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Genetics

A new *Salmonella* tester strain (TA102) with A·T base pairs at the site of mutation detects oxidative mutagens

(carcinogens/multicopy plasmid/hydroperoxides/aldehydes/psoralens)

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ABSTRACT **A new tester strain, TA102, is described as an addition to the set of strains for the *Salmonella*/microsome mutagenicity test. This strain contains A·T base pairs at the site of the mutation (determined by DNA sequence analysis) in contrast to the other *Salmonella* tester strains that detect mutagens damaging G·C base pairs. This strain differs from previous tester strains in that the mutation has been introduced into a multicopy plasmid, so that ≈30 copies of the mutant gene are available for back mutation. The new strain detects a variety of oxidative mutagens, including x-rays, bleomycin, hydrogen peroxide and other hydroperoxides, streptonigrin and other quinones, and phenylhydrazine; a variety of aldehydes, including formaldehyde, glyoxal, kethoxal, glutaraldehyde, and malondialdehyde; a number of psoralens (in the presence of near-UV light), mitomycin C, neocarzinostatin, and UV light. Some of these mutagens have been previously shown to damage thymine in DNA. Several auxiliary tester strains also are described, including TA96, a frameshift tester strain with a hot spot for mutation at a run of five A·T base pairs with a specificity similar to that of TA102. The importance of oxidative mutagens is discussed.**

Damage to DNA is likely to be a major cause of cancer and other diseases (1, 2). The *Salmonella* mutagenicity test (3), along with other short-term assays (4), is being extensively used to survey a variety of substances in our environment for mutagenic activity. The test measures back-mutation in several specially constructed mutants of *Salmonella*. A homogenate of rat liver (or other mammalian tissue) is added to the bacterial suspension as an approximation of mammalian metabolism (3). By using this system, over 80% of the organic carcinogens tested have been detected as mutagens (5–7).

All of the histidine-requiring mutants in the standard set of *Salmonella* tester strains have G·C base pairs at the critical site for reversion: -C-C-C- in the base-pair substitution strain TA100,* -C-C-C-C-C-C- in the frameshift strain TA97 (8), and -C-G-C-G-C-G-C-G- in the frameshift tester strain TA98 (9). The present study describes tester strains that have A·T base pairs at the critical site for reversion. These strains detect a variety of oxidants and other agents as mutagens which were not detected in the standard tester strains.

MATERIALS AND METHODS

Chemicals. Chemical sources were as follows: bleomycin sulfate, *t*-butyl hydroperoxide, glyoxal, paraquat, diquat, and phenylhydrazine from Sigma; mitomycin C from Aldrich; streptonigrin from Flow Laboratories (McLean, VA); formaldehyde and hydrogen peroxide from Mallinckrodt; cumene hydroperoxide from Pfaltz and Bauer (Stamford, CT); glutaraldehyde from Baker; kethoxal from U.S. Biochemical (Cleveland, OH); malondialdehyde, synthesized from 1,1,3,3-tetraethoxypropane (10), was a gift of L. J. Marnett; psoralen, 5- and 8-methoxypsoralen, and angelicin were gifts of J. Hearst and M. Ashwood-Smith; neocarzinostatin was a gift of M. Hofnung; restriction enzymes were from New England BioLabs, T4 DNA ligase was from P-L Biochemicals, [*methyl*-^{3}H]thymine was from New England Nuclear, [α-^{32}P]deoxycytidine triphosphate was from Amersham, and *Escherichia coli* DNA polymerase was from Boehringer Mannheim.

Cloning and Sequence Analysis of *hisG428*. The ochre mutation, *hisG428* (11), was cloned by *in vivo* recombination into a derivative of phage M13Hol76 carrying the *hisD6610* mutation (8). M13Hol76 contains the histidine operator, *G*, *D*, and part of the *C* genes from *Salmonella*. The male derivative of *hisG428*, TA2894, was infected with M13Hol76*hisD6610* and recombinant phage were selected by their ability to complement the *hisΔ(D)2121* deletion host, TA2891, and the inability to complement the *hisΔ(G)8476* deletion host, TA2892. Single-stranded DNA from this HisD$^+$ HisG$^-$ recombinant phage was isolated (12) and subjected to sequence analysis (13).

Plasmid pAQ1. M13Hol76*hisG428* DNA was isolated in double-stranded replicative form after infection of TA2892 and was digested with *Eco*RI and *Pst* I restriction endonucleases. The two resulting fragments were separated by electrophoresis in a horizontal 1% agarose gel. The smaller, 3,053-base pair fragment, containing *hisOGD* and part of *hisC*, was isolated from the gel by insertion of a small piece of Whatman DE81 paper into the gel and electrophoresis in a second dimension such that the DNA fragment adsorbed to the paper (14). The paper was removed from the gel, washed four times with 50 μl of TE (10 mM Tris, pH 8/1 mM EDTA) with 0.1 M NaCl, and the fragment was eluted with four washes of TE with 1 M NaCl (50 μl each). The DNA then was extracted with phenol and ether, precipitated with ethanol, and resuspended in ligation buffer. Plasmid pBR322 DNA (grown in *E. coli*) was digested with *Eco*RI and *Pst* I and the 3,609-base pair fragment containing the tetracycline resistance gene was isolated as described above. Equimolar amounts of the two fragments were ligated for 2 hr at 16°C and then overnight at 4°C by using T4 DNA ligase. Ligated DNA (100 ng) was used to transform *E. coli* 294 to tetracycline resistance (25 μg/ml of tetracycline). Plasmid DNA from 10 tetracycline-resistant colonies was purified by the minilysate technique (15) and screened for the presence of a single *Bgl* II site. One of these, designated pAQ1, was extensively analyzed by restriction mapping.

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* Barnes, W., Tuley, E. & Eisenstadt, E. (1982) 13th Annual Meeting of the Environmental Mutagen Society Program and Abstracts, Boston, Feb. 26 to Mar. 1, 1982, No. Aa-1, p. 59.

Strain TA102. The plasmid pAQ1 was used to transform *Salmonella* ST422 (*galE*, restriction$^-$, modification$^+$). Tetracycline-resistant transformants were grown in the presence of the generalized transducing phage P22, and these phage were used to transduce TA2655 [*his*Δ(*G*)*8476*, *galE*] to tetracycline resistance. The resultant strain was designated TA2656. Restriction mapping of plasmid DNA from TA2656 showed that the plasmid had not been altered by the transduction process. A spontaneous deep rough (*rfa*) mutant (16) of TA2656 was isolated to give strain TA2657. The R-factor resistance plasmid pKM101 was introduced (17) into TA2657, to yield strain TA102 [*his*Δ(*G*)*8476 rfa*/pAQ1/pKM101].

Mutagenicity Assays. Compounds were tested for mutagenic activity in the *Salmonella* mutagen assay as described (3). Mutagens were dissolved in distilled water, except for streptonigrin, cumene hydroperoxide, and the psoralens (in dimethylsulfoxide), and phenylhydrazine (in 95% ethanol). X-ray mutagenesis was done on standard pour plates already seeded with bacteria. The x-ray source was a Machlett (Springdale, CT) OEG 60 tube with a beryllium window, operated at 50-kV peak and 25 mA, to give a dose rate of 250 rad/sec (1 rad = 0.01 gray). Plates were irradiated in 5-sec increments to a maximum of 30 sec. In photomutagenesis with psoralens, the bacteria were treated as follows before the standard assay. Bacterial cultures in phosphate-buffered saline in a 6-cm Petri dish were irradiated for 5 min at 10 cm with near-UV light (Gates–Raymaster from Thomas, Philadelphia; 320–400 nm, 4.6 J/m^2/sec at 10 cm) in the presence or absence of mutagen (18). UV mutagenesis was similar, except that a germicidal UV source (General Electric, 15 W) was used at a distance of 33 cm from the dish (3.3 J/m^2/sec) and cultures were irradiated in 3-sec increments to a maximum of 18 sec.

Copy Numbers of pAQ1 and pKM101. The copy numbers of pAQ1 and pKM101 were determined by the relative incorporation of [*methyl*-^{3}H]thymine (23 Ci/mmol, 1 mCi/ml; 1 Ci = 3.7 × 10^{10} becquerels) into plasmid versus chromosomal DNA (19). DNAs were separated by CsCl/ethidium bromide density centrifugation. Gradient fractions were assayed for incorporation of trichloroacetic acid-precipitable ^{3}H.

RESULTS

Selection of *hisG428*, a Mutation Reverted by Chemical Oxidants. We have used three oxidizing mutagens that are not appreciably mutagenic in the standard tester strains in a search for histidine mutations reverted by oxidative DNA damage. The oxidants were streptonigrin, cumene hydroperoxide, and hydrogen peroxide. These mutagens were weakly detected in strain TA92 (*hisG46*/pKM101), a relative of the standard tester strain TA100 (*hisG46 uvrB rfa*/pKM101) with a normal UV-excision repair system. The slight activity of these mutagens on the histidine mutation, *hisG46*, requires the presence of the R-factor plasmid pKM101. Seventy previously mapped and characterized histidine mutations (about half missense and half nonsense) in the *hisGDC* region of the histidine biosynthetic operon (11) were combined with the pKM101 plasmid (17), and each strain then was tested for reversion. Some missense and all the nonsense mutations were reverted. Of the strains that detected these compounds most efficiently, we chose an ochre mutant, TA103 (*hisG428*/pKM101), for further development as a tester strain. Fig. 1 compares the mutagenicity of streptonigrin in TA103 and TA92 (*hisG46*/pKM101). The *hisG428* mutation without the R factor is not appreciably reverted by streptonigrin (<2 revertants per μg), cumene hydroperoxide (<0.1 revertant per μg), or hydrogen peroxide (<0.1 revertant per μg).

Revertants of *hisG428* (in TA103) were shown to be at the *hisG428* site rather than the result of ochre suppressor mutations outside the *his* operon. Revertants of *hisG428*, derived spontaneously, or induced by cumene hydroperoxide, UV light, or psoralen were used as donors in transducing a *hisG* deletion strain to histidine prototrophy. Each of 64 revertants tested yielded transductants, indicating reversion at the *hisG428* site.

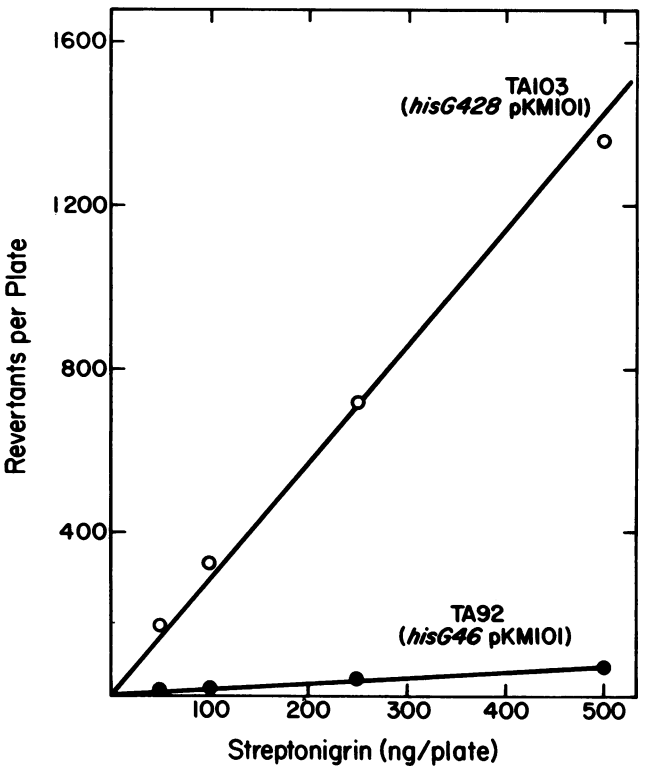

FIG. 1. Reversion of TA103 and TA92 by streptonigrin. The numbers of spontaneous revertants per plate of 58 and 50 for TA103 and TA92, respectively, were subtracted. Each point was the average of three plates.

DNA Sequence of the *hisG428* Mutation. The *hisG428* mutation was cloned onto M13Hol76 and subjected to sequence analysis as described. The mutant sequence at positions 839–853 was determined to be A-G-A-G-C-A-A-G-T-A-A-G-A-G-C-, as compared to the wild-type sequence A-G-A-G-C-A-A-G-C-A-A-G-A-G-C- at the same positions determined by W. M. Barnes and R. N. Husson (personal communication). This confirms that the mutation is an ochre and is located at position 847 from the beginning of the *his* mRNA.

Strains TA2638 and TA104. Strain TA103 (*hisG428*/pKM101) was converted to the *rfa* (deep rough) derivative TA2638 as well as the *uvrB rfa* (UV-excision repair deficient) derivative TA104 by methods described previously (16, 17). The *uvrB* mutation increased the sensitivity to reversion by streptonigrin and cumene hydroperoxide (Fig. 2). The *uvrB* mutation in TA104 also increased the sensitivity to killing (Fig. 2) and the spontaneous mutation frequency (to about 400), as expected (16, 17). It also eliminated the mutagenicity of the crosslinking agents mitomycin C and the psoralens (e.g., with 5-methoxypsoralen and near-UV: 216 revertants per μg/ml on TA103 and <10 revertants per μg/ml on TA104). These crosslinking agents require intact UV-excision repair for mutagenic activity and were quite effective at reverting TA103 and TA2638. Rather than utilize a combination of these tester strains (i.e., TA104 and TA2638), we attempted to increase the sensitivity of the *hisG428* mutation to reversion in a *uvrB*$^+$ background by markedly increasing the number of copies of the mutation in the cell.

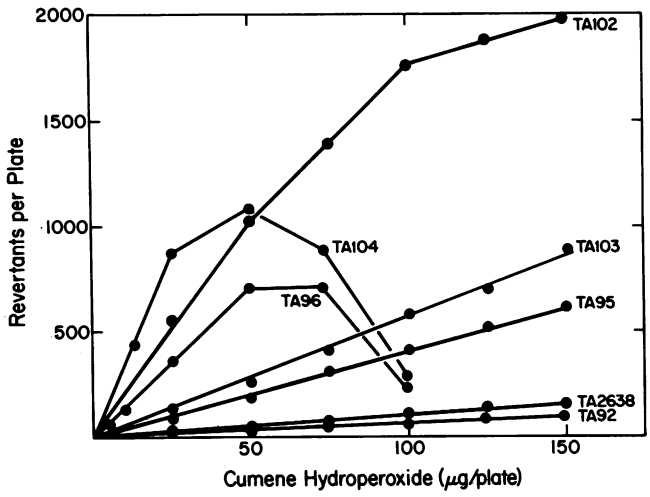

FIG. 2. Reversion of new tester strains by cumene hydroperoxide. The numbers of spontaneous revertants per plate were subtracted: TA102 = 240, TA2638 = 38, TA103 = 63, TA104 = 388, TA95 = 48, TA96 = 60. Each point was the average of at least three plates.

Plasmid pAQ1 in Strain TA102. The plasmid cloning vehicle pBR322 normally exists in multiple copies per cell (20). We have cloned the *hisOGDC* region from *Salmonella* with the *hisG428* mutation into this plasmid to produce the plasmid pAQ1, which also carries a selectable marker for tetracycline resistance. pAQ1 was introduced into a *Salmonella* strain with the *hisΔ(G)8476* deletion on the chromosome, from which the tester strain, TA102 [*hisΔ(G)8476 rfa*/pAQ1/pKM101], was constructed (see *Materials and Methods*). Fig. 3 shows the structure of the pAQ1 plasmid as determined by restriction analysis.

Copy Number of pAQ1 and pKM101 Plasmids. Copy numbers for the pAQ1 and pKM101 plasmids were determined as described. For reasons concerned with strain construction, copy numbers were determined in strain TA2667, which is isogeneic to TA102 except that it contains a *galE* mutation replacing the *rfa* mutation in TA102, a substitution which should not affect plasmid copy numbers. We determined that there are 30 copies of pAQ1 and 7 copies of pKM101 per cell based on the following: (*i*) a ratio of mass of plasmid to chromosomal DNA of 0.120 in TA2667; (*ii*) a control determination of 41 copies of pAQ1 (0.072 ratio of plasmid to chromosomal DNA) in an isogeneic strain (TA2656) containing pAQ1, but not pKM101; (*iii*) a control determination of 10 copies of pKM101 (0.095 ratio of plasmid to chromosomal DNA) in an isogeneic strain (TA2666) containing pKM101, but not pAQ1; (*iv*) the assumption that the *relative* copy numbers of pAQ1 and pKM101 do not change when they are in the same strain; this was necessary because the amount of plasmid DNA is somewhat less than additive when both plasmids are together. It is not possible to separate the two plasmids by CsCl/ethidium bromide density centrifugation as they have approximately the same buoyant density under these conditions. Our estimate of about 30 copies of pAQ1 per cell is consistent with copy number estimates of 20–50 copies for other small plasmids in *Salmonella* that contain the pBR322 origin of replication (20).

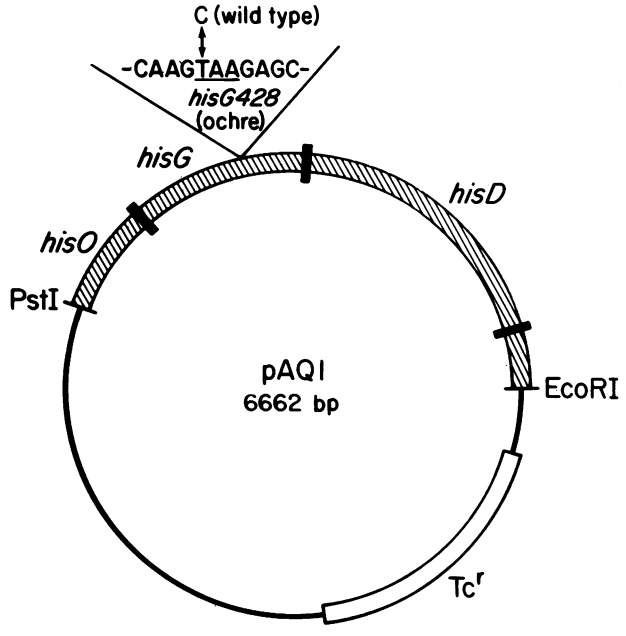

FIG. 3. Structure of plasmid pAQ1.

Mutagenicity Assays with TA102. TA102 was tested for reversion by chemical oxidants and carcinogens that were previously negative in the standard tester strains. TA102 was compared to strains TA2638 (Table 1 and Fig. 2) and TA104, which carry the *hisG428* mutation on the chromosome rather than on a multicopy plasmid. The spontaneous reversion frequency was increased 6-fold in TA102 over TA2638, presumably as a result of having *hisG428* in multiple copies. All of the mutagenic agents tested that were mutagenic on TA2638 were more active on TA102. Most agents reverted TA102 between 5 and 12 times better than TA2638. Kethoxal, glyoxal, glutaraldehyde, and UV light were about 4 times as mutagenic on TA102, and streptonigrin was only 2 times as active on TA102. Mitomycin C and bleomycin were about 25 and 70 times as mutagenic, respectively, on TA102 as on TA2638. Daunomycin, another quinone, also reverts TA102 quite effectively (592 revertants per 6 μg)

Table 1. Mutagens that revert TA102

Mutagen	His⁺ revertants per plate	
	TA102	TA2638
(Spontaneous)	(240)	(38)
Bleomycin (1 μg)	1,245	18
Streptonigrin (100 ng)	1,130	557
Hydrogen peroxide (100 μg)	746	91
t-Butyl hydroperoxide (100 μg)	3,073	266
Cumene hydroperoxide (100 μg)	1,780	119
Neocarzinostatin (1 μg)	1,946	209
Phenylhydrazine (250 μg)	1,184	160
Mitomycin C (500 ng)	2,772	118
UV light (19.8 J/m²)	1,942	571
X-rays (2.5 krad)	1,132	145
Psoralen (1 μg/ml and near-UV)	2,512	257
8-Methoxypsoralen (1 μg/ml and near-UV)	4,171	997
Angelicin (20 μg/ml and near-UV)	1,832	160
Formaldehyde (100 μg)	369	44
Glyoxal (1 mg)	637	128
Kethoxal (200 μg)	1,374	402
Glutaraldehyde (25 μg)	389	93
Malondialdehyde (2 mg)	141	24
Paraquat (10 ng)	40	0
Diquat (10 ng)	36	0

The numbers of spontaneous revertants per plate were subtracted. Each value was the average of at least three plates and was taken from the linear region of dose–response curves. Psoralen-, x-ray-, and UV-mutagenesis experiments were conducted as described in *Materials and Methods*.

as does methylmethane sulfonate (6,586 revertants per μl) (21). TA104 also was reverted by the quinones and some hydroperoxides with about the same sensitivity as TA102, but as can be seen in Fig. 2, TA104 is considerably more sensitive to killing. TA104 was reverted somewhat better than TA102 by some of the aldehydes. TA102 was superior to TA104 for detecting the crosslinking agents and bleomycin. Paraquat and diquat were not appreciably mutagenic in any of the strains, though they were very toxic. All of the mutagens tested require a strain with the R factor to show a mutagenic response (data not shown), with the exception of malondialdehyde, which was slightly more mutagenic in the absence of the R factor. A *uvrB* derivative of TA102, TA2664, was constructed, but its high spontaneous reversion frequency (about 800) made it inconvenient for use in mutagen assays.

Strains TA95 and TA96. In addition to *hisG428*, which has A·T base pairs at the site of the mutation, we have constructed a frameshift tester strain with a run of A·T base pairs, a sequence not present in any of our standard tester strains. The +1 frameshift mutation *hisD6580*, isolated by Bossi and Roth, has an added A·T base pair near a run of five A·T base pairs (22). We have constructed TA95, a pKM101 derivative of TR2705 (*hisO1242 hisD6580*), as well as a complete tester strain, TA96, which is deep rough, *uvrB*, and carries pKM101. TA95 and TA96 have mutational spectra very similar to that of TA103 and TA104, the isogeneic strains with the *hisG428* mutation (Fig. 2). TA104 is somewhat more sensitive than TA96 to reversion by all of the mutagens in Table 1 that reverted TA96 (data not shown). However, malondialdehyde is detected better (116 revertants per mg; 15 spontaneous revertants) by *hisD6580* than by *hisG428*. Crosslinking agents such as the psoralens and mitomycin C are lethal to *uvrB* strains (23, 24) and therefore, were not detected as mutagens by either TA96 or TA104.

DISCUSSION

Oxygen radicals may be the most important class of mutagens contributing to aging and cancer (25), yet a number of oxidants that have been tested are not detected as mutagens by the standard *Salmonella* tester strains. We have screened a large number of histidine mutations to select strains that are reverted efficiently by three oxidants: (*i*) streptonigrin, a quinone that is reduced intracellularly by one electron to the reactive semiquinone radical, which, in turn, can participate in redox cycling with oxygen to generate superoxide radicals (26). Streptonigrin was chosen as a model for the many quinones to which humans are exposed, such as natural toxic compounds in plants or metabolites of drugs and polycyclic hydrocarbons (25); (*ii*) cumene hydroperoxide, a strong oxidant used in plastics manufacturing to catalyze radical polymerization reactions. Cumene hydroperoxide was chosen as a model for the numerous lipid and cholesterol hydroperoxides that are ingested in oxidized fat and also are generated *in vivo* as part of rancidity reactions in cell membranes and which may be a major source of mutagenic oxygen radicals (25); (*iii*) hydrogen peroxide, one of the major endogenous carcinogens generated from normal metabolism (25). The mutations we have selected from this screening, when incorporated into tester strains, effectively detect all three of these oxidants as mutagens, as well as a variety of other mutagens not previously detected in the *Salmonella* mutagen assay.

Construction of Tester Strains Revertible by Oxidants. We selected *hisG428*/pKM101 (TA103), an ochre mutant (11), from the 70 histidine mutants screened, as the most sensitive to reversion by the oxidants, taking into account the desirability of a low spontaneous reversion frequency and a uniform colony size for revertants. The tester strains we built from this strain, TA2638 (*hisG428 rfa*/pKM101) and TA104 (*hisG428 rfa uvrB*/pKM101), detected a variety of oxidants and other mutagens but had some disadvantages. TA104, which is isogeneic to the standard tester strains except for the histidine mutation, was quite sensitive to reversion by most of the agents in Table 1 but was very sensitive to killing by these mutagens, so that only a narrow range of concentrations was suitable for testing. In addition, it did not detect crosslinking agents, such as the psoralens (with near-UV) and mitomycin C, which require a $UvrB^+$ background. TA2638 detected the crosslinking agents but was not nearly as sensitive as TA104 for detecting the oxidative mutagens. As an alternative to using the two strains together as new tester strains, we explored a new approach to constructing a tester strain, which involved increasing the number of copies of the histidine mutation in the cell. This was accomplished by putting the mutated gene on a multicopy plasmid in a $UvrB^+$ background.

The plasmid, pAQ1, which contains the *hisG* gene with the *hisG428* mutation, was constructed and introduced into a cell that carries a deletion of the *hisG* gene on the chromosome and also contains the *rfa* (deep rough) marker and the pKM101 plasmid. This strain, TA102, has proved to combine the best attributes of both TA2638 and TA104. TA102 also detects bleomycin, which is not detected well by either TA2638 or TA104. This differential sensitivity to bleomycin mutagenesis could be due to some aspect of plasmid supercoiling or an ineffective repair system for plasmid DNA. Studies on copy number indicate that there are about 30 copies of pAQ1 and about 8 copies of pKM101 in TA102. We note that the presence of about 30 copies of *hisG428* (on pAQ1) in TA102 only increases the spontaneous reversion frequency 6-fold over that observed in the isogeneic strain, TA2638, with one copy of the mutation on the chromosome. We recommend that TA102 be added to the set of standard tester strains. TA2638 and TA104 could be used as auxiliary strains for special purposes.

We also have constructed frameshift tester strains TA95 (*hisO1242 hisD6580*/pKM101) and TA96 (*hisO1242 hisD6580 rfa uvrB*/pKM101) that contain the sequence -A-A-A-A-A- at the site of reversion. As these strains detect the same spectrum of agents that revert TA102, but are less sensitive, we recommend that they not be used as part of the standard tester strains, but only as auxiliary strains.

DNA sequence analysis shows that the *hisG428* mutation (contained in TA102, TA103, TA2638, and TA104) contains the sequence A-G-A-G-C-A-A-G-T-A-A-G-A-G-C- at positions 839–853, whereas the wild type contains the sequence A-G-A-G-C-A-A-G-C-A-A-G-A-G-C- at the same positions. This confirms the classification of this mutant as an ochre type and the presence of only A·T base pairs in the mutated triplet.

Mutagens Active on the New Tester Strains Damage A·T Base Pairs. The mutagens in Table 1 are detected poorly, or not at all, by the standard tester strains, which contain G·C base pairs at the site of reversion. These mutagens are active in the new strains because they act preferentially at A·T base pairs. Bleomycin and neocarzinostatin are antitumor antibiotics that interact with DNA, causing strand scission and preferential release of free thymine, probably through a mechanism involving oxygen radicals (27–30). Bleomycin preferentially causes strand scission at thymines adjacent to guanines in DNA (28). The thymine at the mutated site in *hisG428* (changed from a cytosine) is, in fact, next to a guanine. Neocarzinostatin was previously shown to revert the ochre mutation in the *E. coli* strain, WP2, and several ochre and amber mutations in *Salmonella* (all of which contain A·T base pairs at the mutated sites) (29). The pKM101 plasmid is required for mutagenesis by neocarzinostatin (29). UV light forms thymine dimers and this may account

for its activity on *hisG428*, which has two adjacent thymines at the mutated site available for dimer formation. Psoralens are light-activated carcinogens that, in the presence of near-UV light, make monoadducts to thymine and interstrand crosslinks (with the exception of angelicin) in the presence of an adjacent thymine in the opposite strand (31, 32), as is the case with *hisG428*. In the present study, psoralen, 5-methoxypsoralen, and 8-methoxypsoralen (derivatives capable of crosslinking DNA) were considerably more mutagenic than angelicin in a $UvrB^+$ background, suggesting that the formation of interstrand crosslinks is the major mutagenic lesion induced, as was previously suggested (23, 32). The psoralens and mitomycin C (a quinone that causes crosslinks in DNA) required a $UvrB^+$ background for mutagenic activity, which was one of the reasons for introducing TA102 (which is $UvrB^+$) as a new tester strain. The various mono- and dialdehydes tested in the present study also appear to induce mutations at A·T base pairs. Formaldehyde has been previously shown to be a mutagen in higher organisms (33), as well as having very weak activity on TA100.† Recently, it has been shown to be a carcinogen in rats (34). The other oxidizing mutagens—x-rays, hydrogen peroxide, cumene hydroperoxide, *t*-butyl hydroperoxide, phenylhydrazine, streptonigrin, and daunomycin—are all known to generate oxygen radicals and most have been shown previously to be mutagens or carcinogens (or both) (3, 25, 35, 36). One of the known products of radiation damage and oxidative damage to DNA is thymine glycol, for which a base-specific repair glycosylase has been described (37). This could be an important DNA lesion formed by oxidative mutagens. Two other oxidants, paraquat and diquat, were not detected as mutagens, though they are thought to be active through formation of a semiquinone which generates superoxide radicals. They are quite toxic to the bacteria and it must be determined whether there is some other toxicity factor that masks mutagenicity. A report that paraquat and mitomycin C are mutagenic in TA100 (38) is in contrast to our experience. Mitomycin C is well known not to be mutagenic in *uvrB* strains such as TA100 (8, 24).

The attribution of the mutagenic event to mutation at an A·T base pair depends on the assumption that the revertant is not due to a suppressor, a possibility in the case of the *hisG428* ochre mutation. Our analysis of 64 revertants of *hisG428*/pKM101 (TA103), induced by a variety of mutagens, showed that none of them was due to suppressors. This is evidence that the revertants are indeed due to mutations at the A·T base pairs in the ochre triplet.

Note Added in Proof. TA102 can be stored in the refrigerator on a "master" plate (containing histidine, ampicillin, and tetracycline in minimal glucose agar) for up to 2 months for inoculating daily cultures for mutagenicity testing (3, 21). When the master plate contained very high levels of tetracycline approaching the inhibitory concentration (>25 μg/ml), we observed up to a doubling of the spontaneous mutation rate and a corresponding increase in sensitivity to mutagens (presumably caused by an increased copy number of the pAQ1 plasmid). We suggest that a level of tetracycline at 10 μg/ml be used in the master plate, as this gives a reproducible spontaneous frequency of about 240–320 revertants per plate (the conditions used in this study).

Glyoxal has been shown previously to be mutagenic on TA100 (39).

† Couch, D. B., Allen, P. F. & Eales, H. C. (1982) 13th Annual Meeting of the Environmental Mutagen Society Program and Abstracts, Boston, Feb. 26 to Mar. 1, 1982, No. Bh-7, p. 99.

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