-9}	
KD4032	*mutS1* ($\phi 80d_{I}lacU118$)	5.4×10^{-7}, 6.1×10^{-7}	348
KD4003	*mut*+ ($\phi 80d_{II}lacU118$)	4.5×10^{-9}, 2.8×10^{-9}	
KD4002	*mutS1* ($\phi 80d_{II}lacU118$)	1.3×10^{-7}, 0.8×10^{-7}	29
KD5033	*mut*+ ($\phi 80d_{I}lacX90$)	4.7×10^{-9}, 1.0×10^{-8}	
KD5032	*mutS1* ($\phi 80d_{I}lacX90$)	3.4×10^{-7}, 3.4×10^{-7}	46
KD5026	*mut*+ ($\phi 80d_{II}lacX90$)	8.9×10^{-9}, 6.7×10^{-9}	
KD5025	*mutS1* ($\phi 80d_{II}lacX90$)	2.3×10^{-7}, 2.9×10^{-7}	33
265,288	*mut*+ ($\phi 80d_{I}lacX90$)	4.5×10^{-8}, 8.0×10^{-8}	
398,402	*mutS1* ($\phi 80d_{I}lacX90$)	5.4×10^{-6}, 7.6×10^{-6}	104
229,230	*mut*+ ($\phi 80d_{II}lacX90$)	2.9×10^{-8}, 1.5×10^{-8}	
252,245	*mutS1* ($\phi 80d_{II}lacX90$)	2.0×10^{-6}, 1.7×10^{-6}	84

Two independently isolated strains of each genotype were grown from single colony revertant-free inocula in L-broth, washed and plated for revertants (supplemented VB lactose plates) and for total cells (*U118* on VB glucose plates, *X90* on VB melibose plates 42°). Averaged results from each pair were used to calculate *mutS1/mut*+ ratios (column 4).

TABLE 6

MutS1 *reversion of* lacX90

Strain	Lac+ reversion frequency	*mutS1/mut+*
KD4041	6×10^{-7}, 3×10^{-6}	
KD4042	6×10^{-4}, 5×10^{-4}	300

Independent isolates were checked for primary site reversion by P1 transduction: 13/20 *mut+* Lac+ and 15/17 *mutS1* Lac+ revertants gave Lac+ recombinants when MX90 and MX74 were used as recipients, indicating that most of the Lac+ revertants were primary site revertants.

reversion frequency at the wild-type *lac* position, where the sense strand is parallel to the *trp* sense strand (SIGNER and BECKWITH 1966). Again, we find that the *X90* ochre codon reverts at approximately the same frequency although located in two widely separated regions of the chromosome.

In both of these analyses we have shown that the Lac+ strains induced by the *mutS1* allele are primary site revertants, since the conclusions that we wish to draw from these data would be invalid if reversion were occurring by suppression. This was shown in two ways: first, Lac+ revertants of strains KD4041 and KD4042 were shown by genetic criteria to be largely primary site revertants (footnote, Table 6); second, $\phi80d_{I}Lac^{+}$ and $\phi80d_{II}Lac^{+}$ revertants were purified and assayed for β-galactosidase and β-galactoside transacetylase activity (Table 7). In these experiments the levels of both enzymes, although seldom as high as a fully-induced wild-type control, were five to ten times higher than could be accounted for by suppression of *U118* or *X90* by an ochre suppressor (CARTER and NEWTON 1971).

These results suggest strongly that the A:T pair in the ochre codon reverts in both orientations relative to the *trp* operon. The difference between KD4032 and KD4002 is unexplained, but could be due to selection, since these strains are not

TABLE 7

β-galactosidase and β-galactoside transacetylase levels in $\phi80d_{I}$ and $\phi80d_{II}$ Lac+ lysogens

Source of Lac+ strain	β-galactosidase 1 2 3 4	Transacetylase 1 2 3 4
KD4033	44, 53, 47, —	55, 60, 74, —
KD4032	84, 66, 68, —	82, 64, 90, —
KD4003	68, 48, 46, 73	82, 48, 74, 73
KD4002	44, 65, 54, 69	68, 46, 88, 103
KD5032	99, 78, 70, 76	63, 64, 53, 47
KD5025	64, 77, 61, 81	61, 71, 53, 66

Independently arising Lac+ revertants obtained from the experiments summarized in Table 5 were purified and grown in M63 glycerol medium containing 5×10^{-4} M isopropyl-β-D-thiogalactoside. The specific activities of β-galactosidase and β-galactoside transacetylase were determined on extracts of each culture (WILLSON *et al.* 1964; LEIVE and KOLLIN 1967). The results are presented here as per cent of the values obtained with a Lac+ strain (Mo) assayed at the same time by the same methods. The values reported in columns 1–4 were run on corresponding extracts. Thus KD4033 isolate No. 1 gave values of 44% and 55% of the Mo standard for β-galactosidase and transacetylase, respectively; isolate No. 2, 53% and 60%; and so on.

TABLE 8

Mutation frequencies in mutS1/mut+ *merodiploids*

Strain†	Clone*	Sm^R/Sm^S	Nal^R/Nal^S	$T5^R/T5^S$
KD1001	1	$<1.2\times10^{-9}$		0.46×10^{-6}
	2	$<1.1\times10^{-9}$		1.3×10^{-6}
KD1002	1	1.4×10^{-7}		1.5×10^{-4}
	2	0.5×10^{-7}		0.4×10^{-4}
KD1001/F143	1	$<.83\times10^{-8}$		
	2	$<2.6\times10^{-8}$		
KD1002/F143	1	$<1.1\times10^{-8}$	$<1.1\times10^{-8}$	
	2	$<1.3\times10^{-8}$	$<1.3\times10^{-8}$	
KD1002′	1	$<6\times10^{-9}$	2.0×10^{-8}	4×10^{-7}
	2	$<5\times10^{-9}$	$<0.5\times10^{-8}$	2×10^{-7}
	3	1.70×10^{-7}	70×10^{-8}	100×10^{-7}

* Independently isolated colonies were grown overnight with aeration in supplemented VB medium. Each culture was titered for total cells on VB plates, and for streptomycin resistant (Sm^R), nalidixic acid resistant (Nal^R) and T5 resistant ($T5^R$) cells as described in MATERIALS AND METHODS.

† Clones KD1002′ 1, 2 and 3 are segregants isolated from KD1002/F143 following overnight growth in H-broth. All three are Lys^- Arg^-; 1 and 2 are clearly Mut^+, while 3 is Mut^-.

isogenic for the *ara* region of the chromosome (KONRAD and BECKWITH, personal communication). In any case, the difference that we have found for *lacU118* in the two orientations is the opposite of that predicted from the *trpA223* results, and does not apply to a different ochre codon in the *lacZ* gene, *X90*. These observations, and the results of Table 6 with *X90* in the wild-type position and orientation, suggest that the failure of *trpA223* to revert at a significant rate is a consequence of position on the genome and not a preference of the *mutS1* allele for one strand over the other.

Merodiploid mutS1 *strains:* We have constructed *F⁻mutS1/F′mut⁺* merodiploids. The mutation rates in these strains are compared to haploid *mut⁺* and *mutS1*, and to merodiploid *mut⁺* isolates (Table 8). The results clearly show that the *mutS1* allele is recessive in the configuration studied. Furthermore, we can recover both *mut⁺* and *mutS1* alleles from putative merodiploids by growing them in acridine orange. Thus a single colony of KD1002/F143 yields Arg^-Lys^- segregants: one class is Mut^+ (KD1002′ clones 1 and 2), the other is Mut^- (KD1002′ clone 3), as shown in Table 8. These data do not place a lower limit on the mutant frequency in these strains, since our measurements of Sm^R and Nal^R frequencies failed to find any resistant colonies. However, the Sm^R data suggest that the diploid mutation frequency is at least one or two orders of magnitude lower than that in haploid Mut^- segregants, inferring, but not proving, that the gene is completely recessive.

Reversion of an F′lac codon by MutS1: The *mutS1* allele reverts *lacX90* in *trans*. This conclusion follows from the experiments summarized in Table 9. The Lac^+ reversion frequency was increased 10 to 100 fold by the *mutS1* allele. Lac^+ revertants were further checked to insure that the strains were diploid for the *lac* region by growing four revertants in H-broth from each of the eight cultures

TABLE 9

Mutation of F'lac by mutS1

Strain	Isolate	F'Lac+ reversion frequency
KD3010	1	1.0×10^{-6}
	2	3.0×10^{-6}
KD3011	1	2.0×10^{-5}
	2	1.5×10^{-5}
KD3012	1	1.0×10^{-7}
	2	5.0×10^{-7}
KD3013	1	6.0×10^{-5}
	2	4.0×10^{-5}

* Two colonies of each strain (isolates 1 and 2) were inoculated into tryptone broth and grown to saturation at 37°. Lac+ reversion frequencies were calculated from the total number of Lac+ colonies determined on VB lactose and from the total number of cells, determined by plating on VB glucose plates. Several Lac+ isolates from each plate were shown to segregate with the episome, as described in the text.

used to gather data in Table 9. Each culture was then spread on TZ-lactose plates. All 32 isolates continued to segregate Lac$^+$ and Lac$^-$ clones. In addition, eight independently arising Lac$^+$ revertants from KD3010 and K3012, and 23 from KD3011 and KD3013 were further tested as donors in a cross with MX74, a Lac$^-$ deletion strain. All of the *mut*$^+$ Lac$^+$ revertants, and 12 of the *mutS1* Lac$^+$ revertants, were good donors. Both of the above criteria convince us that at least 50% of the *mutS1* Lac$^+$ reversion events occurred on the exogenote, and therefore that the *mutS1* allele can act in *trans*.

DISCUSSION

The mutS1 *region of the genome:* Our mapping data suggest the gene order *prd-11 cysC mutS recA* (Figure 1). This order is based on (a) the co-transduction frequency of *prd-11* and *cysC* (b) the observation that *mutS1* rarely, if ever, cotransduces with *prd-11* and (c) the frequency of double and single recombinants in a three-factor Hfr × F$^-$ cross. These observations are in general agreement with the map of TAYLOR (1970), except that his map places *prd* between *cysC* and *mutS1*. It is possible that our *prd-11* isolate differs from others, but more likely that our order is the correct one, since the order given by TAYLOR was of necessity based on two-factor crosses. It is also interesting, and perhaps significant that three genes involved in DNA replication—*mutS1*, *recA* and *nalB*—appear to be clustered in this small region of the chromosome.

Specificity of the MutS1 *allele:* The codon and amino acid interchanges between the *trpA* mutants used in this study and all their known full revertants are listed in Table 10. YANOFSKY and coworkers have shown by comparing the amino acid sequences that each of these revertants results from the replacement of the mutant amino acid residue by a functional one at the mutated site (YANOFSKY *et al.* 1966a,b). Recall that the *mutS1* allele strongly increases the frequency of full revertants of *trpA23*, *trpA46*, *trpA58* and *trpA446* (Figure 2 and Table 3).

TABLE 10

Possible full revertants of the trpA alleles used in this study (YANOFSKY *et al.* 1966)

Allele *trpA*	Codon	Known full revertants
A23	AGA (arg)	GGA (gly)
		AGC (ser)
A46	GAA (glu)	GGA (gly)
		GCA (ala)
A58	GA$^{U}_{C}$ (asp)	GG$^{U}_{C}$ (gly)
A446	UG$^{U}_{C}$ (cys)	UA$^{U}_{C}$ (tyr)
A223	AU$^{U}_{C}$ (ile)	AC$^{U}_{C}$ (thr)
A78	UG$^{U}_{C}$ (cys)	GG$^{U}_{C}$ (gly)

On the other hand the frequency of full revertants of *trpA78* is essentially unchanged, and that of *trpA223* is only slightly elevated relative to the *mut*$^{+}$ levels. These results should be contrasted with the result obtained with another mutator gene, *mutT1*, whose reversion pattern is quite different from *mutS1*. It is known that *mutT1* causes unidirectional A:T→C:G transversions (YANOFSKY *et al.* 1966; Cox and YANOFSKY 1967). Clearly then *mutS1* must cause other base-pair changes and our data show that this mutator causes transition mutations. Moreover, because the full reversion frequency of *trpA446* (G:C→A:T). is increased, as well as that of *trpA23*, *trpA46* and *trpA446* (A:T→G:C), this mutator causes *reversible* transitions (A:T↔G:C), an effect similar to 2-aminopurine. It is worth mentioning in this context that the one transition which is only weakly enhanced by *mutS1*, the full revertant of *trpA223* (further discussed below), is however strongly enhanced by 2-aminopurine (YANOFSKY *et al.* 1966a,b).

A slight ambiguity exists in the interpretation of these results, which could be removed by amino acid sequence analysis; namely, that there are two different kinds of full revertants known to occur at both *trpA23* and *trpA46*. (YANOFSKY *et al.* 1966a,b). Note however that the non-transition class at both sites is an A:T→C:G transversion (Table 10). Since our data show that this class does not increase in a *mutS1 trpA78* strain, we reason that the full revertants induced by *mutS1* from *trpA23* and *trpA46* are also not of this transversion class, and by elimination we conclude they represent transitions. This argument may be applied as well to the results obtained with *trpA58*. Although both a full revertant and a partial revertant are known to occur at the *trpA58* site (YANOFSKY *et al.* 1966a,b) our observations show that *mutS1* increases only the full revertant class. The partial revertant of *trpA58* that does not increase in a *mutS1* background is an A:T→C:G transversion, the same base-pair change which would lead to full revertants of *trpA78*. The fact that two easily recognized A:T→C:G transversions are *not* found in *mutS1* stocks thus argues that the full revertants arising in *mutS1 trpA23* and *trpA46* stocks are A:T→G:C transitions.

Effect of neighboring base sequences on mutS1 *action:* Having shown above that G:C and A:T pairs are mutated by *mutS1*, we will now discuss evidence that

some A:T pairs are immune to its action. The different full revertant frequencies of *trpA23*, *trpA46*, *trpA58* and *trpA446* (Table 3), while striking, are probably due to varying amounts of plate reversion, and may or may not be attributable to variable *mutS1* specificity. In all these cases (excluding *trpA446*) the effect of *mutS1* has been not only to raise the frequency of full revertants at least 50-fold above the *mut*$^+$ level, but to shift strongly the mutational spectrum in favor of full revertants. This shift in the type of revertant is of course the evidence that *mutS1* increases transitions only. However, *mutS1* increases the *trpA223* reversion frequency only four-fold, (Tables 3 and 4, Figure 2), small in comparison with the other three A:T pairs, despite the fact that *trpA223* is also very prone to plate reversion. Moreover, the ratio of full to partial revertants (calculated from Table 4) is hardly different: 0.69 (27/39) for *mut*$^+$ and 0.56 (41/73) for *mutS1*. Application of a χ^2 test of significance to these data using a 2×2 contingency table shows that this difference is not significant ($0.15<P<0.20$). If anything, the spectrum has been shifted slightly away from full revertants. We conclude therefore from two kinds of data that the A:T base pair of *trpA223* is being mutated only feebly if at all by *mutS1*, and in this respect it behaves very differently from the A:T pairs of *trpA23*, *trpA46* and *trpA58*.

To what might this difference be due? One possibility is that the *mutS1* allele is specific for one DNA strand because of some asymmetry in the replicating complex leading to strand specificity in the resulting transitions. Since the four reverted base pairs all have purines in the sense strand, but *trpA223* has a pyrimidine, this could explain the data. However, we rule out this hypothesis from our results on the reversion of UAA (ochre) codons in the *lac* operon, since they are reverted at high frequency when the sense strand is both parallel and antiparallel to the *trp* sense strand, as shown with $\phi 80 d_I lac$ and $\phi 80 d_{II} lac$ lysogens (Tables 5, 6 and 7). This system would detect certain asymmetries in DNA replication, but the mutator gene studied here does not possess them.

Another possibility is that large segments of the genome are differently affected by *mutS1*, with some regions being immune to it. However any such large scale model will not account for the data here, for all the *trpA* codons studied lie within a single gene, separated by no more than 59 amino acid residues in the enzyme, or less than 180 base pairs. (YANOFSKY *et al.* 1964). In fact, the anomalous A:T base pair of *trpA223* is bracketed by mutable sites. It is only 23 bases from the susceptible G:C of *trpA446* on its left and by 53, 54, and 152 bases, respectively, from the susceptible A:T's of *trpA23*, *trpA46*, and *trpA58* on its right.

Since we have ruled out strand specificity and long-distance differences (down to 20–50 base pairs) in the *mutS1*-controlled mutation rate, we conclude that the *mutS1* product fails to act on the *trpA223* A:T pair because of some difference in the adjacent DNA, less than 20–50 base pairs away. Unfortunately, not enough is known of the base sequence to decide if there exist sequences necessary to permit (or block) *mutS1* action. Even though the base sequence of the *trpA23–trpA46* region of the genome is known from frame-shift studies (BERGER, BRAMMER and YANOFSKY 1968), not enough is known about the regions immediately

adjacent to the other *trpA* mutants used in this study to say anything definitive about the influence of base sequence on *mutS1* action. However it is clear that the *mutS1*-revertible base on the *A23* and *A46* antisense strand is in a run of purines: CAGGAATTT and CAGAGATTT are the respective sequences, where we have written the reverted base in bold face print. This does not allow us to formulate a rule about *mutS1* specificity, but does suggest that neighboring purines do not *prevent mutS1* action.

The possibilities numerated above make it obvious that precise definition of the *mutS1* site of action will require more base sequence data. It is also possible that the A:T base pair of *trpA223* is protected by some secondary modification of the DNA nearby, for example, by methylation.

These results confirm the inference drawn by SIEGEL and BRYSON (1968) on the base specificity of the *mutS1* allele. In addition, they represent the second clear example of sequence effects on mutation rate, the other being the study by KOCH (1971) using T4.

MutS1/mut$^+$ *diploids:* These results show that this mutator allele is recessive in *F$^-$mutS1/F'mut$^+$* merodiploids. It is therefore similar in this regard to the *mutT1* allele of *E. coli* (COX and YANOFSKY 1969), the recently isolated mutators in yeast (VON BORSTEL *et al.* 1971), and the antimutator activity of some gene 43 mutants in T4 (DRAKE *et al.* 1969). In *E. coli* and yeast, this presumably reflects the absence of a functional product, or a decrease in the activity of a component of DNA replication or repair. Both possibilities are consistent with the observation that *mutS1* acts in *trans* on F'*lac* codons (Table 9), on the assumption that the F' genome utilizes the *mutS*$^+$ product during replication or repair. The observation that T4 antimutators are recessive probably does not supply a useful model for the interpretation of these results, because here it would seem that the higher, wild-type mutation rate would simply mask the lower, even though some molecules would be replicated with greater fidelity. Such an effect might be expected to appear as partial dominance, but if it is assumed that wild type and antimutator T4 polymerase have an equal probability of replicating a T4 genome, then the mutation rate would decrease by about one-half, a change that would be very difficult to detect.

Further speculation on *mutS1* function does not seem warranted, since it could be involved in any of the steps of DNA replication or repair shared by endo- and exogenote, including control elements, nucleotide metabolism, and so on.

Mutation and evolution: The results presented here are interesting because they show that not all A:T pairs are susceptible to *mutS1* action. This has the following consequences for the evolution of genetic fine-structure: First, if it is A:T pairs only that are resistant to *mutS1* action, then at mutational equilibrium a *mutS1* strain will be a high A:T organism (SUEOKA 1962). Second, in such a strain selection will act to organize strongly conserved nucleotide sequences in areas of low mutation rate. This is because if an A:T→G:C transition is lethal, then there is a clear selective advantage for those members of a population that have a low intrinsic mutability for this A:T pair. As we have shown here, this is at least possible in a *mutS1* population. Consequently the mutation rate will not

be constant for each base-pair within a gene. This conclusion, if it can be generalized to include other classes of spontaneous mutants that arise in wild-type strains, and in other organisms, might cast new light on problems of chromosome structure, gene organization on chromosomes and the problem of mutational load (OHTA and KIMURA 1971).

LITERATURE CITED

BERGER, H., W. J. BRAMMAR and C. YANOFSKY, 1968 Analysis of amino acid replacements resulting from frameshifts and missense mutations in the tryptophan synthetase *A* gene of *Escherichia coli*. J. Mol. Biol. **34**: 219–238.

CARTER, T. and A. NEWTON, 1971 New polarity suppressors in *Escherichia coli*: suppression and messenger RNA stability. Proc. Natl. Acad. Sci. U.S. **68**: 2962–2966.

COX, E. C., 1970 Mutator gene action and the replication of bacteriophage λ DNA. J. Mol. Biol. **50**: 129–135.

COX, E. C. and C. YANOFSKY, 1967 Altered base ratios in the DNA of an *Escherichia coli* mutator strain. Proc. Natl. Acad. Sci. U.S. **58**: 1895–1902.

DEMEREC, M., E. A. ADELBERG, A. J. CLARK and P. E. HARTMAN, 1966 A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**: 61–76.

DRAKE, J. W., 1970 *The Molecular Basis of Mutation*. Holden-Day, San Francisco.

DRAKE, J. W., E. F. ALLEN, S. A. FORSBERG, R. M. PREPARATA and E. O. GREENING, 1969 Spontaneous mutation: genetic control of mutation rates in bacteriophage T4. Nature, **221**: 1128–1132.

GREEN, M. M., 1970 The genetics of a mutator gene in *Drosophila melanogaster*. Mutation Research **10**: 353–363.

HIROTA, Y., 1960 The effects of acridine dyes on mating type factors in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **46**: 57–64.

IVES, P. T., 1950 The importance of mutation rate genes in evolution. Evolution **4**: 236–252.

KOCH, R. E., 1971 The influence of neighboring base pairs upon base-pair substitution mutation rates. Proc. Natl. Acad. Sci. U. S. **68**: 773–776.

LEIVE, L. and V. KOLLIN, 1967 Synthesis, utilization and degradation of lactose operon mRNA in *Escherichia coli*. J. Mol. Biol. **24**: 247–259.

LENNOX, E. S., 1955 Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**: 190–206.

LIBERFARB, R. M. and V. BRYSON, 1970 Isolation, characterization and genetic analysis of mutator genes in *Escherichia coli* B and K-12. J. Bacteriol. **104**: 363–375.

LOW, B. and T. H. WOOD, 1965 A quick and efficient method for interruption of bacterial conjugation. Genet. Res. **6**: 300–303.

NEWTON, W. A., J. R. BECKWITH, D. ZIPSER and S. BRENNER, 1965 Nonsense mutants and polarity in the *Lac* operon of *Escherichia coli*. J. Mol. Biol. **14**: 290–296.

OHLSSON, B. M., P. F. STRIGINI and J. R. BECKWITH, 1968 Allelic amber and ochre suppressors. J. Mol. Biol. **36**: 209–218.

OHTA, T. and M. KIMURA, 1971 Functional organization of genetic material as a product of molecular evolution. Nature **233**: 118–119.

PARDEE, A. B., F. JACOB and J. MONOD, 1959 The genetic control and cytoplasmic expression of "Inducibility" in the synthesis of β-galactosidase by *Escherichia coli*. J. Mol. Biol. **1**: 165–178.

PLOUGH, H. H., 1941. Spontaneous mutability in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **9**: 127–137.

SIEGEL, E. C. and V. BRYSON, 1964 Selection of resistant strains of *Escherichia coli* by antibiotics and antibacterial agents: role of normal and mutator strains. pp. 629–634. In: *Anti microbial Agents and Chemotherapy 1963*. ——, 1967 Mutator gene of *Escherichia coli* B. J. Bacteriol., **94**: 38–47.

SIGNER, E. R. and J. R. BECKWITH, 1966 Transposition of the *Lac* region of *Escherichia coli* III. The mechanism of attachment of bacteriophage ϕ80 to the bacterial chromosome. J. Mol. Biol., **22**: 33–51.

SPEYER, J. F., J. D. KARAM and A. B. LENNY, 1966 On the role of DNA polymerase in base selection. Cold Spring Harb. Symp. Quant. Biol. **31**: 693–697.

TAYLOR, A. L., 1970 Current linkage map of *Escherichia coli*. Bacteriol. Res. **34**: 155–175.

TAYLOR, A. L. and C. D. TROTTER, 1967 Revised linkage map of *Escherichia coli*. Bacteriol. Rev. **31**: 332–353.

VOGEL, H. J. and D. M. BONNER, 1956 Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**: 97–106.

VON BORSTEL, R. C., K. T. CAIN and C. M. STEINBERG, 1971 Inheritance of spontaneous mutability in yeast. Genetics **60**: 17–27.

WALLACE, B., 1970 Spontaneous mutation rates for sex-linked lethals in the two sexes of *Drosophila melanogaster*. Genetics **64**: 553–557.

WILLSON, C., D. PERRIN, M. COHN, F. JACOB and J. MONOD, 1964 Non-inducible mutants of the regulator gene in the "Lactose" system of *Escherichia coli*. J. Mol. Biol. **8**: 582–592.

WITKIN, E. M., 1969 The role of DNA repair and recombination in mutagenesis. Proc. 12th Int. Cong. Genetics **3**: 222–245.

WU, T. T., T. M. CHUSED and E. C. C. LIN, 1967 A dehydrogenase enabling mutants of *Escherichia coli* to grow on 1,2-propanediol. Bacteriol. Proc. p. 52.

YANOFSKY, C. and E. S. LENNOX, 1959 Transduction and recombination study of linkage relationships among the genes controlling tryptophan synthesis in *Escherichia coli*. Virology **8**: 425–447.

YANOFSKY, C., E. C. COX and V. D. HORN, 1966 The unusual mutagenic specificity of an *Escherichia coli* mutator gene. Proc. Natl. Acad. Sci. U.S. **55**: 274–281.

YANOFSKY, C., J. ITO and V. D. HORN, 1966 Amino acid replacements and the genetic code. Cold Spring Harb. Symp. Quant. Biol. **31**: 151–162.

YANOFSKY, C., B. C. CARLTON, J. R. GUEST, D. R. HELINSKI and V. HENNING, 1964 On the colinearity of gene structure and protein structure. Proc. Natl. Acad. Sci. U.S. **52**: 266–272.