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A set of *lacZ* mutations in *Escherichia coli* that allow rapid detection of each of the six base substitutions

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ABSTRACT **We describe the construction of six strains of *Escherichia coli* with different mutations at the same coding position in the *lacZ* gene, which specifies the active site glutamic acid residue at position 461 in β-galactosidase. Each strain is Lac⁻ and reverts to Lac⁺ only by restoring the glutamic acid codon. The strains have been designed so that each reverts via one of the six base substitutions. The set of strains allows detection of each transition and transversion simply by monitoring the Lac⁻ to Lac⁺ frequency, as demonstrated here with characterized mutagens and mutator alleles. These strains are useful for rapidly determining the mutagenic specificity of mutagens at a single site, for detecting low levels of stimulation of certain base substitutions, for monitoring specific base changes in response to various experimental conditions or strain backgrounds, and for isolating new mutator strains.**

One of the first steps toward elucidating mutagenic pathways is to determine the specificity of mutations generated spontaneously or by treatment with mutagens. In many cases, identifying the type of mutations produced yields important information on the nature of the premutagenic lesion, as well as the role of cellular systems in processing damaged DNA (reviewed in ref. 1). Toward this end, systems have been developed that allow the rapid determination of mutagenic specificity by genetic methods. For instance, reversion of *trpA* alleles was utilized by Yanofsky and coworkers (2) to examine mutagenic specificity in *Escherichia coli*. In yeast, Sherman and coworkers (3, 4) utilized the reversion of characterized mutations in the isocytochrome *c* system to deduce the base substitution specificity of different mutagens. Ames and colleagues (5, 6) monitored the reversion of specific mutations in the *his* operon of *Salmonella typhimurium* to allow the rapid screening of base substitution and frameshift activity of a large number of mutagens. This work was important because it established a strong correlation between carcinogens and mutagens (6). Levin and Ames (7) have modified this system to allow the determination of specific base substitutions, utilizing the reversion of nonsense codons, followed by analysis of the revertants, a principle first used by Person and coworkers (8). The *lacI* system (9, 10) provides a more detailed look at mutagenic specificity by examining the forward spectrum of nonsense mutations produced in the *lacI* gene of *E. coli*. Mutations at >80 sites can be monitored. In addition to these genetic methods, the direct DNA sequence determination (11–16) and colony hybridization with specific probes (17) have also been used for the determination of the nature of spontaneous and induced mutations.

All of the above systems require further analysis to determine the ability of a mutagen to produce each of the six possible base substitutions. A system that allows the determination of each base substitution, simply by measuring the number of revertant colonies that appear on a plate, would greatly simplify the study of mutagenesis. Foster and coworkers (18) have described a strain that allows the unique determination of the G·C → T·A transversion by measuring reversion at a specific site in the plasmid-encoded *bla* gene in *E. coli*. We report here the design of six strains with different mutations at the same coding position in the *lacZ* gene, which detect, individually, all six types of base substitution mutations.

The *E. coli lacZ* gene encodes the enzyme β-galactosidase (19). Cells producing active enzyme can use lactose as a carbon source. One amino acid presumed to be in the active site (20), Glu-461, has been found to be essential for enzyme activity (21). We have shown that substitution of any other amino acid at this position lowers enzyme activity sufficiently to prevent growth on lactose minimal medium (21). We have constructed six strains that have point mutations in the Glu-461 codon and are therefore Lac⁻. Only one specific base substitution will restore the glutamic acid codon in each strain and restore the Lac⁺ phenotype. The set of strains provides a method of rapidly characterizing the mutagenic specificity of mutagens at a single site. The *lacZ* reversion system is capable of detecting extremely low rates of each of the base substitutions. In this paper, we outline the construction of the system and demonstrate its use on a set of known mutagens and mutator strains.

MATERIALS AND METHODS

Bacterial Strains. Strains CC101–CC106 (see also ref. 22) are derivatives of the strain P90C [*ara*Δ(*lac proB*)$_{XIII}$] carrying an F *lacI*⁻ *Z*⁻ *proB*⁺ episome. Each strain carries a different *lacZ*⁻ mutation affecting residue 461 in β-galactosidase. The mutation carried in each strain is depicted in Fig. 1. The *mutY* strain is a *rpsL* (*strA*), *mutY* derivative of P90C, and the *mutT* strain (supplied by E. Eisenstadt, Harvard School of Public Health) is a *leu*::Tn*10*, *mutT* derivative of P90C. K. B. Low (Yale University Medical School) supplied the *mutH* strain (KL862: *his, leu, rpsL*). The *lac proB* deletion *XIII* was introduced into each of these last two strains by crossing with strain CSH63 (23), resulting in a Val^r Leu⁺ Pro⁻ derivative of each strain. The episomes from CC101–CC106 were introduced into the (*lac pro*) derivatives of each of the above-mentioned strains.

The methods used to introduce mutations into *lacZ*, and the procedures for transferring the mutant genes to the F′ *lac proB* episome are described elsewhere (21).

Mutagenesis. Mutagenesis with 2-aminopurine (2AP), *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), ethyl methanesulfonate (EMS), and UV is essentially as described by Coulondre and Miller (24), and ICR-191 mutagenesis was described by Calos and Miller (12). After mutagenesis, cul-

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Abbreviations: MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; EMS, ethyl methanesulfonate; 2AP, 2-aminopurine; 5AZ, 5-azacytidine.

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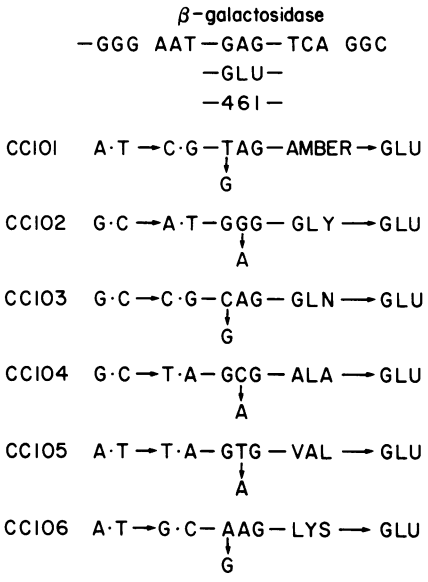

FIG. 1. Altered codon at position 461 in *lacZ*. Coding position 461 in *lacZ* has been altered in each of six different strains. The base substitution required to restore the codon to GAG is shown here, together with the amino acid change that results. Only glutamic acid at position 461 in β-galactosidase restores the wild-type phenotype.

tures were plated to determine survival and then grown in LB to allow expression and plated for Lac$^+$ revertants and also for rifampicin-resistant (Rif^r) mutants to monitor the mutagenesis. Table 1 gives representative survival and Rif^r mutant data, which was similar in all strains, CC101–CC106. Table 2 shows the Lac$^+$ revertant frequencies. Briefly, the protocols are as follows:

2AP. The strain to be treated was diluted so that 100–1000 cells were inoculated into separate tubes containing the desired concentration of 2AP in LB. These were grown for 20 hr at 37°C and plated for mutants and viable cells. The best results were obtained with 2AP at 700 μg/ml.

EMS. A fresh overnight culture was subcultured and grown until it reached a density of 2–3 × 10^8 cells per ml. The cells were chilled on ice, spun down, washed twice in A buffer, and then resuspended in half the original volume of A buffer (23). EMS was added in the cold by pipeting 0.07 ml of EMS into 5 ml of resuspended cells. The test tubes were closed with masking tape (or when glass screw-capped tubes were used, the tops were fastened tightly), mixed in a Vortex, and rotated on a roller drum at 30 rpm for different times at 37°C. After mutagenesis, the cells were spun down, washed twice in A buffer, and then resuspended in the same volume of A buffer and titered for viable cells. Samples (0.5 ml) were added to 10 ml of broth and the cultures were grown overnight and plated for mutants and viable cells.

MNNG. An overnight culture was subcultured and grown to a density of 2–3 × 10^8 cells per ml. The cells were spun down, washed twice in 0.1 M sodium citrate buffer (pH 5.5), and resuspended in the original volume of citrate buffer. The cells were chilled on ice for 10 min and then placed in a 37°C water bath; 0.5 ml of a 1 mg/ml solution of MNNG in distilled water was added to 4.5 ml of resuspended cells. (The stock solution can be prepared by dissolving 10 mg of MNNG per ml in acetone and then diluting 1:10 in distilled water.) The final concentration of MNNG was therefore 100 μg/ml. The cells were then spun down, after different times of exposure, washed twice in 0.1 M potassium phosphate buffer (pH 7.0), and resuspended in the original volume of phosphate buffer. The cultures were plated for viable cells at this point. Different overnight cultures were generated by adding 5 ml of

Table 1. Survival and Rif^r mutant frequency

Mutagen	Condition	% survival	Rif^r revertants per 10^8 cells
EMS	0 min	100	15
	15 min	71	16,100
	30 min	56	36,900
	45 min	44	85,200
	60 min	36	78,700
MNNG	0 min	100	61
	5 min	49	64,400
	10 min	45	91,800
	15 min	28	94,700
	30 min	5.2	106,000
UV	0 sec	100	9
	30 sec	14	2,150
	45 sec	2	3,100
	60 sec	0.75	5,140
	75 sec	0.025	1,960
5AZ	0 μg/ml	—	41
	1 μg/ml	—	105
	5 μg/ml	—	370
	10 μg/ml	—	1,223
	50 μg/ml	—	1,369
	70 μg/ml	—	2,340
	100 μg/ml	—	3,960
2AP	0 μg/ml	—	57
	10 μg/ml	—	72
	50 μg/ml	—	194
	100 μg/ml	—	361
	500 μg/ml	—	1,037
	700 μg/ml	—	1,380
	1000 μg/ml	—	2,033
mutT		—	350
mutY		—	150
mutH		—	500

Strains CC101–CC106 behaved identically in response to mutagens with respect to survival and the frequency of Rif^r mutants after treatment. Representative values are shown here. See Table 2 and *Materials and Methods* for further details.

washed suspension to 10 ml of broth. These were grown overnight at 37°C and plated for mutants and viable cells.

UV light. A fresh overnight culture was subcultured and grown to a density of 2 × 10^8 cells per ml. These were centrifuged and resuspended in half the original volume of 0.1 M $MgSO_4$ and placed on ice for 10 min. UV irradiation was carried out for different times with a Westinghouse Sterilamp fixture (type SB-30) using a glass Petri dish as a container set 86 cm from the lamp. Directly after irradiation, 0.5-ml samples were inoculated into separate 10-ml broth tubes and grown overnight, and samples were plated directly for survivors.

5-Azacytidine (5AZ). 5AZ was prepared directly before use by dissolving it in distilled water to a concentration of 1 mg/ml, filter-sterilizing, and then adding to bacterial medium at the appropriate concentration (see Table 2). As with 2AP treatment, 100–1000 cells were inoculated into LB + 5AZ and grown for ≈24 hr, until the density was between 10^8 and 10^9 cells per ml. Outgrowth cultures for each mutagenic treatment were plated on lactose minimal medium, and the number of colonies was counted after 36 and 48 hr and compared with the viable titer on glucose minimal medium or LB medium. Each value represents the average of several determinations.

Recovery and Sequencing of Lac$^+$ Revertants. The region around coding position 461 of the *lacZ* gene from Lac$^+$ revertants was transferred to an F1 bacteriophage by recombination with the *lacZ* region of the phage as a prelude for sequencing. Cells containing the mutant episome were grown

Table 2. Reversion of Lac⁻ strains in response to different mutagens

Mutagen	Condition	Strain (reversion event) CC101 (A·T → C·G)	CC102 (G·C → A·T)	CC103 (G·C → C·G)	CC104 (G·C → T·A)	CC105 (A·T → T·A)	CC106 (A·T → G·C)
EMS	0 min	1.3	1.2	0.4	4.0	5.4	0.5
	15 min	0.8	242	—	13	3.9	4.3
	30 min	8.7	2,400	0.9	13	—	16
	45 min	29	5,200	2.3	25	19	34
	60 min	24	7,000	2.2	52	14	39
MNNG	0 min	3.8	3.0	<0.5	8.7	1.2	<0.5
	5 min	16	15,400	1.1	7.5	8.4	48
	10 min	38	23,000	5.7	—	20	87
	15 min	21	25,000	14	5.5	47	230
	30 min	130	—	23	5.1	28	130
UV	0 sec	<0.5	0.8	<0.5	2.4	1.0	<0.5
	30 sec	<0.5	15	1.7	14	11	8.3
	45 sec	3.2	46	0.8	31	13	150
	60 sec	20	67	1.3	12	33	240
	75 sec	8.7	47	1.3	7	—	130
5AZ	0 μg/ml	—	—	0	1	—	—
	1 μg/ml	—	—	20	1	—	—
	5 μg/ml	—	—	107	9	—	—
	10 μg/ml	—	—	313	15	—	—
	50 μg/ml	<0.5	<0.5	524	25	4	2
	70 μg/ml	—	—	731	40	—	—
	100 μg/ml	—	—	972	25	—	—
2AP	0 μg/ml	—	27	—	—	—	—
	10 μg/ml	—	31	—	—	—	—
	50 μg/ml	—	67	—	—	—	—
	100 μg/ml	—	88	—	—	—	—
	500 μg/ml	—	415	—	—	—	—
	700 μg/ml	<0.5	457	2	15	2	160
	1000 μg/ml	—	320	—	—	—	—
mutT		500	5	<0.5	<0.5	<0.5	<0.5
mutY		<0.5	4	<0.5	160	<0.5	<0.5
mutH		0	320	0	11	3	34

After EMS, UV, and MNNG (100 μg/mg) mutagenesis, cells were grown overnight in broth and titered on glucose minimal medium and lactose minimal medium, as described in *Materials and Methods*. Mutagenesis was monitored for survival and for the generation of Rifʳ mutants (see Table 1). Results are expressed as Lac⁺ revertants per 10^8 cells.

in rich medium to midlogarithmic phase. Cells (100 μl) were mixed with phage (100 μl), diluted to 10^{-6} in 1× minimal A salts. The phage is an F1 derivative that contains a *lacZ* gene with a missense mutation at the site encoding Glu-461 (21). Plaques from this phage are white on plates containing 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal). Phage and cells were incubated at 37°C for 10 min to allow attachment. LB (2 ml) was added, and the cultures were incubated overnight, with aeration, to allow recombination between the episomal and phage *lacZ* genes. The cells were pelleted, and the phage in the supernatant were used to infect JM801 [F⁺ *kan*/*ara*Δ(*lac proB*) *thi rpsL*]. Phage containing the *lacZ* gene from the episome produced bright blue plaques on plates containing X-Gal. Since the missense mutation, which specifies histidine at position 461, cannot revert to Lac⁺ by a single base change, blue plaques were the result of recombination with the episome that transferred the *lac*⁺ region to the phage and not the result of point mutations in the phage *lacZ* gene. Single-stranded DNA was recovered and sequenced by standard procedures.

RESULTS

Fig. 1 depicts the changes introduced at coding position 461 of *lacZ* in each of six strains. These changes were generated by oligonucleotide-directed, site-specific mutagenesis, as described (21). Each strain can revert to Lac⁺ only by reverting the codon at position 461 to GAG, since this will specify the required glutamic acid at the corresponding position in β-galactosidase. Therefore, as shown in Fig. 1, each strain can revert to Lac⁺ via only one of the six base substitutions. This provides us with a valuable set of indicator strains for rapidly monitoring the base substitution specificity of different mutagens and mutators.

Reversion Tests. We have used strains CC101–CC106 to test a series of known mutators and mutagens in *E. coli*. Mutagenesis was monitored for resulting survival and for the generation of Rifʳ mutants, as reported in Table 1 for a representative set of experiments. Rifʳ mutants were induced at similar levels in each of the six strains CC101–CC106 with each mutagenic treatment. The Lac⁺ revertant frequencies are shown in Table 2.

Two transversion-specific mutators have been examined with this system. Strains that are *mutY* stimulate only the G·C → T·A transversion (22). As Table 1 shows, only CC104, which is diagnostic for the G·C → T·A transversion, reverts in response to the *mutY* background. The *mutT* allele specifically stimulates the A·T → G·C transversion (25). As anticipated, only CC101 responds to the *mutT* background (Table 1). Mutators lacking the mismatch repair system preferentially induce transitions among base substitutions (16, 26–28), and

the *lacZ* system described here verifies this, since only CC102 and CC106 respond to a *mutH* strain (Table 1).

Mutagens known to favor the G·C → A·T transition, such as MNNG and EMS (10), stimulate preferentially CC102, the indicator of this transition. The *lacZ* system is sensitive enough to detect weaker stimulation of the other transition and all four transversions (Table 1) in most cases for these two mutagens, but the levels are 200 to 2000 times lower than for the G·C → A·T transition.

2AP, which has been shown to stimulate both A·T → G·C and G·C → A·T transitions (refs. 9 and 29 and references therein), reverts only CC102 and CC106, the strains that respond to these two events.

Levin and Ames (7) have reported that the base analog 5AZ specifically generates G·C → C·G transversions. CC103 should be the only strain that reverts strongly in response to 5AZ, a result borne out by the data shown in Table 2. We also detect a very low reversion over background of CC104 in response to 5AZ, indicating a slight stimulation of the G·C → T·A transversion. However, this is only 2–3% of the level of the G·C → C·G transversions.

We have also examined UV with the *lacZ* system. UV induces a variety of base substitutions. Although transitions are generally favored over transversions, both are induced, and a considerable variation is seen from site to site (see, for instance, ref. 13 and references therein). One determinant, at least for transitions, is the presence of adjacent pyrimidines on one strand or the other (13–15, 30). However, other aspects of the surrounding sequence clearly play a role in determining the UV mutability for any given site (13, 30), as first demonstrated for spontaneous and mutagen-induced mutations in the *rII* system of phage T4 by Benzer (31). Table 2 reveals that with the *lacZ* system, UV induces several transversions at low rates and the two transitions at higher rates. The A·T → G·C transition at the site present in strain CC106 is induced at approximately a 4-fold higher rate than the G·C → A·T transition in strain CC102.

Sequence Verification of Revertants. To verify the nature of some of the revertants to the Lac$^+$ phenotype, the region surrounding coding position 461 in the *lacZ* gene was sequenced, as detailed in *Materials and Methods*. Table 3 depicts the results. In all cases examined, the revertants indeed resulted from mutations that restored the GAG codon at position 461.

DISCUSSION

We have described a system that will prove helpful for rapidly determining the base substitution specificity of different mutagens and mutators. The system is based on the use of a set of six isogenic strains that carry different mutations at coding position 461 in the *lacZ* gene. Only glutamic acid at the corresponding position in β-galactosidase results in the Lac$^+$ phenotype. Therefore, each strain can only revert via a specific base substitution (Fig. 1). We have not encountered a single exception to this rule. Although one of the strains carries an amber mutation at position 461, only single step mutations that create an efficient glutamic acid inserting nonsense suppressor could result in the Lac$^+$ phenotype by a pathway other than restoring the GAG codon itself at position 461. Such nonsense suppressors have never been observed via a single base change.

Table 3. Sequences of selected Lac$^+$ revertants

Strain	Codon 461	Mutagen	No. of revertants sequenced	Sequence at codon 461	Change
CC102	GGG	EMS	3	GAG	G·C → A·T
CC102	GGG	2AP	3	GAG	G·C → A·T
CC106	AAG	2AP	3	GAG	A·T → G·C
CC104	GCG	5AZ	3	GAG	G·C → T·A
CC103	CAG	5AZ	3	GAG	G·C → C·G

The advantage of this system is that in each strain, the frequency of Lac$^+$ cells is a direct measure of one specific base substitution. Determinations can be carried out without additional genetic or sequence analysis. Although the detailed spectrum of a mutagen ideally requires using numerous sites to monitor each specific event, reversion systems of the type described here can often outline the specificity of a mutagen. Reversion systems are valuable for screening a large number of mutagens (1–6) to pinpoint those that merit more detailed examination. Also, by being able to monitor a specific base substitution at one specific site, it is easy to test the effect of numerous variables on that mutational event. We have calibrated the system by testing a set of characterized mutagens and mutator strains. As revealed in Table 1 and *Results*, each of the six strains behaves as expected with each mutator and mutagen. This *lacZ* detection system is sensitive enough to detect relatively minor stimulation of base substitutions by different mutagens. For example, the low rate of transversions induced by MNNG and EMS is picked up by this system (Table 1). (The stimulation of transversions by these agents is, however, very small compared with the ≈1000-fold greater stimulation of G·C → A·T transitions.)

Of particular interest is the specificity of 5AZ, an analog of cytosine, which was first noted by Levin and Ames (7) to preferentially induce G·C → C·G transversions in *S. typhimurium*. As shown in Table 1, the same specificity is apparent in the *lacZ* system, with a low level of G·C → T·A transversions. At present, it is unclear how 5AZ induces such specific mutations among survivors.

In addition to its use in determining mutagenic specificity, the *lacZ* system described here is particularly useful in detecting new mutator strains. Mutators that induce specific transitions or transversions can be detected by using these strains as part of a papillation assay (22). Increased reversion from Lac$^-$ to Lac$^+$ is detected by increased incidence of blue (Lac$^+$) papillae in a white (Lac$^-$) colony. We have already detected four mutator loci specific for different transversion events (refs. 22 and 32; M. Michaels, C. Cruz, and J.H.M., unpublished results).

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