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Ultraviolet Light Protection, Enhancement of Ultraviolet Light Mutagenesis, and Mutator Effect of Plasmid R46 in *Salmonella typhimurium*

KRISTIEN E. MORTELMANS[1] AND B. A. D. STOCKER*
Department of Medical Microbiology, Stanford University School of Medicine, Stanford, California 94305

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Plasmid R46 partially protected *Salmonella typhimurium*, wild type or *uvrB* or *polA*, against the lethal effect of ultraviolet (UV) irradiation, but did not protect *recA* mutants. The plasmid also increased frequency of UV-induced reversion to His$^+$ in all tested *his* point mutants (wild type for UV sensitivity), including amber, ochre, UGA, missense, and frame-shift mutants. Plasmid R46 also increased UV-induced reversion to His$^+$ in *uvrB* and *polA* strains, but no UV mutagenic effect was detected in R$^-$ or R46-carrying *recA* derivatives of a *his*(amber) mutant. The spontaneous reversion frequency of *his* nonsense mutants of all classes, and of some *his* missense mutants, was increased about 10-fold when the strains carried R46, but the plasmid had no effect on the spontaneous reversion frequency of some other *his* missense mutations or of reversion rate of *his* frame-shift mutants (except for two *uvrB* derivatives of one single-base insertion mutant). The plasmid increased the ability of wild type, *polA*, and *uvrB* hosts to support plaque production by UV-irradiated phage, and made strain LT2 *hisG46* less sensitive to methyl methane sulfonate and to X rays and more responsive to the mutagenic effect of visible-light irradiation. R46 increased spontaneous reversion frequency of a *his*(amber) *rec*$^+$ strain, but had no such effect in its *recA* sublines. Since the plasmid in the absence of host *recA* function fails to produce its mutator effect, or to confer UV protection or to enhance UV mutagenesis, these three effects may be produced via some mechanism involved in *recA*-dependent deoxyribonucleic acid repair, perhaps by an increase in activity of the "error-prone" component of the inducible repair pathway.

Plasmid ColIb-P9 was shown by Howarth (10) to diminish the sensitivity of *Salmonella typhimurium* to ultraviolet (UV) irradiation and to enhance greatly the mutagenic effect of UV in respect to reversion of some auxotrophic mutants (11). Carriage of some other colicin and R factors (6, 15, 21, 27) likewise reduces the lethal effect of UV irradiation—an effect which, for brevity, we hereinafter call UV protection. The mechanism by which plasmids diminish susceptibility to UV killing and enhance the mutagenic effect of UV is not known, but an obvious possibility is that the plasmids produce both effects by increasing the ability of the host to repair UV-damaged deoxyribonucleic acid (DNA) by some error-prone, and therefore mutagenic, process. To test what kinds of errors (base substitution, frame shift, etc.) were produced in irradiated bacteria containing a UV-protective plasmid, we planned to test such a plasmid in *S. typhimurium* auxotrophic mutants of known codon character available among *his* mutants (8). The plasmid we used was R46 (entry no. 79 in the Novick catalog [23], formerly called R-Brighton [6] or R1818 [3, 5], and probably identical with TP120 [7]). R46 falls in incompatibility group N, and confers resistance to ampicillin, streptomycin, tetracycline, and sulfonamides; it also confers UV protection (6). R46, when transferred into mutant LT2 *hisG46*, as expected conferred UV resistance and increased the frequency of UV-induced reversion to His$^+$; unexpectedly, it also caused an increase, by a factor of ca. 10, in the frequency of spontaneous reversion.

In this paper we describe experiments to test what classes of mutation occur at increased spontaneous frequency when the host carries R46, and what classes of mutation revert at increased frequency, for a given UV dose, if R46 is present. Bacterial mutations causing UV sensitivity, viz., *uvrB*, *polA*, and *recA*, were

[1] Present address: Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.

tested for their effect on the ability of R46 to confer UV protection, to enhance UV mutagenesis, and to increase the spontaneous mutation rate. We also describe effects of R46 on the ability of its host to reactivate UV-irradiated phage, on host sensitivity to killing by X rays and by exposure to methyl methane sulfonate, and on susceptibility to visible-light mutagenesis.

(This paper is based on the Ph.D. thesis [Stanford University, 1975] of K. E. Mortelmans.)

MATERIALS AND METHODS

Bacterial strains and phages. The strains used, except where otherwise stated, are derivatives of *S. typhimurium* LT2, LT7, or M7471 (14). Strains with single mutations are referred to by mutation number, e.g., LT2 *trpD1* (see reference 8 for the origins of the *his* mutants). Table 3, footnote *a*, lists the multiply marked lines used. For tests of the ability of strains to reactivate UV-irradiated phage, we used phages P22.*c2*HT13/4, derived from phage P22 (26), and ES18 (13).

Media. The nutrient broth and nutrient agar used were Oxoid nutrient extract broth no. 2 (CM67) and blood agar base (CM55). The defined medium used was Davis minimal medium, with glycerol, 0.5% (vol/vol), supplemented when appropriate with required amino acids or other growth factors. Prototrophic revertants of *trp* or *his* auxotrophs were selected on defined medium supplemented with nutrient broth, 1% (vol/vol), hereinafter called enriched minimal medium.

Genetic methods. To transfer R46 to a strain, it and an auxotrophic donor, usually SL1127 [= LT2 *pur proA strA* (R46)], were grown together in broth, unshaken, at 37°C for ca. 18 h. The mixture was then streaked out on defined medium selective for the recipient strain, supplemented with ampicillin, 25 μg/ml; streptomycin, 100 μg/ml; and tetracycline, 25 μg/ml. Resistant clones were reisolated from single colonies on the same medium and checked for possession of the four drug resistance traits of R46. To determine rates of spontaneous reversion of His^- or Trp^- strains, cells from an overnight, unshaken broth culture were washed and resuspended, at ca. 10^9/ml, in inorganic salts solution; 0.1-ml samples were spread on plates (ca. 25 ml) of enriched minimal medium supplemented with any additional growth factors required. Colonies were counted after 2 days at 37°C. (In several experiments on strain *hisG46*, very few additional revertant colonies were detected on longer incubation. In experiments with other strains, the counting of colonies only after 2 days of incubation may have resulted in failure to detect some revertants growing only slowly in absence of histidine. All the *his* mutants used gave about the same small amount of growth, as judged by eye, on the minimally supplemented defined medium; i.e., none was significantly leaky. The calculation of mutation rate from number of revertant colonies, described below, would not be valid for a leaky mutant.) To determine UV survival, serial decimal dilutions, in broth, were made from an overnight, 37°C, nonshaken nutrient broth culture, or from a washed-cell suspension made from such a culture. Drops, of volume 0.01 ml, from appropriate dilutions were delivered by standard dropper (23-gauge Luer-Stub adapter, Clay-Adams, Inc., New York) to the surface of nutrient agar plates. After the drops had dried, the plates were exposed for varying times under an 8-W germicidal lamp (General Electric G85T), calibrated by use of a photovoltaic dose-rate meter (12). Irradiated plates were wrapped in aluminum foil, to prevent possible photoreactivation. Colonies were counted after overnight incubation at 37°C. (Control experiments showed that the survival of irradiated *S. typhimurium* LT2 was about the same on nutrient agar and on simple defined medium.) To measure rates of UV-induced reversion, 0.1-ml samples of washed-cell suspensions prepared as described above (i.e., ca. 10^8 cells) were spread on plates of selective medium, usually enriched minimal medium, three plates for each UV dose. The surface-inoculated plates were irradiated, and then wrapped in foil; colonies were counted after 2 days at 37°C. To calculate the number of UV-induced revertants per 10^8 survivors, the mean number of colonies per irradiated plate, if significantly greater than the mean per nonirradiated plate, was corrected for spontaneous revertants by subtraction of the mean number per control plate, and then corrected for fraction of irradiated bacteria surviving, determined by viable counts as described above.

To test whether His^+ revertants resulted from mutation at *his* or from external suppressor mutation, they were crossed by transduction to *his* deletion recipients and, as a control, to their *his* parent. A "high-transducing" variant, P22.*c2*HT13/14 (26), was used, because the frequency of transduction of some suppressor loci by wild-type P22 is expected to be much lower than the frequency of transduction of *his*. Since this phage is a lytic variant, the strains to be used as recipients were made lysogenic for P22.*sie*, therefore immune to killing by P22.*c2* but still susceptible to transduction. His^+ transductants were sought by the "drop-on-lawn" method (13). All the lysates used evoked many His^+ colonies, 20 or more per drop area, from the control, his^- parent, recipient; each evoked either many colonies, indicating reversion at *his*, or no colonies, indicating external suppressor mutation, from the *his* deletion recipient.

To test host cell reactivation (HCR) character, phage P22.*c2*HT13/14 or phage ES18, of titer greater than 5×10^{10} plaque-forming units/ml, was diluted 1/100 in saline and exposed to UV, for doses between 200 and 3,600 ergs/mm^2. The irradiated and nonirradiated samples were titrated by the drop-on-lawn method (13) on nutrient agar plates inoculated by flooding with the indicator strain to be tested. Plaques were counted after overnight incubation at 37°C.

RESULTS

Effect of R46 on LT2 *trpD1* and *hisG46*. The presence of plasmid R46 in the LT2 mutants

trpD1 and *hisG46* conferred UV resistance (Fig. 1), to about the degree previously observed for this plasmid (6) and for plasmid ColIb-P9 (10) in other LT2 lines. In strain *trpD1*, it caused about 12-fold increases in the number of Trp$^+$ revertants (calculated per survivor) evoked by UV irradiation, 100 ergs/mm^2 (Table 2) or 200 ergs/mm^2 (data not shown), comparable to that produced by plasmid ColIb-P9 in the same strain (11). No Trp$^+$ revertants were obtained from nonirradiated *trpD1* or *trpD1*(R46). In LT2 *hisG46* the presence of plasmid R46 similarly caused an increased yield of UV-induced revertants (Table 2). An unexpected effect was the larger number of His$^+$ colonies on plates inoculated with *hisG46*-(R46) and incubated 2 days (without irradiation), compared with the number on plates inoculated with the parent strain, *hisG46*: in several experiments, the average number of His$^+$ colonies per plate of enriched minimal medium was about 3 for *hisG46* and about 30 for *hisG46*(R46). Exactly the same effect was observed in each of six independently prepared clones of *hisG46*(R46). Two clones of *hisG46* lacking all the four drug resistance traits of R46 were isolated from a culture of *hisG46*-(R46) grown in broth with sodium dodecyl sulfate, 10% (wt/vol); both cured clones were similar to the original *hisG46* in yield of spontaneous revertants (and also in UV sensitivity and yield of UV-induced revertants). Thus, an increase in number of spontaneous His$^+$ revertant colonies is a regular consequence of the presence of R46 in strain LT2 *hisG46*. All of several spontaneous revertants of *hisG46* and of *hisG46*(R46) tested by transduction (see Materials and Methods) had reverted by mutation at *his*, not at some external suppressor locus.

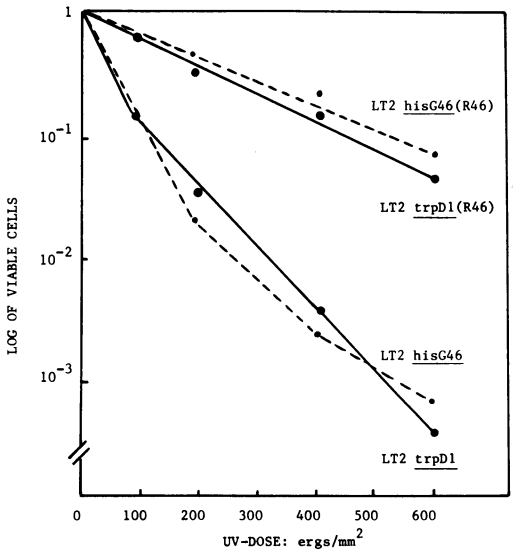

FIG. 1. *UV dose/log survival for strains LT2 trpD1 and trpD1(R46), and also for LT2 hisG46 and hisG46(R46).*

The number of revertant colonies per plate of enriched minimal medium, inoculated with *hisG46* or *hisG46*(R46), showed at most only a small increase when plates were incubated for longer than 2 days. It therefore seemed that the number of His$^+$ colonies counted after 2 days of incubation represented all or nearly all the mutations, at *his* or by suppressor mutation, occurring during the limited growth permitted by the available L-histidine content of the broth supplement. The broth content (1%, vol/vol) of the enriched minimal medium supported multiplication of a nonreverting *his* mutant to a final population of 2.9×10^9 colony-forming units per plate; comparison with histidine-limited grown in liquid defined medium showed this to correspond to a content of 9.7 μg of available L-histidine per plate. The experiments recorded in Table 1 showed that the number of revertant colonies of strain LT2 *hisG46* per plate of histidine-supplemented defined medium was linearly related to the amount of the histidine supplement, in the range of 1 to 30 μg of L-histidine per plate. Other experiments (Table 1) showed that a 100-fold variation in inoculum size did not affect the number of revertant colonies per plate of enriched minimal medium. These results correspond to theoretical prediction for reversion, at constant frequency per bacterium per generation, of a nonleaky auxotrophic mutant growing from a relatively small inoculum on plates of medium containing a growth-limiting amount of the required growth factor. For each of the two strains, the number of revertant colonies per plate on defined medium supplemented with L-histidine, 10 μg per plate, was about the same as the number on plates supplemented with broth, 1% by volume, and calculated to contain about 10 μg of available L-histidine. Thus, the frequency of spontaneous mutation per bacterium per generation is about the same on these two media (for calculated values see Table 1).

Effect of R46 on *his* point mutations of known codon character. Plasmid R46 was transferred into various *S. typhimurium* strains with *his* point mutations of known character, nonsense (amber, ochre, or UGA), missense, or frame shift, but otherwise wild type. The R46-carrying derivatives and R$^-$ parents were tested for spontaneous reversion and for survival and yield of UV-induced revertants after exposure to UV, 60 ergs/mm^2 (Table 2). R46 conferred UV protection in all the mutants: the median survival of the R$^+$ forms after expo-

TABLE 1. *Number of His⁺ colonies/plate, and calculated frequency of spontaneous mutation per bacterium per generation, for strains hisG46 and hisG46(R46) incubated on defined medium supplemented with L-histidine or with nutrient broth, 1% by volume*

Medium supplement[a]	Inoculum size	Final His⁻ population[b]	Colonies/plate[c]		Mutation rate[d]	
			R⁻	R⁺	R⁻	R⁺
L-Histidine, 1 μg	10^8	3×10^8	0.3	1.1		
L-Histidine, 3 μg	10^8	9×10^8	1.0	6.6	6.9×10^{-10}	4.5×10^{-9}
L-Histidine, 10 μg	10^8	3×10^9	3.6	31	8.3×10^{-10}	7.2×10^{-9}
L-Histidine, 30 μg	10^8	1×10^{10}	8.6	85	5.9×10^{-10}	5.9×10^{-9}
Broth, 1%, vol/vol	4×10^7	3×10^9	3.3	29	7.6×10^{-10}	6.7×10^{-9}
Broth, 1%, vol/vol	4×10^5	3×10^9	4.0	23	9.2×10^{-10}	5.3×10^{-9}

[a] Each plate contained ca. 25 ml of defined medium supplemented either with indicated amount of L-histidine or with nutrient broth (Oxoid, CM67), 1% by volume, calculated to contain ca. 9.4 μg of available L-histidine.

[b] Final population calculated from amount of L-histidine supplied, or from yield of a *his* deletion mutant grown on defined agar medium with broth, 1%, vol/vol.

[c] Mean number of colonies/plate for three plates of indicated medium, after 2 days at 37°C. R⁻, Strain *hisG46*; R⁺, strain *hisG46*(R46).

[d] Probability of mutation to prototrophy per bacterium per generation, m, calculated from equation $m = \log_e 2(M/N)$, where M = number of mutations (i.e., of His⁺ colonies) and N = final population (9).

sure to 60 ergs/mm² was 0.90 and that of their R⁻ parents was 0.42. The survivals of the R⁻ and R46-carrying forms of the two mutants derived from LT7, which in its wild-type form carries a ColI plasmid (24), did not differ significantly from survivals of derivatives of strain LT2.

The six *his* amber mutants behaved alike. Their R⁻ forms produced 3 to 12 spontaneous revertants/plate, and their R⁺ forms produced about 10 times as many (range, 28 to 82/plate). UV exposure evoked 20 to 38 revertants per 10^8 survivors from the R⁻ mutants, and 200 to 460 per 10^8 survivors from the R46 derivatives. To test its effect in an *E. coli* genetic background, R46 was transferred to strain LS730, a K-12 stock with several genetic markers including *his-29*(amber); the plasmid in this *E. coli* strain diminished the killing and increased the mutagenic effect of UV irradiation (60 ergs/mm²), and increased the rate of spontaneous reversion to His⁺, to about the same extent as in *his*(amber) mutants of *S. typhimurium*. The four *his*(ochre) mutants of *S. typhimurium* behaved like the amber mutants. The number of spontaneous revertants varied from 1.6 to 3.0 per plate for the R⁻ strains and from 20 to 42 for the R46 derivatives: the number of UV-induced revertants per 10^8 survivors ranged from 38 to 65 for the R⁻ and from 100 to 353 for the R⁺ strains. The four *his* UGA mutants in general behaved like the amber and ochre mutants. However, UGA mutants *hisG200* and *hisG611*, both in R⁻ and R⁺ form, gave more spontaneous revertants than the corresponding forms of any other nonsense mutant, and irradiation of UGA mutant *hisD3725* in its R⁻ form did not evoke any detectable number of UV-induced revertants (<2 per 10^8 survivors). Thus, qualitatively, R46 produced the same effects in all three classes of nonsense mutants: increase in spontaneous reversion frequency and increases in survival and in frequency (per survivor) of induced reversion on exposure to UV.

Revertants of amber mutant *hisC527* and of ochre mutant *hisG534*, and of their R46-carrying derivatives, were examined (see Materials and Methods) to see whether they resulted from mutation at *his* or from external suppressor mutation. Both spontaneous and UV-induced revertants were tested; where available, both small and normal-sized colonies were picked, a total of three to nine for each combination. In each set, some or all of the revertants proved to result from mutation within the *his* cluster. Some external suppressor mutations were detected among spontaneous revertants of *hisC527*(R46), induced revertants of *hisC527* and *hisC527*(R46), and spontaneous and induced revertants of *hisC354*(R46).

The five additional *his* missense mutants tested (Table 2), together with the first tested mutant, *hisG46*, also of type missense, fall into two groups. Three mutants (including *hisC104*, derived from strain LT7) gave few spontaneous revertants (1 to 2 colonies/plate), whether R⁻ or carrying R46. UV exposure (60 ergs/mm²) had no detectable mutagenic effect on these three mutants in their R⁻ form (<6 revertants/10^8 survivors) but a significant effect (ca. 12 revertants/10^8 survivors) on their R46-carrying derivatives. By contrast, the presence of R46 in the other three missense mutants (including *hisG46*) caused about 10-fold increases in their spontaneous reversion rates (even in mutant *hisC899*, whose spontaneous reversion rate

TABLE 2. *Effect of R46 on UV sensitivity and on frequency of spontaneous and UV-induced reversion of trpD1 and various his mutants*

Strain	Nonirradiated (revertant colonies per plate[a])			Irradiated (60 ergs/mm²)						
				Survival (%)[b]		Revertant colonies per plate[c]		Induced mutations per 10^8 survivors[d]		
	R⁻	R⁺	R⁺/R⁻	R⁻	R⁺	R⁻	R⁺	R⁻	R⁺	R⁺/R⁻
LT2 *trpD1*[e]	0	0		20	84	5	272	25	320	13
LT2 *hisC50*(amb)	4.3	39.3	9.1	41	87	17.3	213	32	200	6.2
LT2 *hisC340*(amb)	7.3	64.6	8.8	45	91	22.0	439	30	412	13.7
LT2 *hisC501*(amb)	11.6	81.6	7.0	47	90	29.6	363	38	312	8.2
LT2 *hisC434*(amb)	3.3	35.6	10.8	45	85	20.3	213	38	209	5.5
LT2 *hisC508*(amb)	8.0	62.3	7.8	46	83	17.2	317	20	307	15.3
LT2 *hisC527*(amb)	3.6	28.0	7.7	49	90	21.0	442	35	460	13.1
K-12 *his-29*(amb)[f]	8.3	81.0	9.8	71	89	68.0	563	84	542	6.5
LT7 *hisC117*(och)	3.0	20.0	6.6	37	86	20.0	156	45	158	3.5
LT2 *hisC342*(och)	1.6	42.0	26.2	45	91	31.0	363	65	353	5.4
LT2 *hisC502*(och)	3.0	26.0	8.6	43	88	25.0	114	51	100	1.9
LT2 *hisC354*(och)	2.3	36.0	15.6	41	87	18.0	171	38	155	4.1
LT2 *hisG200*(UGA)	47.0	197.0	4.2	41	94	117	705	170	540	3.2
LT2 *hisD3725*(UGA)	1.6	28.0	17.5	39	92	2.0	203	—[g]	190	2+[h]
LT2 *hisI570*(UGA)	2.0	20.0	10.0	38	88	6.0	243	10	253	25.3
LT2 *hisG611*(UGA)	18.0	160.0	8.8	43	93	25.0	667	16	545	34.1
LT2 *hisG46*(ms)	3.6	31	8.6	40	85	28	192	60	182	3.0
LT2 *hisC8*(ms)	1.0	1.3	1.3	41	91	0.6	12.0	—	12	2+
LT7 *hisC104*(ms)	1.3	1.0	0.8	36	87	0.6	11.0	—	11	2+
LT2 *hisC890*(ms)	2.3	2.6	1.1	42	90	1.3	14.0	—	13	2+
LT2 *hisC496*(ms)	2.3	26.3	11.4	40	85	8.6	116	16	105	6.6
LT2 *hisC899*(ms)	20.0	186.0	9.3	35	85	206	960	531	921	1.7
LT2 *hisC207*(fs,−1)	3.6	5.0	1.3	40	90	7.6	49.3	10	50	5.0
LT2 *hisD3052*(fs,−1)	12.6	11.6	0.9	60	100	25.6	77.0	22	65	2.9
LT2 *hisC3076*(fs,+1)	2.3	2.0	0.9	60	100	2.3	23.2	—	21.2	2+
LT2 *hisF3704*(fs,−1)	14.6	17.0	1.2	40	95	14.3	245	—	240	2+
LT2 *hisC3737*(fs,+1)	19.2	17.6	0.9	38	90	21.0	116	—	109	2+

[a] Entries in columns headed R⁻ and R⁺ are mean numbers of revertant colonies/plate for three plates of defined medium with broth, 1% by volume, each inoculated with ca. 10^8 of, respectively, the indicated *his* or *trp* mutant and its R46-bearing derivative. R⁺/R⁻, Ratio of these means.

[b] Ratios of colony counts on nutrient agar plates, irradiated/nonirradiated, for mutant and for R46-bearing derivative.

[c] Mean number of revertant colonies/plate for three plates of defined medium with broth, 1% by volume, each inoculated with ca. 10^8 washed cells of the indicated *his* or *trp* mutant or its R46-carrying derivative and irradiated (60 ergs/mm²) before incubation.

[d] The mean number of revertant colonies per irradiated plate was corrected for spontaneous mutations by subtracting the mean number of revertant colonies per nonirradiated plate; the mean number of UV-induced mutations per plate inoculated with ca. 10^8 bacteria, thus calculated, was divided by the proportion of bacteria surviving irradiation to calculate the number of UV-induced mutations per 10^8 survivors.

[e] For strain LT2 *trpD1*, the UV dose was 100 ergs/mm² instead of 60 ergs/mm².

[f] Note that this strain, LS730, is a derivative of *E. coli* K-12. Its *his* mutation, *his-29*, is amber. All other strains are *S. typhimurium*.

[g] Mean number of revertant colonies per irradiated plate not significantly greater than mean per nonirradiated plate, i.e., no mutagenic effect of UV irradiation detected.

[h] 2+, Mutagenic effect of UV greater for R46-carrying strain but no ratio calculable, because no measurable mutagenic effect in R⁻ strain.

was about 10-fold higher than those of the other five missense mutants). UV irradiation had an obvious mutagenic effect on these three strains in their R⁻ form and the presence of R46 increased this mutagenic effect, about eightfold for two mutants. For the third mutant, *hisC899*, the number of induced revertants per survivor was the highest observed among the

R46 derivatives, but the factor increase over the number in the R⁻ parent was only 1.7 times, since this mutant even in its R⁻ form produced many revertants on irradiation.

The five frame-shift mutants tested comprised three that were believed to have single-base deletions and two with single-base insertions. All five produced spontaneous revertants at detectable frequency (2.3 to 19 colonies/plate); this frequency was not altered by introduction of R46. UV irradiation evoked revertants from the R⁻ forms of two of the three single-base-deletion mutants, but had no detectable mutagenic effect on the other three frame-shift mutants. UV treatment induced reversion in the R46-carrying forms of all five frame-shift mutants. (We describe below tests of the effect of R46 on spontaneous and UV-induced reversion rates of *uvrB* derivatives of several of the frame-shift mutants.) In summary, the presence of R46 in five frame-shift mutants of wild-type UV sensitivity had no effect on their spontaneous reversion rates but increased the frequency of UV-induced reversion of all five of them.

Effect of R46 in UV-sensitive mutants. We transferred plasmid R46 into various UV-sensitive sublines of *his* mutants of *S. typhimurium*. As representatives of classes deficient in excision repair, we used the *uvrB* deletion (therefore excision-negative) derivatives of *his* strains, developed for tests of mutagenic activity of chemicals (1, 2). The presence of R46 in strain TA1950, *hisG46 uvrB*, reduced its UV sensitivity but did not bring resistance to the level of the wild-type (*uvr*⁺) parent (Fig. 2). The *hisG46 uvrB* strain showed a rate of spontaneous reversion of His⁺ about sixfold higher than its *hisG46 uvr*⁺ parent (Table 3); the cause of this increase, previously observed by Bruce Ames (personal communication), is unknown. The introduction of R46 into *hisG46 uvrB* resulted in further increase in spontaneous reversion frequency (from 19 to 340 colonies/plate). The lethal and mutagenic effect of irradiation on *hisG46 uvrB* was tested at a dose of 5 ergs/mm². The survivals of the R⁻ and R46 forms of *hisG46 uvrB* were 15 and 80%, and the numbers of induced reversions per 10^8 survivors of this dose, 63 for the R⁻ and 395 for the R46 form of *hisG46 uvrB*, were of the same order as the numbers per 10^8 survivors of 60 ergs/mm² for the R⁻ and R46 forms of the *hisG46 uvr*⁺ parent (Table 3). Similar tests in four *uvrB* (deletion) sublines of three *his* frame-shift mutants showed that in all four R46 reduced the killing effect and increased the mutagenic effect of exposure to 5 ergs of irradiation per mm² (Table 3). The *uvrB* defects did not change the frequency of spontaneous reversion of any of the three frame-shift mutants. Introduction of R46 did not cause any increase in spontaneous reversion frequency in the *uvrB* forms of two frame-shift mutants *hisC207* and *hisD3052*. The third frame-shift mutant tested was *hisC3076*, with a single base insertion. Two *uvrB* derivatives were tested, the deletion of one extending through *chl*, *bio*, and *uvrB*, and that of the other covering also *gal*. R46 caused an increase in spontaneous reversion frequency, about 15-fold, in each of these two *uvrB* derivatives.

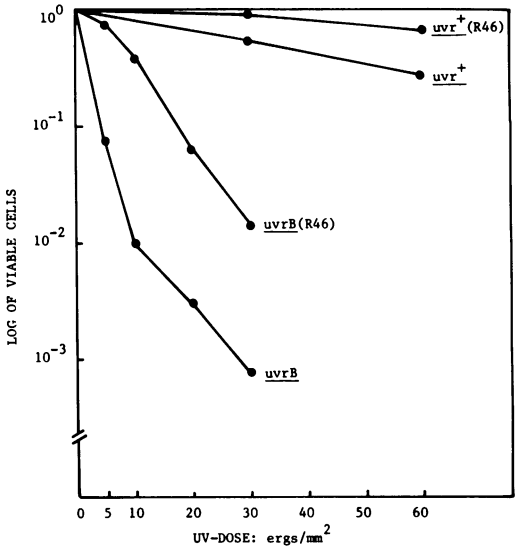

FIG. 2. *UV dose/log survival for strain LT2 hisG46 (indicated uvr⁺) and its uvrB mutant, TA1950, and also for their R46-carrying derivatives.*

A UV-sensitive *S. typhimurium*, SL4702, deficient in broken-cell DNA-polymerizing activity, is inferred to lack polymerase I and is numbered *polA1* (14, 19, 20). We introduced R46 into SL4702 and into its *polA*⁺ parent, SL4525. As expected, the *polA* mutant without R46 was much more sensitive to UV than its *polA*⁺ R⁻ parent (Fig. 3). The presence of R46 in the *polA* line considerably reduced its sensitivity, bringing it almost exactly to the level of the *polA*⁺ R⁻ parent (Fig. 3); but the *polA*(R46) strain was considerably less resistant than the *polA*⁺(R46) control strain. The rates of spontaneous reversion of *hisC527* (amber) in the *polA* mutant and in the *polA*⁺ parent were about the same, 4 and 3.3 colonies/plate, respectively (and about the same as the rate for the same *his* allele in an LT2 background, Table 2). The presence of R46 caused about a 10-fold increase in spontaneous reversion frequency, both in the *polA* mutant and in its *polA*⁺ parent (Table 3).

TABLE 3. *Effect of R46 on spontaneous reversion, UV survival, and UV-induced reversion of uvr⁺ and uvrB forms of one missense and three frame-shift his mutants as well as wild-type, polA, and recA forms of hisC527(amber)*

Strain[a]	Nonirradiated			UV dose (ergs/mm²)	Irradiated						
	His⁺ colonies per plate[b]				Survival (%)		His⁺ colonies per plate		Mutations per 10^8 survivors[b]		
	R⁻	R⁺	R⁺/R⁻		R⁻	R⁺	R⁻	R⁺	R⁻	R⁺	R⁺/R⁻
uvr⁺ and *uvrB* forms of missense and frameshift mutants											
hisG46(ms)	3.6	31.0	8.6	60	40	85	28	192	60	182	3.0
hisG46 uvrB	18.6	340	18	5	15	80	28	656	63	395	6.0
hisC207(fs)	3.6	5.0	1.3	60	40	90	7.6	49	10	49	4.9
hisC207 uvrB	3.0	3.6	1.2	5	12	80	4.6	21	–	22	2+
hisC3052(fs)	12.6	11.6	0.9	60	60	100	26	77	22	65	3.0
hisC3052 uvrB	10.3	10.6	1.0	5	13	80	11	36	–	32	2+
hisC3076(fs)	2.3	2.0	0.9	60	60	100	2.3	23	–	21	2+
hisC3076 uvrB#1	2.6	31.6	12	5	12	72	13	580	83	762	9.1
hisC3076 uvrB#2	2.0	30.3	15	5	15	75	17	492	102	578	5.6
Wild-type, *polA*, and *recA* forms of *hisC527*(amber)											
hisC527(am)	3.3	33.0	10	50	60	80	35	565	53	604	11
hisC527 polA	4.0	38.6	9.6	50	14	55	14	446	71	744	10
hisC527 rec-280	2.6	2.0	0.7	1	9	12	2.0	2.3	–	–	–
hisC527 rec-281	2.6	3.0	1.1	1	8	7	2.0	2.6	–	–	–

[a] Suffixes (ms), (fs), and (am) indicate missense, frame-shift, and amber *his* mutations. Strains indicated *uvrB*, all with deletions through *chl* and *uvrB* (1, 2), are: TA1950, *hisG46 chl(bio uvrB)1001* Δ; TA1951, *hisC207 chl(bio uvrB)1002*Δ; TA1534, *hisD3052 chl(bio uvrB)1004*Δ; TA1952, *hisC3076 chl(bio uvrB)1003*Δ (indicated above as *uvrB* no. 1); and TA1532, *hisC3076 chl(gal bio uvrB)1007*Δ (indicated above as *uvrB* no. 2). The amber *his* mutant of wild-type UV sensitivity is SL4525, *S. typhimurium* M7471 (ColE1-30) *leu-1051 malB479 cysI1173 hisC527 gal-459* (14). Its UV-sensitive derivatives (14) are: SL4702, SL4525 *polA1*; SL4691, SL4525 *recA280*; and SL4700, SL4525 *recA281*.

[b] For experimental conditions and method of calculation see footnotes of Table 2.

The mutagenic effect of UV was tested at 50 ergs/mm², which allowed 15% survival of the *polA* R⁻ strain and survivals of >50% for the other three strains (Table 3). The number of UV-induced mutants per 10^8 survivors was about the same for the *polA* R⁻ and the *polA⁺* R⁻ strains and about 10-fold greater for the *polA*(R46) and *polA⁺*(R46) lines. Thus, the three investigated effects of R46 were obtained to about the same extent in a *polA* host as in its *polA⁺* parent.

Mutants of *E. coli* termed *recA* are extremely sensitive to UV and unable to support recombination; the molecular basis of the defect is unknown. We tested two phenotypically similar mutants in *S. typhimurium*, isolated by MacPhee (14) in strain SL4525, described above. Plasmid R46 was transferred to the two *recA* mutants, and the survival of the *recA* R⁻ mutants and their R46 derivatives was determined at UV doses from 1 to 5 ergs/mm². The *recA* mutants were, as expected, extremely sensitive to UV, the 10% survival dose being ca. 1 erg/mm² compared with ca. 150 erg/mm² for the *recA⁺* parent. The survival curves for the R46-bearing derivatives of the two *recA* mutants were indistinguishable from those of their *recA* R⁻ parents. The number of spontaneous His⁺ revertants per plate was between 2 and 3, both for the two *recA* R⁻ strains and for their R46 derivatives; this is about the same as the rate in the *recA⁺* R⁻ parent, SL4525 [and also about the same as in the R⁻ form of strain LT2 *hisC527*, with the same *his*(amber) allele as the two *recA* strains]. A dose of 1 erg/mm² was used to test for UV mutagenesis; survival was about 9% for all four strains. No UV-induced reversion to His⁺ was detected in any of the four *recA* strains (Table 3). Thus, in two *recA* hosts, plasmid R46 neither conferred UV protection nor increased the spontaneous frequency of reversion of an amber *his* mutation, nor did the plasmid restore the mutagenic effect of UV irradiation which, as previously reported, is not detectable in *recA* mutants (30).

Effect of R46 on ability of host to reactivate UV-irradiated phage. To test whether R46 altered the ability of its host to support plaque production by UV-irradiated phage (i.e., affected HCR character), samples of irradiated phage were titrated on the R⁻ and R46 forms of LT2 *hisG46 uvrB* and its *uvr⁺* parent, and of

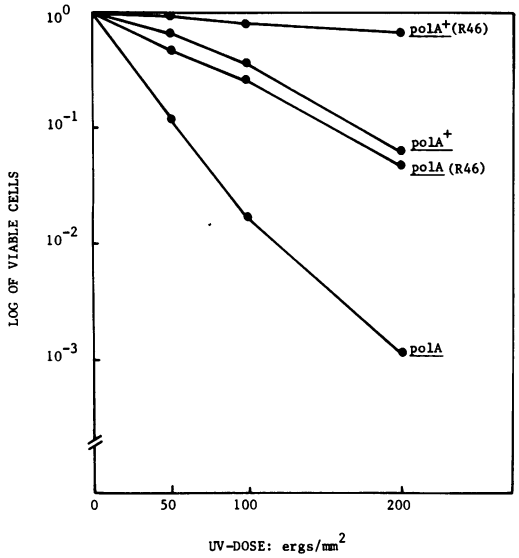

FIG. 3. *UV dose/log survival for strain SL4525 (indicated polA$^+$) and its polA mutant, SL4702, and also for their R46-carrying derivatives.*

the *polA1* mutant, SL4702, and its *pol*$^+$ parent. A P22 variant, P22.*c2*HT13/14, was used for the experiments on the *hisG46* strains. Phage ES18, which has the same UV sensitivity and susceptibility to reactivation as phage P22 (14), was used in the experiments with the *polA* and *pol*$^+$ strains, which are resistant to P22 because of altered lipopolysaccharide (24). The presence of R46 in the *uvrB* strain increased its ability to support plaque formation by irradiated phage (Fig. 4) but did not bring it to the level of the *uvr*$^+$ R$^-$ strain. Furthermore, irradiated phage P22 gave higher counts on the R46-bearing *uvr*$^+$ strain than on its R$^-$ *uvr*$^+$ parent; the plasmid could therefore be said to raise the HCR ability of the *uvr*$^+$ strain above the wild-type level. The presence of R46 in the *pol*$^+$ control strain similarly resulted in increased reactivation of irradiated phage ES18 (Fig. 5). The HCR defect of the R$^-$ form of the *polA* mutant was, as expected, less severe than that of the *uvrB* mutant (Fig. 5). Presence of R46 in the *polA* mutant caused a considerable increase in ability to support plaque production by irradiated phage, bringing it almost exactly to the level observed in the R$^-$ form of the *pol*$^+$ control strain.

Effect of R46 on survival of *S. typhimurium* exposed to methyl methane sulfonate or to X rays, and on visible-light mutagenesis. The presence of R46 in LT2 *hisG46* greatly increased the survival of a culture exposed to methyl methane sulfonate, 0.36%, vol/vol, for various times (data not shown). Only about 10% of the *hisG46* cells survived a 60-min treatment, compared with about 90% survival for its R46-carrying derivative. The survival of *hisG46*(R46) cells exposed to X rays (under aerobic conditions) for various periods was like-

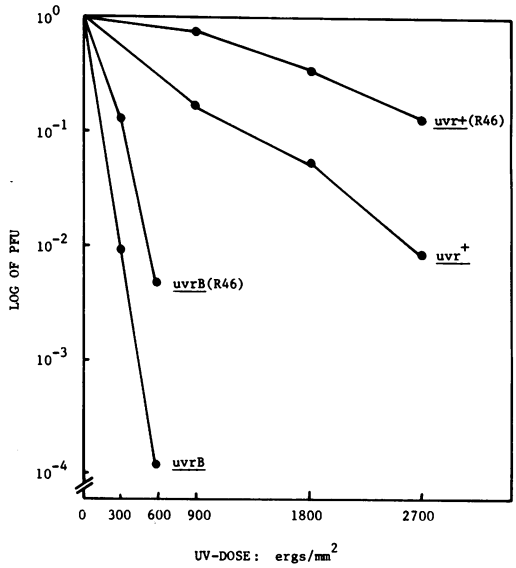

FIG. 4. *Test of ability of UV-irradiated phage P22.c2 HT13/4 to produce plaques on strain hisG46 (indicated uvr$^+$) and on its uvrB mutant, TA1950, as well as on their R46-carrying derivatives. Plaque counts are expressed as proportion of count of nonirradiated phage.*

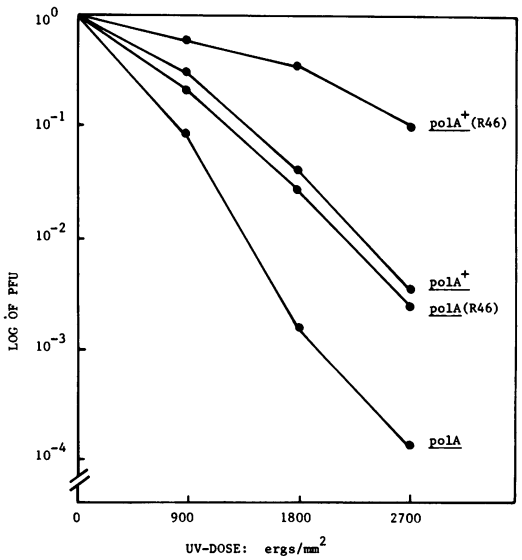

FIG. 5. *Test of ability of UV-irradiated phage ES18 to produce plaques on strain SL4525 (indicated polA$^+$) and on its polA1 mutant, SL4702, as well as on their R46-carrying derivatives. Plaque counts are expressed as proportion of count of nonirradiated phage.*

wise greater than that of similarly treated cells of *hisG46* (data not shown). Thus, only about 30% of *hisG46* survived a dose of 5 krad, compared with about 90% for *hisG46*(R46).

In the course of an experiment on photoreversal of the effects of UV irradiation of strain LT2 *hisG46*, we observed that exposure to the photoreactivating visible light (without prior exposure to UV) itself had a mutagenic effect. To measure this effect, and to test whether R46 modified it, plates of defined medium supplemented with L-histidine, 10 μg/plate, were surface inoculated with ca. 10^7 washed bacteria of strain *hisG46* or strain *hisG46*(R46). The plates were exposed (with plastic lids on) for 18 h at room temperature to the light from two 40-W fluorescent lamps, at a distance of circa 1.5 meters. Control plates were similarly treated, except that they were shielded from the light by being wrapped in aluminum foil. The plates were then incubated in the dark at 37°C, and colonies were counted after 2 days. For *hisG46* exposed to visible light, the mean number of colonies per plate was 81, compared with 2.6 for the control plates of the same strain not exposed to visible light. For *hisG46*(R46) the mean number of His^+ revertants per irradiated plate was 429, compared with 28 for the control, nonirradiated plates of the same strain. Thus, visible light induced His^+ revertants of *hisG46*, as was also observed by Speck and Rosencrantz (28), and the presence of R46 in the strain caused a more than fivefold increase in the number of revertants induced by the exposure to visible light.

DISCUSSION

Plasmid R46 reduces the bactericidal effect of UV irradiation but enhances its mutagenic effect, and enhances the ability of its host to reactivate UV-irradiated phage. We suppose that the plasmid produces all these effects by increasing the ability of its host to repair damaged DNA. Of the known pathways of DNA repair, the photoreactivation mechanism seems not relevant, since the effects of R46 were obtained without exposure to visible light. The first step in one dark repair process, excision repair, is cutting of one DNA strand by an endonuclease (determined by genes *uvrA* and *uvrB*, so that this kind of repair is thought to be absent in strains in which *uvrA* or *uvrB* is deleted). This is followed by excision and by polymerization, using the complementary strand as template, a process believed to be error free and therefore not concerned in UV mutagenesis. Other pathway(s) of dark repair are not well understood. In strains of wild-type UV sensitivity, the short strands of DNA resulting from replication past uncorrected dimers are joined into strands of normal length, as is inferred by sister-strand exchange. This *recA*-dependent rejoining is one component of dark repair. There may be, in addition, a process by which gaps are filled by base addition without use of a template strand. At least some parts of the dark repair pathway(s) other than excision repair, including the error-prone mechanism postulated to explain UV mutagenesis, are components of a set of functions that in rec^+ cells are induced by interruption of DNA synthesis, e.g., by UV irradiation (25, 31).

R46 reduced the bactericidal effect of UV irradiation to about the same extent in wild-type, *uvrB* (deletion), and *polA* strains (Tables 2 and 3, Fig. 1–3), but gave no protection in *recA* hosts (Table 3), and enhanced reactivation of UV-irradiated phage to about the same extent in wild-type, *uvrB*, and *polA* hosts (Fig. 4, 5). We infer that R46 produces these effects by enhancing DNA repair via a pathway other than excision repair and not dependent on host *polA* function. R46 produced about the same degree of enhancement of the mutagenic effect of UV irradiation in *uvrB* and *polA* strains as in strains of wild-type UV sensitivity, but did not restore UV mutagenesis in *recA* strains (Table 3). This also is compatible with our hypothesis that R46 acts by enhancing the activity of (a component of) the *recA*-dependent, inducible, error-prone pathway for repair of UV-damaged DNA.

The presence of plasmid R46 produced only about the same degree of UV protection and of enhancement of UV mutagenesis and phage-reactivating ability in a *polA* strain as in its $polA^+$ parent. We infer that R46 does not produce these effects by providing an activity functionally equivalent to that of the product of gene $polA^+$, since if it did one would expect to observe much greater effects in a *polA* host than in a wild-type host. In this respect our results and inferences about R46 differ from those of MacPhee (19) about R-Utrecht, another UV-protecting plasmid of incompatibility group N. In his experiment, R-Utrecht did not alter the ability of a $polA^+$ strain to reactivate UV-irradiated phage ES18 but caused both an increase in reactivating ability and a partial recovery of in vitro DNA polymerase activity in the *polA* mutant, SL4702. He inferred that R-Utrecht probably confers UV protection, at least in part, by determining production of a plasmid-specified polymerase.

Our observations on the undiminished ability of plasmid R46 to confer UV protection in excision-deficient mutants and failure to pro-

tect *recA* mutants correspond to most other observations recorded for UV-protecting plasmids, in *Escherichia coli* or *S. typhimurium* (18, 21, 27, 29). However, Siccardi (27) reported some UV protection in two *recA* mutants in *E. coli*. In a recent study, Tweats and his colleagues (29) show that R46 confers UV protection in *uvr*, *polA*, *lig*, *recB*, and *recC* mutants of *E. coli* but not in *recA* mutants: the UV-protecting property of the plasmid was prevented by exposure of irradiated cells to chloramphenicol for 180 min before testing viability. Their conclusion, that R46 protects *E. coli* against UV killing by increasing the efficacy of a *recA*-dependent, inducible repair pathway, agrees with our conclusions for the same plasmid tested in *S. typhimurium*.

R46 reduced the killing effect of X irradiation and of exposure to methyl methane sulfonate. These treatments are thought to result in production of single-strand gaps in DNA, so that increased ability to effect repair by the inducible, *recA*-dependent pathway, postulated above, might account for the protecting effect of R46.

In our experiments (Tables 1 and 2) involving 25 different *his* point mutants, the presence of R46 caused in all cases an increase in the number of His^+ revertants per 10^8 survivors of UV irradiation, 50 erg/mm^2: from too few to detect (<5) to between 11 and 90 in seven mutants, and by factors of 1.9 to 34 in the other 18 mutants. Increase in number of UV-induced revertants was observed for point *his* mutations of all classes tested, i.e., nonsense (amber, ochre, and UGA), missense, and frame-shift (+1 and −1 classes) (Table 2). Most of the tested revertants of two nonsense and of one missense mutant were shown to result from reversion at *his*, not by external suppressor mutation. The ability of R46 to enhance the frequency of UV-induced reversion for all classes of point mutation suggests that the presence of R46 in UV-irradiated bacteria increases the frequency of both frame-shift and base-change mutations, including several, perhaps all, of the six possible base-change mutations (i.e., from adenine-thymine [AT] pair to guanine-cytosine [GC], TA or CG, and from GC pair to AT, TA or CG). We think this is the result expected if R46 increases the activity of the inducible, error-prone pathway for repair of UV-irradiated DNA without qualitative alteration of the spectrum of errors that it makes. R46 enhanced the mutagenic effect of appropriate doses of UV irradiation on *uvrB* and *polA* derivatives of *his* point mutants (Table 3) but did not restore UV-mutagenic effect in the case of two *recA* derivatives of a *his*(amber) strain (Table 3). These results are compatible with the hypothesis that R46 enhances UV mutagenesis by causing increased activity of the error-prone repair mechanism, known to depend on *recA* but not on *uvrB* or *polA* function. An incomplete form of R46, plasmid pKM101 (K. E. Mortelmans, Ph.D. thesis, Stanford Univ., Stanford, Calif., 1975; Mortelmans and Stocker, manuscript in preparation) greatly increases the mutagenic effect of exposure to certain chemicals (22), an effect also produced by R-Utrecht (16, 17). The mechanism here postulated for enhancement of UV mutagenesis by R46 might also explain enhancement of chemical mutagenesis and/or enhancement of UV mutagenesis by incomplete R46 (22) and other UV-protecting plasmids (11, 16, 17). The mechanism of reversion of missense mutation *hisG46* caused by exposure to visible light, observed by us and by Speck and Rosencrantz (28), is unknown. If visible-light exposure causes DNA lesions correctable by the error-prone repair pathway, the postulated ability of R46 to enhance such repair would explain its enhancement of the mutagenic effect of visible-light irradiation, observed by us.

When plasmid R46 was present in missense mutant *hisG46*, the final number of spontaneous His^+ revertant colonies obtained per plate of selective medium was about 10 times greater than the number obtained from the R^- parent strain (Tables 1 and 2); most of the additional mutants proved to result from mutation at *his*. The number of revertant colonies, of *hisG46* or *hisG46*(R46), was directly proportional to the amount of histidine supplement per plate and independent of the inoculum size (Table 1); these observations support the inference that the plasmid causes a 10-fold increase in frequency of mutation to His^+ per bacterium per generation. The mutator effect of R46 in respect to spontaneous reversion to His^+ was observed in all of 14 nonsense *his* mutants and in two of five additional missense *his* mutants, but in none of five frame-shift *his* mutants tested in strains of wild-type UV sensitivity (Table 2). The limited range of mutations whose spontaneous reversion rate was affected by R46 contrasts with the ability of the plasmid to increase UV-induced reversion rate for all 25 *his* point mutants (Table 2). Almost any base change will convert a nonsense codon into a sense codon, which will usually result in regain of enzymatic function; by contrast, the range of base-change mutations that restore enzymatic activity to a missense mutant is limited for missense mutants of known codon character in the *trpA* gene of *E. coli* (32). We therefore interpret our data as indicating that the pres-

ence of R46 in *S. typhimurium* increases the frequency of spontaneous occurrence of only some of the six possible base-change mutations (e.g., perhaps only of transitions) and that it does not increase the frequency of spontaneous frame-shift mutations in strains of wild-type UV sensitivity.

Plasmid R46 increased the spontaneous reversion frequency in *uvrB* and *polA* derivatives of, respectively, missense mutant *hisG46* and amber mutant *hisC527* to about the same extent as in their parent strains, of wild-type UV sensitivity, but had no effect on the spontaneous reversion frequency of *recA* derivatives of the amber *his* mutation (Table 3). Thus, the mutator effect of R46, like its UV-protecting effect and its enhancement of UV mutagenesis, requires host *recA* but not host *uvrB* or *polA* function.

(The *uvr* deletions tested had two surprising effects in respect to spontaneous mutation rate [Table 3]. [i] The spontaneous reversion frequency for the R^- and R46 forms of the *uvrB* derivative of missense mutant *hisG46* were, respectively, 5 times and 11 times those of the corresponding uvr^+ strains. [ii] The four *uvrB* deletion derivatives of three frame-shift mutants tested in R^- form reverted at the same rate as their uvr^+ parents, but introduction of R46 into either of two different *uvrB*(deletion) sublines of the one-base insertion mutant *hisC3076* caused a 10-times increase in spontaneous reversion. By contrast, R46 did not affect reversion rate of the uvr^+ parent strain, nor of the *uvrB* deletion derivatives of two other frame-shift mutations. These two effects remain unexplained.)

The dependence of the mutator property of R46 on host *recA* function suggests that this property may result from the same mechanism as the *recA*-dependent UV-protecting and mutagenesis-enhancing properties; this mechanism, we surmise, is increased activity of some component(s) of the inducible, *recA*-dependent, error-prone repair pathway. Perhaps the pathway is used, to a minor extent, in normal replication of chromosomal genes in nonirradiated bacteria, or in repair of some kind of spontaneously arising defect in DNA. Witkin (31) reports that two treatments believed to cause induction of the error-prone repair system, viz., growth of a *tif* strain at 42°C and exposure of a temperature-sensitive *dnaB* mutant at 42°C for 90 min, cause increases in spontaneous mutation rate. The observations of Bleichrodt and Verheij (4) suggest that the inducible repair process acts on the DNA of nonirradiated phage ϕX174 to increase the fraction of spontaneous mutants. Witkin (31) discusses the possibility that the inducible, error-prone, *recA*-dependent repair reflects the activity of an error-prone DNA polymerase not normally used for replication of chromosomal genes. R46 might produce its mutator and other effects by specifying such an error-prone polymerase or by increasing the activity of one specified by a bacterial gene. However, the difference in range of mutations responding to the mutator effect and to enhancement of UV mutagenesis by the plasmid argues against this hypothesis. Furthermore, plasmid pKM115, an incomplete variant of R46, has a strong mutator effect (in respect of *hisG46*) but does not UV-protect, whereas various unrelated plasmids that confer UV protection and enhance UV mutagenesis have no, or little, mutator effect (K. E. Mortelmans, Ph.D. thesis, Stanford University, Stanford, Calif., 1975; Mortelmans and Stocker, manuscript in preparation).

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