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Genetics

Spontaneous and mutagen-induced deletions: Mechanistic studies in *Salmonella* tester strain TA102

(single-strand breaks/mutagenic gyrase inhibitors/DNA repair/multicopy plasmid)

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ABSTRACT ***Salmonella*** **tester strain TA102 carries the *hisG428* ochre mutation on the multicopy plasmid pAQ1. DNA sequence analysis of 45 spontaneous revertants of *hisG428* on the chromosome in the presence of pKM101 (strain TA103) indicates that *hisG428* revertants fall into three major categories: (*i*) small, in-frame deletions (3 or 6 base pairs) that remove part or all of the ochre triplet; (*ii*) base substitution mutations at the ochre site; (*iii*) extragenic ochre suppressors. Deletion revertants are identified in a simple phenotypic screen by their resistance to the inhibitory histidine analog thiazolealanine, which feedback inhibits the wild-type *hisG* enzyme but not the enzyme resulting from the deletions. The effect of various genetic backgrounds on the generation of spontaneous deletion revertants was examined. The error-prone repair system encoded in the pKM101 plasmid markedly increased the frequency of total spontaneous reversion events in all genetic backgrounds except *recA* but did not affect the frequency of spontaneous deletion revertants in any background except *polA*. The presence of a *polA* mutation increased the frequency of spontaneous deletion revertants by 2-fold in the absence of pKM101 and by 20-fold with pKM101. The presence of a *uvrB* mutation or a *recA* mutation suppressed the generation of spontaneous deletion revertants to approximately 1/2.5. When *hisG428* was in multiple copies on pAQ1, the frequency of spontaneous deletion revertants increased by 40-fold, which is the approximate copy number of pAQ1. Mutagenic agents that induce single-strand breaks in DNA (e.g., x-rays, bleomycin, and nalidixic acid) induced deletion revertants in TA102. These agents induced deletion revertants only in *hisG428* on pAQ1 and only in the presence of pKM101. Deletion revertants were not induced by frameshift mutagens (i.e., ICR-191 and 9-aminoacridine). These results indicate that different pathways exist for the generation of spontaneous and mutagen-induced deletion revertants of *hisG428*.**

Genetic rearrangements such as deletions and insertions constitute an important class of mutations. Although it has been suggested that they are important as initiating events in tumor production (1, 2), their study has been largely neglected. Several systems have been described for detecting large deletions (0.7–40 kilobases) in bacteria (3–6). Large deletions are induced by the mutagens mitomycin C, 4-nitroquinoline 1-oxide, nitrous acid, nitrogen mustard, x-rays, and UV light (4–6), although there is variation among different systems.

In this study we present a simple system for detecting a class of small deletions [3 or 6 base pairs (bp)] in the *hisG* gene of *Salmonella*, using the recently described tester strains TA102 and TA104 (7, 8). The occurrence of spontaneous and mutagen-induced deletions was studied in a variety of DNA repair backgrounds.

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MATERIALS AND METHODS

Materials. *N*-(2-Thiazolyl)-DL-alanine, methyl methanesulfonate, nitrogen mustard, and 2,7-dinitro-9-fluorenone were from Aldrich; bleomycin sulfate, nalidixic acid, oxolinic acid, methyl glyoxal, mitomycin C, 9-aminoacridine, and 4-nitroquinoline 1-oxide were from Sigma; cumene hydroperoxide was from Pfaltz & Bauer (Stamford, CT); sodium nitrite was from Mallinckrodt; ICR-191 was from Polyscience (Warrington, PA); AM715 was from N. Cozzarelli; *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide was from the laboratory of L. J. Marnett; deoxycytidine [α-^{32}P]triphosphate was from Amersham; and *Escherichia coli* DNA polymerase large fragment was from Boehringer Mannheim. Bacterial strains are listed in Table 1.

Cloning and DNA Sequence Analysis of Spontaneous *hisG428* Revertants. Histidine-independent (His$^+$) revertants of *Salmonella typhimurium* strains carrying the *hisG428* ochre mutation (9) were selected on minimal-glucose plates supplemented with a trace of histidine (11, 12). The *hisG* gene in revertants of TA103[*hisG428*/pKM101] was cloned by *in vivo* recombination into a derivative of phage M13Hol76. M13Hol76 contains the histidine operator, *hisG*, *hisD*, and part of the *hisC* gene from *Salmonella* (13); the derivative, M13Hol76*his*Δ(*OG*)*8473* (10), is missing part of the operator and the *hisG* gene. Male derivatives of revertants were constructed to make them sensitive to infection with M13, a male-specific phage. Revertants were infected with M13Hol76*his*Δ(*OG*)*8473* and HisG$^+$ recombinant phage were selected by their ability to complement the *his*Δ(*G*)*8476* host, TA2892 (10). Single-stranded DNA from these HisG$^+$ recombinant phage was isolated (14) and subjected to sequence analysis (15) using the 90-bp *Taq I* restriction fragment from M13Hol76 as a primer (16).

Thiazolealanine Resistance. His$^+$ revertants of strains carrying the *hisG428* mutation were characterized by their sensitivity to the histidine analog *N*-(2-thiazolyl)-DL-alanine. Revertants were picked directly from minimal-glucose plates and streaked in a radial fashion (approximately 30 revertants per plate) onto fresh minimal-glucose plates by using a fine platinum wire. Minimal plates were supplemented with biotin (2 μM) for strains with *gal bio uvrB* deletions. An aqueous solution of thiazolealanine (25 μl of 20 mg/ml) was applied to filter paper discs (6 mm, Becton Dickinson), which were then placed in the center of streaked plates. Plates were incubated at 37°C for 24 hr.

Mutagenicity Assays. Compounds were tested for mutagenic activity in the *Salmonella* mutagen assay as described (11, 12). X-ray mutagenesis was conducted as described (7). Nitrous acid mutagenesis was done by incubating 0.1 ml of

Abbreviations: bp, base pair(s); His$^+$, histidine-independent; TA^r, TA^s, and TA^{hs}, thiazolealanine resistant, sensitive, and hypersensitive.

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Table 1. *Salmonella* strains

Strain	Genotype	Source
LT2	Wild type	N. Zinder
SA2197	*purC7*/F'42 *fin-301* lactose-utilizing	K. Sanderson
hisG428	*hisG428*	Ref. 9
TA2892	*hisΔ(G)8476*/F'42 *fin-301* lactose-utilizing	Ref. 10
TA2665	*hisΔ(G)8476*	This work
TA2661	*hisΔ(G)8476*/pAQ1	This work
TA2662	*hisΔ(G)8476*/pAQ1/pKM101	This work
TA2898	*hisG428 recA1 srl-2*::Tn*10*	This work
TA2899	*hisG428 recA1 srl-2*::Tn*10*/pKM101	This work
TA1890	*zec-2*::Tn*10 hisG428 polA2 ara-9*	This work
TA1891	TA1890/pKM101	This work
TA2659	*hisG428 ΔuvrB gal bio chl-1057 rfa-1028*	This work
TA104	TA2659/pKM101	Ref. 7
TA103	*hisG428*/pKM101	Ref. 7
TA2657	*hisΔ(G)8476 galE503 rfa-1027*/pAQ1	Ref. 7
TA102	TA2657/pKM101	Ref. 7
TA2638	*hisG428 galE531 rfa-1026*/pKM101	Ref. 7

tester strain cultures with nitrous acid in 0.5 ml of 0.1 M sodium acetate (pH 4.7) for 5 min at 37°C. Mutagens were dissolved in dimethyl sulfoxide, except for bleomycin sulfate, mitomycin C, sodium nitrite, nalidixic acid, oxolinic acid, and AM715, which were dissolved in distilled water.

RESULTS

DNA Sequences of Spontaneous Revertants of *hisG428*. The *hisG* gene from 72 spontaneous revertants of TA103[*hisG428*/pKM101] was recombined into phage M13Hol-76*hisΔ(OG)8473* and His$^+$ recombinant phage were selected. Of these, 41 (57%) failed to produce His$^+$ phage, indicating that these revertants are His$^+$ by virtue of an extragenic ochre suppressor. DNA from the remaining 31 His$^+$ recombinants was sequenced in the region of the *hisG* gene known to carry the *hisG428* mutation (7). These results are shown in Fig. 1. Revertants of the ochre (TAA) mutation fell into the four following categories: 7 isolates were a T·A → C·G transition to the wild-type sequence CAA (glutamine); 7 isolates were a T·A → A·T transversion to AAA (lysine); 15 isolates were a T·A → A·T transversion to TTA (leucine); and 2 isolates were a 6-bp deletion (Δ2) that maintains the reading frame and removes part of the ochre triplet.

Phenotypic Screen for Deletion Revertants: Thiazolealanine Resistance. The four revertant classes at the *hisG428* site are indistinguishable from each other by growth rate in minimal medium (data not shown). To distinguish among these classes, the inhibitory histidine analog thiazolealanine was used. Thiazolealanine inhibits the *hisG* gene product, phosphoribosyl-ATP synthetase, which catalyzes the first step in the biosynthesis of histidine, at the histidine feedback inhibition site (17). Inhibition studies using thiazolealanine in radial streak assays demonstrated that the 6-bp deletion mutant described above was resistant to thiazolealanine, whereas all of the point mutation revertants were sensitive to thiazolealanine inhibition to the same extent as the wild-type strain, LT2.

Additional spontaneous revertants of TA103 were screened for thiazolealanine resistance and 14 of 316 (4%) were resistant. Each of these 14 revertants was sequenced and all contained a small, in-frame deletion (3 or 6 bp) that removed part or all of the ochre triplet (Fig. 1; 3 of Δ1; 3 of Δ2 plus the 2 original isolates; 7 of Δ3; and 1 of Δ4). The *hisG428* site is apparently a critical determinant for feedback inhibition of the encoded enzyme, and deletion of one or two amino acids from the protein at this position results in an enzyme that is not subject to feedback inhibition by thiazolealanine. Previously identified *Salmonella* mutants resistant to feedback inhibition by histidine have been mapped to areas of the *hisG* gene surrounding the *hisG428* site (18).

A major class (195/316) of revertants was identified as hypersensitive to thiazolealanine inhibition. These revertants contained extragenic ochre suppressor mutations. The frequency of thiazolealanine hypersensitivity (62%) was similar to the frequency of extragenic ochre suppressors (41/72, or 57%: all thiazolealanine-hypersensitive) uncovered by genetic analysis in the DNA sequencing experiments.

These observations were the basis for a simple phenotypic screen to distinguish among three general classes of *hisG428* revertants: Strains with small deletions are resistant to thiazolealanine (TA^r), strains with point mutations at the *hisG428* site are sensitive (TA^s), and strains with extragenic ochre suppressors are hypersensitive (TA^{hs}). These results are shown in Fig. 2. This class of ochre suppressors has been further characterized (unpublished results).

Frequency of Spontaneous *hisG428* Deletion Revertants in Different Genetic Backgrounds. The effects of a *polA* mutation, a *uvrB* mutation, and a *recA* mutation on the frequency of spontaneous deletion revertants were determined. Reversion of *hisG428* on the multicopy plasmid pAQ1 was also studied. The pKM101 plasmid (19), which confers error-prone repair to *Salmonella* (20, 21), was introduced into each of these backgrounds to study its effect. These results are shown in Table 2.

(*i*) The error-prone repair system encoded in the pKM101 plasmid markedly increased the spontaneous frequency of revertants due to point mutations, but not the frequency of spontaneous revertants due to deletions (e.g., TA103 vs.

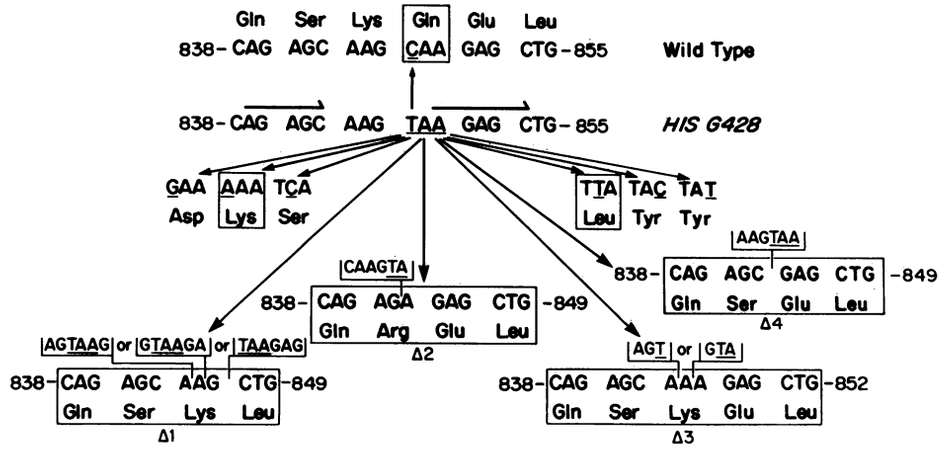

FIG. 1. DNA sequence analysis of spontaneous revertants of TA103[*hisG428*/pKM101]. Base pair substitution mutations were to glutamine (CAA, strain TA2668), leucine (TTA, strain TA2669), and lysine (AAA, strain TA2670). Deletion revertants 1 through 4 were designated TA2671, TA2672, TA2673, and TA2674, respectively.

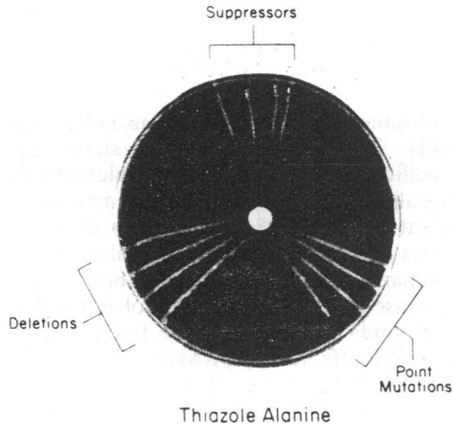

FIG. 2. Thiazolealanine radial streak plate. Revertants of TA103[*hisG428*/pKM101] are TA^r (small deletions), TA^s (point mutations at *hisG428*, one of which, TA2668, results in a wild-type gene), or TA^{hs} (extragenic ochre suppressors).

hisG428; TA104 vs. TA2659). The increase in spontaneous reversion frequency due to the presence of pKM101 is abolished in the *recA* derivative (TA2899 vs. TA103). This observation is in accord with the fact that the *recA* gene is also required for error-prone repair processes, and has been directly implicated in SOS-dependent mutagenesis (22). It is interesting to note, however, that the pKM101-dependent increase in *hisG428* spontaneous reversion (TA103 vs. *hisG428*) is also *recA* dependent (TA2898 vs. TA2899). (*ii*) The *polA* mutation (in TA1890) increased the frequency of spontaneous deletion revertants by approximately 2-fold in the absence of pKM101 (TA1890 vs. *hisG428*) and by approximately 20-fold in the presence of pKM101 (TA1891 vs. TA103). (*iii*) The *uvrB* mutation (in TA2659) and the *recA* mutation (in TA2898) suppressed the generation of spontaneous deletion revertants, which were reduced to approximately 1/2.5 in the *uvrB* strain (TA2659 vs. *hisG428*) or the *recA* strain (TA2898 vs. *hisG428*). This reduced frequency was not altered by the presence of pKM101 (TA2659 vs. TA104; TA2898 vs. TA2899). (*iv*) When *hisG428* was in multiple copies on pAQ1 (TA2657), the spontaneous deletion frequency was increased from that of *hisG428* in single copy on the chromosome by approximately 40-fold (TA2657 vs. *hisG428*; TA102 vs. TA103), which is the approximate copy number of pAQ1 in TA2657 (7). By analogy with TA^r revertants of *hisG428* on the chromosome, we interpret this result to mean that the frequency of deletion revertants in pAQ1-containing strains is increased directly with the copy number of the *hisG428* allele. We have considered, and rejected, three alternative explanations to account for this increase in the frequency of TA^r revertants in pAQ1-containing strains (23).

Table 2. Revertants of *hisG428* in various genetic backgrounds: Deletions relative to total revertants

	pKM101 absent		pKM101 present	
Background	Strain	His$^+$ revertants per plate	Strain	His$^+$ revertants per plate
Chromosomal	*hisG428*		TA103	
hisG428	Total	6.0	Total	65
	Deletions	2.4	Deletions	2.6
Plasmid	TA2657		TA102	
hisG428	Total	183	Total	284
(pAQ1)	Deletions	111	Deletions	105
Chromosomal	TA1890		TA1891	
hisG428,	Total	13	Total	449
polA	Deletions	4.2	Deletions	51
Chromosomal	TA2659		TA104	
hisG428,	Total	12	Total	350
uvrB	Deletions	1.1	Deletions	1.0
Chromosomal	TA2898		TA2899	
hisG428,	Total	6.0	Total	6.3
recA	Deletions	1.1	Deletions	1.2

Values represent the average number of total revertants and deletion revertants per plate. The frequency of spontaneous deletions per plate was calculated from the percent deletions and the total number of revertants per plate. Deletion revertants were identified as TA^r. Approximately 300 revertants were analyzed from TA2657 and TA102; 350 revertants from *hisG428*, TA2659, TA1890, and TA1891; 500 revertants from TA103, TA2898, and TA2899; and 850 revertants from TA104.

Mutagen-Induced Deletion Revertants of *hisG428*. A variety of mutagens that revert strains carrying the *hisG428* mutation were tested for their ability to induce deletion revertants in a strain with *hisG428* on the chromosome (TA2638) and in one with *hisG428* on the pAQ1 plasmid (TA102). These results are shown in Table 3. Several mutagens induced a high frequency of deletions in TA102, particularly bleomycin, x-rays, and the DNA gyrase inhibitors, nalidixic acid, oxolinic acid, and AM715. Neither these agents nor any of the other mutagens tested induced deletion revertants in the chromosomal *hisG428* strain, TA2638. The agents in Table 3 were also incapable of inducing deletion revertants of *hisG428* on the chromosome in a *recA*, *polA* or *uvrB* background (data not shown).

Bleomycin, x-rays, and the DNA gyrase inhibitors were not appreciably mutagenic on TA2657, the derivative of TA102 that lacks pKM101 (<10 revertants per μg for bleo-

Table 3. Mutagen-induced deletion revertants of TA102

	TA102		TA2638	
Mutagen	His$^+$ revertants per plate	% deletions	His$^+$ revertants per plate	% deletions
(Spontaneous)	(244)	(37)	(53)	(4)
Bleomycin (2 μg)	1719	76	23	NT
X-rays (2.5 krad)	987	50	127	0
Nalidixic acid (6 μg)	394	54	12	NT
Oxolinic acid (1 μg)	1571	51	45	0
AM715 (1 μg)	2103	59	96	0
MMS (250 μg)	1972	13	1646	0
MG (40 μg)	1604	10	678	0
DNFone (25 μg)	2548	6	226	0
NQNO (5 μg)	2344	0	217	0
CHP (150 μg)	2587	20	290	0
BPDE (500 ng)	3798	11	560	0
MMC (500 ng)	3650	26	156	0
Nitrous acid (5 mg/ml)	983	25	115	0
NM (1 mg)	2828	26	445	0

The numbers of spontaneous revertants per plate were subtracted. Values for induced reversion were taken from the linear portion of dose–response curves except for the gyrase inhibitors, which gave sigmoidal curves. Each value is representative of several experiments, in which approximately 100 revertants were tested for thiazolealanine resistance. The percent deletions induced was calculated from the frequency of TA^r revertants after spontaneous occurrences had been subtracted. MMS, methyl methanesulfonate; MG, methyl glyoxal; DNFone, 2,7-dinitro-9-fluorenone; NQNO, 4-nitroquinoline 1-oxide; CHP, cumene hydroperoxide; BPDE, benzo[*a*]pyrene-7,8-diol-9,10-epoxide; MMC, mitomycin C; NM, nitrogen mustard; NT, not tested due to insufficient mutagenic activity. One rad = 0.01 gray.

mycin, nalidixic acid, and oxolinic acid; <10 revertants per 5 krad of x-rays). Since these agents induced primarily deletion revertants in TA102, and their mutagenic activity is dependent on pKM101, it follows that pKM101 is required for the generation of mutagen-induced deletion revertants. This is in apparent contrast to the observation that pKM101 has no effect on the frequency of spontaneous deletion revertants (except in a *polA* background). The frameshift mutagens 9-aminoacridine and ICR-191 did not revert any of the *hisG428*-containing tester strains (<10 revertants per 100 μg of 9-aminoacridine or 5 μg of ICR-191 in TA102).

DISCUSSION

The most recent additions to the *Salmonella* mutagenicity test are tester strains that carry the *hisG428* ochre (TAA) mutation (7). The strain suggested for use in general mutagen screening was TA102, which carries the *hisG428* mutation on the multicopy plasmid pAQ1 (7). Since it is of interest to know the types of mutations that revert *hisG428* and which of these are induced by various mutagens, we set out to characterize revertants of *hisG428*. The class of *hisG428* revertants described in detail in the present study is a set of small, in-frame deletions (3 or 6 bp) of the ochre triplet. Several systems have been described previously for detecting large deletions (3–6); however, a positive selection system for the detection of small deletions has not been reported previously.

Characterization of Spontaneous Revertants of *hisG428* on the Chromosome. We characterized 72 spontaneous revertants of TA103 [*hisG428*/pKM101]. Forty-one of these were extragenic suppressor mutations, residing in the anticodons of four suppressor tRNA species (unpublished results). Twenty-nine of the revertants arose from base-pair substitution mutations at the *hisG428* site, resulting in replacement of the ochre triplet with a glutamine, lysine, or leucine codon, as determined by DNA sequence analysis. Two small deletions were identified among the sequenced *hisG428* revertants. As outlined in *Results*, these deletions lie in a portion of the *hisG* gene that makes the *hisG* protein resistant to inhibition (at the histidine feedback-inhibition site) by the histidine analog thiazoleälanine. Fourteen additional TA^r revertants (= 4% of 314 screened) were sequenced and all were deletions. Thus 16 deletions fell into four classes either 3 or 6 bp in length that removed part or all of the ochre triplet (Fig. 1). Three more possible deletions that would result in amino acid sequences identical or very similar to one of the identified deletions were not found among our collection.

The *hisG428* site is flanked by a 5-bp direct repeat (5′ A-G-A-G-C 3′) which may be important in the generation of small deletions through base mispairing. Albertini *et al.* (3) have reported that large deletions which arise in the *lacI* gene of *E. coli* occur almost exclusively between short direct repeats. Two classes of models have been proposed to explain the spontaneous generation of deletions. One involves single-stranded intermediates that form during DNA replication, and the other invokes recombinational events mediated by enzymes that recognize short homologies (3, 24, 25). The finding that spontaneous deletions often occur at short repeated DNA sequences prompted the suggestion that slipped-base mispairing during DNA synthesis might be involved in deletion formation (24), as had been proposed for the generation of frameshift mutations (26). Alternatively, the repeated sequences might act as substrates for homologous but unequal recombination. DNA secondary structure resulting from palindromic sequences has recently been proposed to be involved in the generation of some deletions by juxtaposing deletion endpoints (25). The repeated sequence flanking *hisG428* may also allow the persistence of a single-stranded region of DNA at this site. Such a single-stranded region may make this site more sensitive to damage by mutagens and may be an important factor in the sensitivity of *hisG428*-containing strains to reversion by some mutagens (7).

Characterization of *hisG428* Revertants in Different Genetic Backgrounds. The development of a simple phenotypic screen to facilitate the identification of deletion revertants of *hisG428* has allowed us to screen large numbers of spontaneous revertants of *hisG428* in a variety of genetic backgrounds. This analysis has led to several observations that can be summarized as follows. (*i*). Although the pKM101 plasmid increased the total frequency of *hisG428* revertants in all backgrounds except *recA*, the frequency of deletion revertants was unaffected by pKM101 in every background except *polA*. pKM101 increased the frequency of spontaneous deletion revertants in a *polA* background. The pKM101-encoded *muc* genes (19–21, 27), which have been implicated in error-prone repair (20), enhance the frequency of base-pair substitution mutations but not frameshift mutations (19, 28). Since the known ochre suppressors in *E. coli* and *Salmonella* arise from base substitution mutations (29), the frequencies of point mutations at the *hisG428* site and extragenic ochre suppressors are expected to be enhanced by pKM101. If spontaneous deletion revertants arise through a mechanism common to the generation of frameshift mutations, their frequency would not be expected to be enhanced by pKM101. (*ii*) A *polA* mutation slightly enhanced the frequency of spontaneous deletions; the frequency was further increased to 20-fold in the presence of pKM101. This is in agreement with the observation that the frequency of spontaneous large deletions in the *tonB–trp* region of *E. coli* is enhanced 20-fold by a *polA* mutation (5). (*iii*) A *uvrB* deletion and a *recA* mutation both decreased the frequency of spontaneous deletions to approximately 1/2.5. Mutants in these related repair systems may be deficient in a common pathway involved in generating deletions, since expression of *uvrB* is under the control of the *recA*, *lexA* regulatory system (30, 31). The frequency of deletions in the *lacI* gene of *E. coli* is reduced to 1/25th in a *recA* mutant (3). Anaerobic growth also results in a preferential reduction in the frequency of deletion revertants of *hisG428* (to approximately 1/3), and the frequency is further reduced (to approximately 1/10) in the *recA* derivative under these conditions (32). This result suggests that at least two independent pathways exist for the generation of the small deletions monitored in this system. (*iv*) The frequency of deletion revertants was increased by approximately 40-fold in a strain that carries *hisG428* on pAQ1. This increase reflects the copy number of the pAQ1 plasmid.

Mutagen-Induced Deletion Revertants of *hisG428* in TA102. Among the SOS-dependent mutagens screened, several induced deletion revertants of *hisG428* in TA102. Bleomycin is an antitumor antibiotic that possesses several reactive functional groups (33) and induces single-strand breaks in DNA, presumably due to the ability to generate hydroxyl radicals (34, 35). X-rays also cause single-strand breaks and double-strand breaks through the generation of hydroxyl radicals (36). Nalidixic acid, oxolinic acid, and AM715 are inhibitors of bacterial DNA gyrase (ref. 37; N. Cozzarelli, personal communication). They, too, induce single-strand breaks, but they do so indirectly through their inhibitory activity on gyrase (37). Their relative mutagenic activity in TA102 corre sponds qualitatively to their ability to inhibit gyrase (Table 3; N. Cozzarelli, personal communication), suggesting that their mutagenicity is related to gyrase inhibition. The sigmoidal dose–response curves for mutagenesis observed with these agents may also be indicative of their indirect mutagenic activity. These agents induced primarily deletion revertants in TA102, whereas deletions were relatively infrequent among the revertants induced by the other SOS-de-

pendent mutagens tested. We believe that single-strand breaks may be the premutagenic lesion responsible for mutagen-induced deletion revertants of TA102. The observation that potent frameshift mutagens do not revert *hisG428* indicates that deletion revertants are not induced through pathways common to frameshift mutagenesis.

Many of the mutagens in Table 3 induce large deletions of the *Salmonella* and *E. coli* chromosomes in other systems (3–6). Although deletion revertants of *hisG428* on the chromosome arise spontaneously, their frequency was not increased by the mutagens tested in this study in any strains used except TA102, which carries *hisG428* on the pAQ1 plasmid. This observation accounts for the relative lack of mutagenicity of bleomycin and the gyrase inhibitors on chromosomal *hisG428* strains (Table 3) and emphasizes the disparate mechanisms of mutagen-induced large deletions and small deletion revertants of *hisG428*. One possible explanation for the difference between the generation of deletion revertants on pAQ1 and on the chromosome is that pAQ1 is derived from pBR322 (7), which carries the ColE1 origin of replication (38) and is, therefore, replicated by DNA polymerase I (39, 40). In contrast, chromosomal replication is more complex, involving DNA polymerase III and numerous other replication factors as well as DNA polymerase I (41). Therefore, differences may exist in replicative DNA repair between the chromosome and pAQ1. Alternatively, the relative degree of DNA supercoiling may be responsible for this difference.

It appears that spontaneous and mutagen-induced deletion revertants are not generated through a common pathway, because deletion revertants arise spontaneously in all genetic backgrounds examined but are induced by mutagens only in TA102. Furthermore, while the induction of deletion revertants by mutagens requires pKM101, generation of spontaneous deletion revertants is not affected by this plasmid. The *muc* genes carried on pKM101 (27) have been shown to be involved in SOS-dependent mutagenesis in *Salmonella* and *E. coli*. SOS-dependent repair and mutagenesis also requires the *recA* function to be induced (22). In the absence of the pKM101 plasmid, *Salmonella* is devoid of error-prone DNA repair (20, 21). This system appears to be necessary for the generation of mutagen-induced deletion revertants.

At least three pathways can lead to the generation of deletion revertants of *hisG428*. (*i*) A *recA*-dependent pathway that generates spontaneous deletion revertants both on the chromosome and on pAQ1. (*ii*) A *recA*-, pKM101-dependent pathway that is inducible by mutagens. The activity of this pathway is limited to *hisG428* on pAQ1 (i.e., it does not function on *hisG428* on the chromosome). (*iii*) A pathway independent of both *recA* and pKM101. This pathway is dependent on aerobic growth and generates spontaneous deletion revertants both on the chromosome and on pAQ1.

We have characterized this system further with respect to the identity of the base-pair substitution mutations in suppressor revertants, devised a simple method to identify the types of mutation induced by any mutagen in *hisG428*-containing tester strains, and analyzed revertants of *hisG428* induced by a variety of mutagens (unpublished results).

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