JOURNAL OF BACTERIOLOGY, June 1987, p. 2476–2481
0021-9193/87/062476-06$02.00/0

Vol. 169, No. 6

Creation of a Test Plasmid for Detecting G · C-to-T · A Transversions by Changing Serine to Arginine in the Active Site of β-Lactamase

PATRICIA L. FOSTER,[1]* GLORIA DALBADIE-McFARLAND,[2] ELAINE F. DAVIS,[1] STEVE C. SCHULTZ,[2] AND JOHN H. RICHARDS[2]

Division of Environmental Health, Boston University School of Public Health, and the Hubert H. Humphrey Cancer Research Center, Boston University School of Medicine, Boston, Massachusetts 02118,[1] and Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125[2]

Received 9 January 1987/Accepted 19 March 1987

Oligonucleotide-directed mutagenesis of the β-lactamase gene, *bla*, on pBR322 was used to change the codon for the active-site serine 70, AGC, to CGC, coding for arginine. *Escherichia coli* cells carrying the mutant plasmid, pGD104, were sensitive to ampicillin, indicating that the arginine-containing enzyme is inactive. We characterized the reversion of the mutant *bla* gene by a number of mutagens and in different genetic backgrounds and demonstrated that full ampicillin resistance can be restored only by a G · C-to-T · A transversion occurring at the first base of the codon. Thus, reversion of the mutant *bla* gene is diagnostic for G · C-to-T · A transversions, and bacteria carrying pGD104 can be used as test strains to detect the occurrence of this mutation.

Studies of mutational specificity have contributed greatly to the understanding of the mutagenic process. The classic determination of the spatial distribution of mutations in the *r*II gene of bacteriophage T4 (5), the elegant genetic analysis of forward mutations in the *lacI* gene of *Escherichia coli* (11), and the use of *trpA* alleles to detect specific base substitution mutations (1) have provided extensive information about the nature of both induced and spontaneous mutagenesis. Recently, *Salmonella typhimurium* strains have been developed that can be used to test for the ability of mutagens to induce each of the six possible base substitution events (33). These and other systems to determine mutational specificity can be utilized to deduce the nature of mutagenic DNA lesions and the biochemical pathways by which they are processed (reviewed in reference 18). However, a comprehensive genetic analysis of mutational pathways is difficult when using the somewhat cumbersome systems developed to date. Consequently, we sought a way to detect easily the unambiguous occurrence of a specific class of mutation in a variety of different genetic backgrounds. We choose to develop a test plasmid for G · C-to-T · A transversions, but the method is applicable to all six possible base substitution events.

To detect specifically a given mutational event, a genetic system has to meet two criteria. First, the mutation must yield a selectable phenotype. Second, to detect a specific base substitution, the resultant phenotype must depend on the identity of only one DNA base pair. To meet these criteria, we drew upon the wealth of knowledge about the enzymatic activity of class A β-lactamases and chose as a mutational target the gene for this enzyme, *bla*, on pBR322. We created by oligonucleotide-directed mutagenesis (12) a specific mutation in the codon for serine 70 (using the numbering of the consensus sequence [2]; residue 70 [2] corresponds to codon 68 [52]). Ser-70, essential for β-lactamase activity, is part of a conserved triad (Ser-Thr-Xaa-Lys) found in class A β-lactamases at the catalytic site (2). Hydrolysis of the β-lactam ring of penam and cephem antibiotics occurs via an acyl-enzyme intermediate formed between the Ser-70 hydroxyl group and the carbonyl carbon of the β-lactam ring (22, 31). Cysteine is the only other residue known to substitute for Ser-70 in this reaction, yielding an enzyme with 1/30th of the wild-type activity (12, 13, 49, 50). The wild-type codon is AGC (52), which we mutated to CGC (arginine), yielding an inactive enzyme. Only a G · C-to-T · A transversion at the first base of this codon can revert the arginine to serine. Other base substitutions at the first base give cysteine and glycine, base substitutions at the second base give leucine, proline, and histidine, and base substitutions at the third base are silent. Based on the mechanism of enzyme action, only a G · C-to-T · A transversion, restoring serine, would be expected to revert the ampicillin-sensitive (Ap^s) phenotype to full ampicillin resistance (Ap^r).

G · C-to-T · A transversions are not well induced by most mutagens commonly used to mutagenize bacteria. The base substitutions induced by direct-acting mutagens, i.e., those that do not require SOS processing for their mutagenicity, are almost exclusively transitions (15). Several SOS-dependent mutagens, such as UV light, 4-nitroquinoline-1-oxide, and neocarzinostatin, also preferentially induce transitions, but can induce transversions at low frequencies (10, 23, 24). However, a number of SOS-dependent mutagens, including several known carcinogens, induce high frequencies of G · C-to-T · A transversions. This mutation is virtually the only base substitution induced by aflatoxin B1 (AFB1) (25); it is a major component of the mutagenic spectrum of benzo[*a*]pyrene, cyclopenta[*cd*]pyrene, and *cis*-diamminedichloroplatinum (II) (6, 19); and it is induced by depurination (48). In addition, it is the most frequent base substitution recovered after the induction of the SOS system in the absence of DNA damage (44). These findings have led to the hypothesis (reviewed in reference 37) that depurination at guanines, or the occurrence of noninformational lesions at guanines, is a common intermediate in the mutagenicity of all these agents and treatments; the induction of

* Corresponding author.

TABLE 1. Plasmids used in this study

Plasmid	Description	Drug resistance	Reference
pGD104	pBR322 with mutant *bla* gene	Tetracycline	This paper
pSE137	*umuDC* cloned on a pSC101 derivative	Spectinomycin	20
pSE155	*mucAB* cloned on a pSC101 derivative	Spectinomycin[a]	S. J. Elledge, personal communication
pGW270*	pKM101 derivative with a nonrevertable mutation in the *bla* gene	Kanamycin	32; this paper

[a] pSE155 has low-level tetracycline resistance that makes pGD104 slightly unstable in strains with both plasmids.

transversion mutations can be explained by the preferential incorporation of dATP opposite such sites. Hence, an easy and rapid method to detect the occurrence of G · C-to-T · A transversions would be useful to: (i) find other mutagens that induce this event; (ii) identify DNA repair functions that may prevent its induction; and (iii) screen for mutants affected in the pathway that processes DNA lesions to give this mutation.

In this paper we report the creation of a mutation in the *bla* gene giving CGC, coding for arginine, at residue 70. We characterize the induction of Ap^r revertants with a number of mutagens and show that the mutation is reverted by agents that induce G · C-to-T · A transversions. Finally, the sequences of a number of revertant plasmids are confirmed to be AGC, serine, at residue 70.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 AB1885 (F^- *uvrB5 thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 strA31 sup-37* λ^- [30]) was used throughout. PF501, a *recA441 sulA11* (= *tif sfi*) derivative, was made by: (i) P1 transduction to *recA430 srl*::Tn*10* from GW2727 (obtained from G. Walker), selecting for tetracycline resistance (Tet^r) and screening for UV sensitivity and the inability to use sorbitol (Srl^-); (ii) P1 transduction to *recA441* from GC3217 (27), selecting for Srl^+ and screening for UV resistance and Tet^s; (iii) P1 transduction to *pyrD34 zcb-222*::Tn*10* from CY307 (obtained from D. Brash), selecting for Tet^r and screening for inability to grow without uracil (Ura^-); (iv) P1 transduction to *sulA11* from GC3217 by selecting for Ura^+ and screening for Tet^s and nonfilamentation at 42°C in minimal medium with 100 μg of adenine per ml. Genetic methods were as described previously (42).

Plasmids. The plasmids used are listed in Table 1. pGW270* is an Ap^s derivative of pGW270 (32), which is a derivative of the mutagenesis-enhancing plasmids R46 and pKM101 (46). pGW270* was constructed by cutting pGW270 DNA with *Eco*RI, filling in the overhang with DNA polymerase I large fragment, and religating with T4 ligase. Ap^s kanamycin-resistant plasmids were then screened for the ability to restore mutability to a *umuC36* strain. Plasmid DNA preparation and manipulations and bacterial transductions were as described previously (39, 45).

Oligonucleotide-directed mutagenesis. pBR322 was mutated in vitro with a 15-base synthetic oligonucleotide (5′-ATGATGCGCACTTTT-3′, antisense of nucleotides 3946 to 3960 [52]), containing the desired mutation (at nucleotide 3954) to prime DNA synthesis as described previously (12). The presence of the desired mutation in the plasmid was confirmed by DNA sequencing. Cells carrying the mutant plasmid, pGD104, were Ap^s Tet^r.

Bacterial mutagenesis. Bacteria were grown in LB broth with drug selection for plasmids until the mid-log phase (50 Klett units). Cells were then centrifuged and suspended at 10× in E salts (54) or, for *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) experiments, 0.1 M citrate buffer (pH 5.5). Approximately 2×10^7 cells were exposed to the mutagen for 5 min at room temperature for MNNG and methyl methanesulfonate (MMS), 20 min at room temperature for dibromoaflatoxin, 60 min at 37°C in the presence of metabolic activation buffer containing 60 μl of rat liver microsomes per ml (3) for AFB1, and the designated period of time for UV light. Chemical mutagen-exposed cells were then centrifuged and washed once with E salts or, for MNNG experiments, 0.1 M phosphate buffer (pH 7). Survival titers of samples were determined on LB plates, and in some experiments, samples were plated on Vogel-Bonner minimal plates (54) supplemented with 4 μM arginine and 10 μg of other required amino acids per ml to select for arginine prototrophy. The remaining cells were inoculated into 5-ml LB broth plus drugs and grown overnight at 37°C. Samples were then plated on LB plus ampicillin (50 mg/liter) and, in some experiments, LB plus rifampin (100 mg/liter), and titers were determined on LB plus tetracycline (20 mg/liter). In two UV experiments and *tif* experiments (see below), a sample from the overnight culture was plated on supplemented Vogel-Bonner minimal plates without arginine to score for arginine prototrophs. The medium was as described previously (42).

Tif induction. Early-log-phase cultures grown in supplemented Vogel-Bonner minimal medium plus tetracycline (10 mg/liter) at 32°C were divided in half and grown for 2 h at 32°C and at 42°C with 100 μg of adenine per ml added. Approximately 10^8 cells were then inoculated into 5 ml of the same medium and grown overnight at 32 and 37°C (42°C is eventually lethal). The rest of the procedure was as for mutagen-treated cells.

Sequencing. Plasmid DNA was prepared from single-colony-purified revertants or nonreverted bacteria and transformed into JM109 (59). After single-colony purification of transformants, plasmid DNA was prepared and cut with *Hin*cII and *Eco*RI. The 450-base-pair fragment containing the mutated site was purified in low-melting-temperature agar and ligated into *Hin*cII-*Eco*RI-cut and phosphatased M13mp8 DNA. The ligated DNA was transformed into JM109, and white plaques were picked and sequenced by the standard dideoxy technique (47). Two to five plaques of each revertant were sequenced. Methods were as described previously (39, 41; or instructions from the suppliers).

RESULTS

By changing the AGC Ser-70 codon to CGC (arginine) we created a G · C base pair as a mutational target. The surrounding sequence is 5′-GCGC-3′/5′-GCGC-3′ (52). Table 2 compares reversion of this mutant *bla* gene with the induction of rifampin resistance (Rif^r) or arginine prototrophy (Arg^+) by several mutagens. Rifampin resistance can arise from a number of mutations in the *rpoB* gene (4, 7). The

argE3 mutation is an ochre that can be reverted by base substitutions at the site or by the creation of several extragenic tRNA suppressors (53). The mutagens used were chosen for their base substitution specificity as follows.

MNNG. At the concentrations used, MNNG induces G · C-to-A · T transitions owing to the strongly mutagenic lesion O^6-methylguanine (10, 38). A · T-to-G · C transitions are induced at 1/10th the frequency of G · C-to-A · T transitions (10) and may be due to miscoding by O^4-methylthymine (51) or the misincorporation of O^6-methyl-dGTP (17). MNNG showed no apparent site specificity in its ability to induce nonsense mutations in the *lacI* gene (10). Reversion of the mutant *bla* gene was not induced by this mutagen.

MMS. MMS has been shown to induce a number of different base substitutions (53). Its mutagenicity is thought to be due, at least in part, to spontaneous or enzymatic depurination of 7-methylguanine and 3-methylpurines (15, 16), which would be expected to yield transversions. As predicted, MMS reverted the mutant *bla* gene approximately 16-fold above background.

UV light. UV light induces transition mutations at adjacent pyrimidines (11). The target site of the mutant *bla* gene does not contain a pyrimidine pair. However, UV light also induces transversions at low frequencies, and these mutations do not require the presence of adjacent pyrimidines (11, 24, 43). It was therefore an unexpected result that UV light did not revert the mutant *bla* gene. The DNA lesion that gives rise to UV light-induced transversions appeared not to be formed at the mutant *bla* target site. Alternatively, UV-induced lesions occurring on a multicopy plasmid may be readily repaired by some recombinational pathway.

TABLE 2. Reversion of the mutant *bla* gene induced by various mutagens compared with induction of rifampin resistance and arginine prototrophy

Strain[a]	Mutagen (concn)	Surviving fraction	Mutants per 10^8 cells		
			Ap^r	Rif^r	Arg^+
AB1885	MNNG				
	0 μg/ml	1.0[b]	11[b]	2	
	50 μg/ml	1.3[b]	13[b]	77	2,078[b,d]
	100 μg/ml	0.85[b]	17[b]	470	6,394[b,d]
AB1885	MMS				
	0%	1.0[c]	6[c]	2	
	5%	0.59[c]	159[c]	136	90[d]
AB1885	UV light				
	0 s	1.0[c]	13[c]	<1	3
	1 s	0.42[c]	8[c]	160	175
	2 s	0.04[c]	8[c]	249	405
AB1885	AFB1				
	0 μM	1.0	4	<1	
	10 μM	0.3	3	1	
	100 μM	0.03	4	5	
AB1885(pSE137)	0 μM	1.0	<1	<1	
	10 μM	0.39	2	5	
	100 μM	0.02	4	16	
AB1885(pSE155)	0 μM	1.0[c]	166[c]	21[c]	
	10 μM	0.85[c]	224[c]	208[c]	
	100 μM	0.02[c]	697[c]	497[c]	
AB1885(pGW270*)	Br_2AFL[e]				
	0 μM	1.0[b]	25[b]		
	100 μM	0.32[b]	168[b]		

[a] All strains also carry pGD104.
[b] Average of three determinations.
[c] Average of two determinations.
[d] Determined on plates containing limiting arginine. Mutations arising spontaneously on the plates have been subtracted.
[e] Br_2AFL, Dibromoaflatoxin.

TABLE 3. Reversion of the mutant *bla* gene and induction of arginine prototrophy under conditions than enhance SOS mutagenesis

Strain[a]	Mutagen (concn)	Surviving fraction	Mutants per 10^8 cells	
			Ap^r	Arg^+
PF501	*tif*			
	32°C	1.0[b]	6[b]	3
	42°C + adenine	0.44[b]	29[b]	27
AB1885(pGW270*)	MNNG			
	0 μg/ml	1.0	15	
	50 μg/ml	1.1	15	1,963[c]
	100 μg/ml	0.83	21	6,537[c]
AB1885(pGW270*)	MMS			
	0%	1.0	15	
	5%	0.66	1,071	
AB1885(pGW270*)	UV light			
	0 s	1.0	71	51
	1 s	0.65	65	1,390
	2 s	0.31	58	1,112

[a] All strains also carry pGD104.
[b] Average of two determinations.
[c] Determined on plates containing limiting arginine. Mutations arising spontaneously on the plates have been subtracted.

AFB1. The base substitutions induced by AFB1 are almost exclusively G · C-to-T · A transversions (25). Like MNNG, metabolically activated AFB1 showed very little site specificity in the induction of nonsense mutations in the *lacI* gene (25). Aflatoxin mutagenicity is dependent on the presence of the mutagenesis-enhancing *mucAB* operon from pKM101 (25); the presence of the analogous operon from *E. coli*, *umuDC*, has little effect (P. L. Foster and E. Eisenstadt, unpublished observations). As predicted, metabolically activated AFB1 reverted the mutant *bla* gene, but only if *mucAB* was present.

Also shown in Table 2 is the frequency of Ap^r revertants induced by dibromoaflatoxin, a direct-acting aflatoxin with the same mutagenic specificity and approximately 1/10th the mutagenic potency of metabolically activated AFB1 (P. L. Foster, J. M. Miller, E. Davis, W. Barnes, and J. Groopman, manuscript in preparation). The DNA sequence of plasmids isolated from Ap^r revertants induced by dibromoaflatoxin was determined in the region of interest (see below).

The results presented in Table 2 and previous studies (44, 48, 53) suggested that an enhanced level of SOS mutagenic processing is necessary for the induction of G · C-to-T · A transversions. In Table 3 are presented reversion frequencies of the mutant *bla* gene under conditions that enhance SOS mutagenesis. The first of these was thermal induction of a strain (*recA441*, previously known as *tif*) that expresses all SOS functions constitutively at 42°C in the presence of adenine (8). This treatment, which has been shown to result in G · C-to-T · A transversions (44), caused a fivefold increase in the frequency of reversion of the mutant *bla* gene.

In the other experiments presented in Table 3, the activities of *mucAB* were enhanced by the presence of this operon on plasmid pGW270*. Since the parent of pGW270,

pKM101, does not appear to have a general effect on the SOS response (28, 36, 57), the rest of the functions of the SOS system presumably remained at normal mutagen-induced levels in these experiments. The frequency of MMS-induced reversion of the mutant *bla* gene was increased 10-fold by the presence of pGW270*, confirming the results of Todd et al. (53) that MMS-induced G · C-to-T · A transversions were enhanced by plasmid R46 (an ancestor of pGW270*). However, pGW270* had no such effect when cells were exposed to MNNG or UV light, mutagens that did not revert the mutant *bla* gene without the plasmid (cf. Table 2). These results indicate that a high level of *mucAB* activity is not of itself sufficient to induce G · C-to-T · A transversions but that a specific lesion in the DNA must also be present. That is, the events that revert the mutant *bla* gene appear to be targeted to the site of a DNA lesion.

*Hin*cII-*Eco*RI fragments from the original mutant plasmid, eight independent dibromoaflatoxin-induced revertants, and eight independent spontaneous Ap^r revertants [from AB1885(pGW270*)] were subcloned in M13mp8, and several independent clones generated from each plasmid were sequenced. The sequence of the nonreverted plasmid was confirmed to be CGC (arginine) at nucleotides 3952 to 3954 with no other sequence differences from pBR322 between nucleotides 3909 and 4055 (52). All eight dibromoaflatoxin-induced and four spontaneous revertants were AGC (serine) at 3952 to 3954, also with no other detected differences from pBR322. Four spontaneous revertants gave both CGC and AGC sequences in approximately a 1:1 ratio. Agarose gel electrophoresis after a very limited DNaseI digestion of these plasmids showed that they were larger than the original pGD104. Thus, they were most likely dimers containing one reverted and one nonreverted site.

DISCUSSION

Replacement of serine 70 by arginine in β-lactamase resulted in an inactive enzyme. The requirement for serine in the β-lactamase catalytic site and the characteristics of the genetic code allow pGD104, carrying the mutant *bla* gene with CGC (arginine) at residue 70, to function as a specific test plasmid for G · C-to-T · A transversions. AFB1 and MMS, both known to induce this base substitution, reverted the mutant *bla* gene, whereas MNNG, which induces transitions, did not. Induced and spontaneous revertants contained the wild-type sequence, which could only arise via a G · C-to-T · A transversion.

Several studies (reviewed in reference 37) have suggested the following hypothetical pathway for the induction of transversion mutations. Certain mutagen-damaged bases are spontaneously or enzymatically converted to apurinic or apyrimidinic (AP) sites in vivo. AP sites also arise spontaneously or after in vitro treatment of DNA with, for example, heat at low pH. AP sites can be bypassed during replication because DNA polymerase inserts nucleotides opposite such sites if the SOS response has been induced. The order of preference of insertion, shown for a number of polymerases in vitro, is A > G > T >> C. Hence, if the missing bases are purines, transversion mutations result. Thus, for example, the induction of G · C-to-T · A transversions by AFB1 (25) can be explained by its strong preference for making adducts to the N-7 of guanines (21, 35, 40), the resultant lability of the glycosylic bond leading to loss of the base (29), and the insertion of adenine by DNA polymerase opposite the AP site. (This hypothesis does not strictly require that depurination or depyrimidination occurs, since it is possible that any noninformational lesion could be similarly bypassed.)

Mutations that arise via this hypothesized pathway can only be recovered if the SOS response is induced. Thus, an alternative hypothesis is that the mutations are untargeted; that is, they may arise not at the sites of missing or damaged bases, but as a result of an error-prone polymerase replicating undamaged DNA. However, the G · C-to-T · A transversions that are well induced by AFB1 and benzo[*a*]pyrene and that result from induction of the SOS response in the absence of DNA damage do not occur at the same sites, and thus must be targeted (reviewed in reference 44). Our finding that UV light, which is a strong inducer of the SOS response (58), does not revert the mutant *bla* gene (Tables 2 and 3) demonstrates that, similarly, the mutations we are detecting here are targeted.

The mutagenicity of SOS-dependent mutagens requires the functions of the *umuDC* operon, which are induced as part of the SOS response (reviewed in reference 56). The analogous operon, *mucAB*, from plasmid pKM101 appears to be more active in promoting both spontaneous and induced mutations (55, 57) (Tables 2 and 3), an effect that is unlikely to be due to enhanced induction of the SOS response (reviewed in reference 56). We had previously observed that mutagenesis by AFB1 was particularly dependent on the presence of *mucAB* (25); *umuDC*, even when present in the same copy number, had little effect (Foster and Eisenstadt, unpublished observations). The results shown in Table 2 confirm this dependence of aflatoxin mutagenicity on *mucAB* activity. However, enhancement of induced mutagenicity by *mucAB* is not nonspecific: the presence of pGW270* had no effect on the induction of either Ap^r or Arg^+ mutations by the SOS-independent mutagen MNNG and had different effects on the frequencies of mutations induced by the SOS-dependent mutagens MMS and UV light (Tables 2 and 3). Specifically, the presence of the plasmid enhanced MMS-induced reversion of the mutant *bla* gene but did not change the inability of UV light to induce this mutation. In contrast, the induction of Arg^+ revertants by both MMS and UV light was increased by pGW270*. Thus, *mucAB*-enhanced mutagenesis after exposure to a mutagen appears to occur only at the sites of DNA lesions that are substrates for SOS processing. We are currently using the mutant *bla* gene to explore the nature of this *mucAB*-enhanced mutagenesis.

As has been previously observed with other mutational targets (26, 46, 55), the presence of *mucAB* also enhanced spontaneous reversion of the mutant β-lactamase gene (Tables 2 and 3). In contrast, spontaneous Ap^r reversions were not increased by the *umuDC* clone, although present on the same vector as pSE155. It would appear that the biochemical activities encoded by the *umuDC* operon are normally limiting for spontaneous as well as induced mutagenic processes but that these activities can be supplied by the *mucAB* gene products (55). Our data confirm those of Fowler et al. (26) that G · C-to-T · A transversions are at least one of these spontaneous events enhanced by *mucAB*. As has been suggested for the transversion mutations that occur after the induction of the SOS response (44), these mutations may be the result of replication past cryptic DNA lesions, possibly spontaneously occurring apurinic sites, that only produce mutations when SOS mutagenic processing is active. Thus, control of the levels or activities or both of the *umuDC* and *mucAB* gene products may be an important determinant of the spontaneous mutation rate of a cell. In this regard, it is interesting that pSE155 was up to 10 times more active than

pGW270 in enhancing both spontaneous and induced reversion of the mutant *bla* gene, although both plasmids occur in approximately the same copy number (9, 34). This result suggests that some control function or antagonistic activity present on pGW270 and its parent, pKM101, was lost when the *mucAB* operon was cloned. Interestingly, a negative control function on plasmid R46 apparently also is missing from its deletion derivative, pKM101 (14).

The techniques we developed are generally applicable to studies of mutagenesis. The plasmid we constructed to detect G · C-to-T · A transversions is just one of a number of test plasmids that can be made by oligonucleotide-directed mutagenesis of the codon for the active-site serine of β-lactamase. Mutants to specifically detect three more base substitutions can be created by making a single base change in the AGC (Ser) codon. For example, changing the AGC to GGC (Gly) would yield a mutant that could be reverted only by G · C-to-A · T transitions. Because serine is also specified by the family of TCX codons, seven more mutant plasmids to detect all six possible base substitution events can be constructed. Once made, these plasmids can be further mutated to alter the sequence context of the target base by changing the base in the wobble position of the codon. Thus, such test plasmids will be useful for studying base substitution specificity, the effects of local DNA sequence on mutagenesis, and the role of various bacterial genes in the induction of mutations. Finally, the techniques are also applicable to other genes and DNA sequences, such as promoters, in which the identity of one base pair is essential to function.

ACKNOWLEDGMENTS

We gratefully acknowledge the contributions of David Botstein to the conception, support, and completion of this project. We thank Eric Eisenstadt for helpful discussions and for critically reading this manuscript. We also thank the people mentioned in Materials and Methods for strains and plasmids, especially Steven Elledge and Graham Walker, and Jeffrey Miller for discussion.

This work was supported by grant 85-16 from the Whitaker Health Sciences Fund to P.L.F. and David Botstein and by Public Health Service grants CA37880 from the National Cancer Institute to P.L.F. and GM16424 from the National Institute of General Medical Sciences to J.H.R. G.D.-M. was supported by NRSA grant GM07626.

LITERATURE CITED

1. **Allen, M. K., and C. Yanofsky.** 1963. A biohemical and genetic study of reversion with the A-gene A-protein system of *Escherichia coli* tryptophan synthetase. Genetics **48**:1065–1083.
2. **Ambler, R. P.** 1980. The structure of β-lactamases. Philos. Trans. R. Soc. London Ser. B **289**:321–331.
3. **Ames, B. N., J. McCann, and E. Yamasaki.** 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. **31**:347–364.
4. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
5. **Benzer, S.** 1961. On the topography of the genetic fine structure. Proc. Natl. Acad. Sci. USA **47**:403–415.
6. **Brouwer, J., P. van de Putte, A. M. J. Fichtinger-Schepman, and J. Reedijk.** 1981. Base-pair substitution hotspots in GAG and GCG nucleotide sequences in *Escherichia coli* K-12 induced by cis-diamminedichloroplatinum (II). Proc. Natl. Acad. Sci. USA **78**:7010–7014.
7. **Burgess, R. R.** 1971. RNA polymerase. Annu. Rev. Biochem. **40**:711–740.
8. **Castellazzi, M., J. George, and B. Buttin.** 1972. Prophage induction and cell division in *E. coli* I. Further characterization of a thermosensitive mutation *tif*-1 whose expression mimics the effect of UV irradiation. Mol. Gen. Genet. **119**:139–152.
9. **Cohen, S. N., and A. C. Y. Chang.** 1977. Revised interpretation of the origin of the pSC101 plasmid. J. Bacteriol. **132**:734–737.
10. **Coulondre, C., and J. H. Miller.** 1977. Genetic studies of the *lac* repressor. IV. Mutagenic specificity in the *lacI* gene of *Escherichia coli*. J. Mol. Biol. **117**:577–606.
11. **Coulondre, C., J. H. Miller, P. J. Farabaugh, and W. Gilbert.** 1978. Molecular basis of base substitution hotspots in the *lacI* gene of *E. coli*. Nature (London) **274**:775–780.
12. **Dalbadie-McFarland, G., L. Cohen, A. D. Riggs, K. Itakura, and J. H. Richards.** 1982. Oligonucleotide-directed mutagenesis as a general and powerful method for studies of protein function. Proc. Natl. Acad. Sci. USA **79**:6409–6413.
13. **Dalbadie-McFarland, G., J. Neitzel, and J. H. Richards.** 1986. Active-site mutants of β-lactamase: use of an inactive double mutant to study requirements for catalysis. Biochemistry **25**:332–338.
14. **Dowden, S. B., and P. Strike.** 1982. R46-derived recombinant plasmids affecting DNA repair and mutation in *E. coli*. Mol. Gen. Genet. **186**:140–144.
15. **Drake, J. W.** 1970. The molecular basis of mutation. Holden-Day, San Francisco.
16. **Drinkwater, N. R., E. C. Miller, and J. A. Miller.** 1980. Estimation of apurinic/apyrimidinic sites and phosphotriesters in deoxyribonucleic acid treated with electrophilic carcinogens and mutagens. Biochemistry **19**:5087–5092.
17. **Eadie, J. S., M. Conrad, D. Toorchen, and M. D. Topal.** 1984. Mechanisms of mutagenesis by O^6-methylguanine. Nature (London) **308**:201–203.
18. **Eisenstadt, E.** 1987. Analysis of mutagenesis in *Escherichia coli* and *Salmonella typhimurium*, p. 1016–1033. *In* F. C. Neidhardt, J. L. Ingraham, D. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
19. **Eisenstadt, E., A. J. Warren, J. Porter, D. Atkins, and J. H. Miller.** 1982. Carcinogenic epoxides of benzo[*a*]pyrene and cyclopenta[*cd*]pyrene induce base substitutions via specific transversions. Proc. Natl. Acad. Sci. USA **79**:1945–1949.
20. **Elledge, S. J., and G. C. Walker.** 1983. Proteins required for ultraviolet light and chemical mutagenesis: identification of the products of the *umuC* locus of *Escherichia coli*. J. Mol. Biol. **163**:175–192.
21. **Essigman, J. M., R. G. Croy, A. M. Nadzan, W. F. Busby, Jr., V. N. Reinhold, G. Buchi, and G. N. Wogan.** 1977. Structural identification of the major DNA adduct formed by aflatoxin B1 *in vitro*. Proc. Natl. Acad. Sci. USA **74**:1870–1874.
22. **Fisher, J., J. G. Belasco, S. Khosla, and J. R. Knowles.** 1980. β-Lactamase proceeds via an acyl-enzyme intermediate. Interaction of the *E. coli* RTEM enzyme with cefoxitin. Biochemistry **19**:2895–2901.
23. **Foster, P. L., and E. Eisenstadt.** 1983. Distribution and specificity of mutations induced by neocarzinostatin in the *lacI* gene of *Escherichia coli*. J. Bacteriol. **153**:379–383.
24. **Foster, P. L., E. Eisenstadt, and J. Cairns.** 1982. Random components in mutagenesis. Nature (London) **299**:365–367.
25. **Foster, P. L., E. Eisenstadt, and J. H. Miller.** 1983. Base substitution mutations induced by metabolically activated aflatoxin B1. Proc. Natl. Acad. Sci. USA **80**:2695–2698.
26. **Fowler, R. G., L. McGinty, and K. E. Mortelmans.** 1979. Spontaneous mutational specificity of drug resistance plasmid pKM101 in *Escherichia coli*. J. Bacteriol. **140**:929–937.
27. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations *sifA* and *sfiB* restore division in *tif* and *lon* strains and permit the expression of mutator properties of *tif*. Mol. Gen. Genet. **140**:309–332.
28. **Goze, A., and R. Devoret.** 1979. Repair promoted by plasmid pKM101 is different from SOS repair. Mutat. Res. **61**:163–179.
29. **Groopman, J. D., R. G. Croy, and G. N. Wogan.** 1981. *In vitro* reactions of aflatoxin B_1-adducted DNA. Proc. Natl. Acad. Sci. USA **78**:5445–5449.
30. **Howard-Flanders, P., R. P. Boyce, and L. Theriot.** 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from

DNA. Genetics 53:1137–1150.
31. **Knott-Hunziker, V., S. G. Waley, B. S. Orlek, and P. G. Sammes.** 1979.Penicillinase active sites: labelling of serine-44 in β-lactamase I by 6β-bromopenicillanic acid. FEBS Lett. 99:59–61.
32. **Langer, P. J., W. G. Shanabruch, and G. C. Walker.** 1981. Functional organization of the plasmid pKM101. J. Bacteriol. 145:1310–1316.
33. **Levin, D. E., and B. N. Ames.** 1986. Classifying mutagens as to their specificity in causing the six possible transitions and transversions: a simple analysis using the *Salmonella* mutagenicity assay. Environ. Mutagen. 8:9–28.
34. **Levin, D. S., M. Hollstein, M. F. Christman, E. A. Schwiers, and B. N. Ames.** 1982. A new *Salmonella* tester strain (TA102) with A · T base pairs at the site of mutation detects oxidative mutagens. Proc. Natl. Acad. Sci. USA 79:7445–7449.
35. **Lin, J. K., J. A. Miller, and E. C. Miller.** 1977. 2,3-dihydro-2-(guan-7-yl)-3-hydroxy-aflatoxin B1, a major acid hydrolysis product of aflatoxin B1-DNA or ribosomal RNA adducts formed in hepatic microsome-mediated reactions in rat liver *in vivo*. Cancer Res. 37:4430–4438.
36. **Little, J. W., and P. C. Hanawalt.** 1977. Induction of protein X in *Escherichia coli*. Mol. Gen. Genet. 150:237–248.
37. **Loeb, L. A.** 1985. Apurinic sites as mutagenic intermediates. Cell 40:483–484.
38. **Loechler, E. L., C. L. Green, and J. M. Essigman.** 1984. *In vivo* mutagenesis by O^6-methylguanine built into a unique site in a viral genome. Proc. Natl. Acad. Sci. USA 81:6271–6275.
39. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
40. **Martin, C. N., and R. C. Garner.** 1977. Aflatoxin B-oxide generated by chemical or enzymatic oxidation of aflatoxin B1 causes guanine substitution in nucleic acids. Nature (London) 267:863–865.
41. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. 101:20–78.
42. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
43. **Miller, J. H.** 1985. Mutagenic specificity of ultraviolet light. J. Mol. Biol. 182:45–68.
44. **Miller, J. H., and K. B. Low.** 1984. Specificity of mutagenesis resulting from the induction of the SOS system in absence of mutagenic treatment. Cell 37:675–682.
45. **Morrison, D. A.** 1977. Transformation in *Escherichia coli*: cryogenic preservation of component cells. J. Bacteriol. 132: 349–351.
46. **Mortelmans, K., and B. A. D. Stocker.** 1979. Segregation of the mutator property of plasmid R46 from its ultraviolet-protecting property. Mol. Gen. Genet. 167:317–328.
47. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463–5467.
48. **Schaaper, R. M., T. A. Kunkel, and L. A. Loeb.** 1983. Infidelity of DNA synthesis associated with bypass of apurinic sites. Proc. Natl. Acad. Sci. USA 80:487–491.
49. **Sigal, I. S., W. F. DeGrado, B. J. Thomas, and S. R. Petteway, Jr.** 1984. A mutant of pBR322 β-lactamase in which the active-site serine has been replaced with cysteine. J. Biol. Chem. 259:5327–5332.
50. **Sigal, I. S., B. G. Harwood, and R. Arentzen.** 1982. Thiol-β-lactamase: replacement of the active-site serine of RTEM β-lactamase by a cysteine residue. Proc. Natl. Acad. Sci. USA 79:7157–7160.
51. **Singer, B., J. Sagi, and J. T. Kusmierek.** 1983. *Escherichia coli* polymerase I can use O^2-methyldeoxythymidine or O^4-methyldeoxythymidine in place of deoxythymidine in primed poly(dA-dT) synthesis. Proc. Natl. Acad. Sci. USA 80:4884–4888.
52. **Sutcliffe, J. G.** 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold spring Harbor Symp. Quant. Biol. 43:77–90.
53. **Todd, P. A., C. Monti-Bragadin, and B. W. Glickman.** 1979. MMS mutagenesis in strains of *Escherichia coli* carrying the R46 mutagenic enhancing plasmid: phenotypic analysis of Arg^+ revertants. Mutat. Res. 62:227–237.
54. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *E. coli*: partial purification and some properties. J. Biol. Chem. 218:97–106.
55. **Walker, G. C.** 1977. Plasmid (pKM101)-mediated enhancement of repair and mutagenesis: dependence on chromosomal genes in *Escherichia coli* K-12. Mol. Gen. Genet. 152:93–103.
56. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. 48:60–93.
57. **Walker, G. C., and P. P. Dobson.** 1979. Mutagenesis and repair deficiencies of *Escherichia coli umuC* mutants are suppressed by the plasmid pKM101. Mol. Gen. Genet. 172:17–24.
58. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. 40:869–907.
59. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequence of the M13mp18 and pUC19 vectors. Gene 33:103–119.