JOURNAL OF BACTERIOLOGY, Sept. 1969, p. 713-719
Vol. 99, No. 3
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Escherichia coli ras Locus: Its Involvement in Radiation Repair

JAMES R. WALKER

Department of Microbiology, The University of Texas at Austin, Austin, Texas 78712

Received for publication 11 June 1969

There are several classes of *Escherichia coli* mutants defective in radiation repair. These include strains defective in pyrimidine dimer excision, in photoreactivation, in recombination, in repair of X-ray damage, and ultraviolet (UV)-conditional mutants which do not divide after UV. Another mutant (*ras*⁻) has been isolated. The *ras*⁻ has increased UV sensitivity, but only slightly increased X-ray sensitivity (1.5-fold increase). Ability to effect genetic recombination, to reactivate irradiated bacteriophage T1, and to be photoreactivated is normal. UV-induced mutation frequency is greatly increased in the mutant. The *ras*⁻ apparently lacks the ability to repair some UV damage in the bacterial cell but can repair UV damage to bacteriophage DNA. The *ras* locus is located between *lac* and *purE* on the chromosome map.

Several classes of radiation-sensitive *Escherichia coli* mutants have been described.

The *uvr*⁻ strains have reduced efficiency for excising pyrimidine dimers (3, 15, 31, 37). The *rec*⁻ mutants have greatly increased ultraviolet (UV) and X-ray sensitivity and perform genetic recombination with low efficiency (5, 14). The *lex*⁻ (or *exr*⁻) locus increases ultraviolet (UV) and X-ray sensitivity without altering genetic recombination or host cell reactivation (6, 14, 27). Conditional cell division mutants which form nonseptate, multinucleate filaments after UV or X ray are *lon*⁻ (1, 2, 8, 17, 33, 34).

An additional UV-sensitive mutant of *E. coli* K-12 has been isolated. This mutant, designated *ras*⁻, is UV-sensitive, but normal in genetic recombination, and host cell reactivation, and only slightly X-ray-sensitive. The frequency of UV-induced mutation is increased greatly in the *ras*⁻ strain. The *ras* locus is located between *lac* and *purE* on the chromosome map.

MATERIALS AND METHODS

Bacteria. The principal strains used, their characteristics, and their source or derivation are listed in Table 1. AX14 is a spontaneous *lon*⁻ mutant of 2e01c (42). AB311 was obtained from E. Cox.

Bacteriophages. Bacteriophage T1 (obtained from A. B. Pardee) was propagated on *E. coli* BB in Yeast Extract Tryptone (YET) broth. A lysogen of λ^+ (obtained from Eugene Goldschmidt) was UV-irradiated in sodium chloride-sodium phosphate buffer (17), shifted to YET broth (plus 0.001 M $MgSO_4$), and incubated at 37 C until lysis. Bacteriophage f2 (obtained from Robert Webster) was grown according to Loeb and Zinder (23). The virulent P1 mutant of Ikeda and Tomizawa (19) was obtained from W. A. Newton.

Media. The YET agar and broth were used (17); in broth the NaCl concentration was modified to 0.5%. Minimal medium base (17) was supplemented with separately autoclaved glucose (0.2%), L-amino acids (50 μg/ml), or thymine (2 or 50 μg/ml, as desired) and thiamine HCl (5 μg/ml) as desired. Streptomycin was filter-sterilized and added separately to 200 μg/ml.

UV irradiation. Cells growing exponentially in YET broth were resuspended in sodium chloride-sodium phosphate buffer at 10^6 cells/ml and irradiated with light from a 15-w General Electric germicidal lamp. Irradiated preparations were plated on YET plates. Intensity was determined by the potassium ferrioxalate actinometry system of Hatchard and Parker as described by Calvert and Pitts (4). After correction for turbidity (29), effective intensity was 13 ergs/mm²/sec. All manipulations were carried out under dim light from General Electric Gold lamps.

X-ray irradiation. Cultures were grown in YET broth plus 0.1% glucose for 20 hr, centrifuged once at room temperature, resuspended in buffer (17) at 10^8 cells/ml, mixed magnetically in a Pyrex beaker for 5 min, and then irradiated while mixing continued. Irradiated samples were plated on YET agar plus 0.1% glucose. A Westinghouse Quadrocondex at 250 kw and 15 ma was the X-ray source. Intensity was about 1.5 krad/min as determined by comparison of data with those of E. L. Powers.

Photoreactivation. UV-irradiated samples were exposed to light from a 100 w General Electric black light (39). A 2-mm layer of Pyrex glass and a 4-mm 0-51 no. 3850 filter (Corning Glass Works, Corning, N.Y.) were used to exclude light below about 360 nm. Samples were maintained at 35 C by a heating mantle

growth medium, cells were elongated during subsequent incubation, but not to a greater extent than those of the wild-type given the same dose. Strain AX83 does not appear to have a defect specific for septum formation.

X-ray sensitivity of AX83 was compared to that of the *ras*$^+$ wild-type strain (Fig. 3). The X-ray sensitivity was increased by a factor of only 1.5.

Host cell reactivation. The ability of AX83 to repair UV lesions in irradiated T1 bacteriophage was determined (Fig. 4). Irradiated T1 was plated on *ras*$^-$, wild-type, and *uvr*$^-$ strains. AX83 reactivated the bacteriophages as well as did wild-type strains. The three *uvr* mutants were identical in their host cell reactivation (Hcr) ability. If λ was the test bacteriophage, AX83 again was Hcr$^+$.

Genetic recombination. Recombination ability in the *ras*$^-$ mutant was comparable to that of wild-type (Table 3). In conjugation experiments which involved different Hfr strains and different selections, the frequency of recombinants was the same as that found with wild-type female strains.

Photoreactivation. The capability of UV-irradiated AX83 to be directly photoreactivated was examined. Cells were irradiated with UV to about 0.5% survival and divided into two portions. One was illuminated with direct photoreactivating light (longer than 360 nm) and plated. The other was held in the buffer in the dark for periods of time greater than that required for illumination for photoreactivation; it was then plated to observe increase in viable cells caused by dark repair (a liquid holding recovery control). A *lon*$^-$ strain [known to be photoreactivatable (21)] similarly was treated to indicate the wild-type level of photoreactivation.

AX83 was directly photoreactivatable (Fig. 5). Liquid holding recovery was negligible during the time required for illumination. The photoreactivation effect was strongly temperature-dependent. Apparently, the *ras*$^-$ strain possesses photoreactivating enzyme, and the UV lesions responsible for inactivation of *ras*$^-$ are reparable by direct photoreactivation.

UV-induced mutagenesis. The frequency of UV-induced mutations from valine sensitivity to valine resistance and from *thyR*$^+$ to *thyR*$^-$ was observed in *ras*$^+$ and *ras*$^-$ strains (Table 4). The *ras*$^-$ strains were much more susceptible to UV mutagenesis than the wild-type strains. At doses too low to induce mutations in the *ras*$^+$ strains, there were numerous mutants produced by *ras*$^-$ strains.

The increased UV-induced mutation frequency of *ras*$^-$ strains was not an artifact of crowded growth on the selective media. The 10-fold increases in numbers of survivors spread had virtually no effect on mutant yield.

Map position of ras. Linkage was detected by

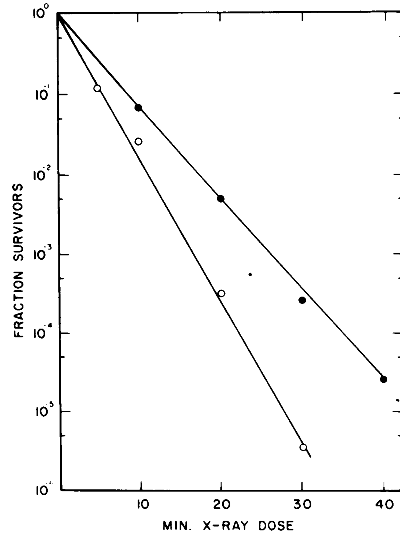

FIG. 3. *X-ray irradiation sensitivity of AX83,* ○ *(ras*$^-$*); AX 100,* ●*, was ras*$^+$*.*

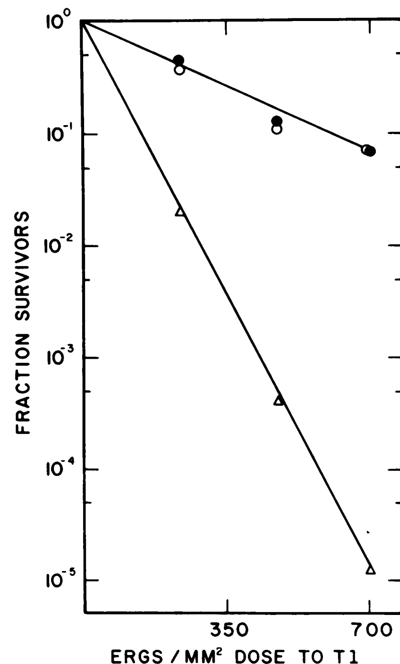

FIG. 4. *Host cell reactivation in AX83,* ○ *(ras*$^-$*); AX100,* ● *(ras*$^+$*); AB1886,* △ *(uvrA*$^-$*). AB1885 (uvrB*$^-$*) and AB1884 (uvrC*$^-$*) gave results identical to AB1886.*

N-methyl-*N'*-nitro-*N*-nitrosoguanidine survivors on glucose minimal medium. The nonmucoid mutants were readily visible although present in small proportions. From 108 nonmucoid mutants, three were significantly more UV-sensitive [as determined by a sensitive, direct screening test (42)] than was the mucoid *lon*$^-$ parent. Two of the three were found to be mutant in a gene for polysaccharide biosynthesis and mutant in known *uvr* loci. One (labeled AX80) of the three was found to be mutant in a gene for polysaccharide biosynthesis and mutant in a UV-sensitivity marker. The UV sensitivity marker of AX80 was found to be a previously undiscovered radiation repair gene and is designated *ras*.

For a genetic analysis of AX80 (Table 2), the first experiment was to determine whether restoration of ability to synthesize polysaccharide would restore UV susceptibility to the parental level. Several genes involved in polysaccharide biosynthesis map near *his* in *Salmonella* (25, 30). To determine whether AX80 was nonmucoid as a result of a mutation near *his*, a *his*$^-$ mutant (labeled AX81) was isolated from AX80 after mutagenesis and penicillin selection. AX81 retained the UV sensitivity and nonmucoidness of AX80.

AX81 then was transduced to *his*$^+$ by using P1 propagated on the mucoid, *lon*$^-$ AX14. The mucoid character was cotransduced with *his*$^+$ with high frequency, 91% (106 of 117). Every *his*$^+$ mucoid transductant retained the extreme UV sensitivity of AX80. Clearly, loss of capsular polysaccharide and increase in UV sensitivity were unrelated. One *his*$^+$ mucoid transductant of AX81 was labeled AX82.

To investigate the properties of the new UV-sensitivity marker, the *lon*$^+$ allele was introduced into AX82 by conjugation with the *lon*$^+$ W1895 (Fig. 1). After a 25-min mating period, *thr*$^+$ *leu*$^+$ *str-r* recombinants were selected. Of the recombinants, 73% were *lon*$^+$.

The *lon*$^+$ recombinants were then scored for UV sensitivity to determine the presence of *ras*$^+$ or *ras*$^-$. Of the *lon*$^+$ recombinants, 95% were *ras*$^+$, since they had the wild-type level of UV resistance; but 5% were *ras*$^-$ (i.e., were sensitive). One *lon*$^+$ *ras*$^-$ recombinant was labeled AX83.

TABLE 2. *Isolation of AX83*

Strain	Derivation
AX14	Spontaneous *lon*$^-$, mucoid by virtue of *lon*$^-$ locus
AX80	Nonmucoid, *ras*$^-$ mutant of AX14 (*lon*$^-$)
AX81	*his*$^-$ mutant of AX80
AX82	*his*$^+$, mucoid transductant of AX81
AX83	*lon*$^+$ derivative of AX82, still *ras*$^-$, and nonmucoid by virtue of the *lon*$^+$

Radiation sensitivity. Ultraviolet sensitivity of AX83 was intermediate between that of wild-type strains and that of most previously described mutants (Fig. 2). Doses sufficient to reduce survivors to 37% for *uvr*$^-$ and *rec*$^-$ strains were 8 ergs/mm^2 and 3 ergs/mm^2, respectively (14). A dose of about 40 ergs/mm^2 was required to yield 37% survivors of AX83.

UV treatment did not initiate filamentous growth of the *ras*$^-$ mutant. When AX83 was irradiated to about 1% survival and diluted in

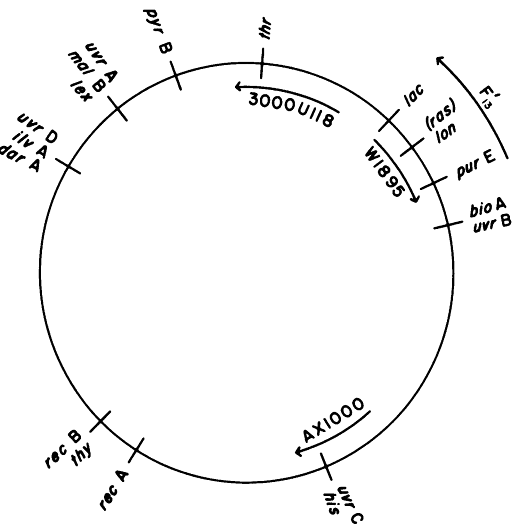

FIG. 1. *Partial genetic map of E. coli, from Taylor and Trotter (40). Parentheses around ras indicate uncertainty of exact location.*

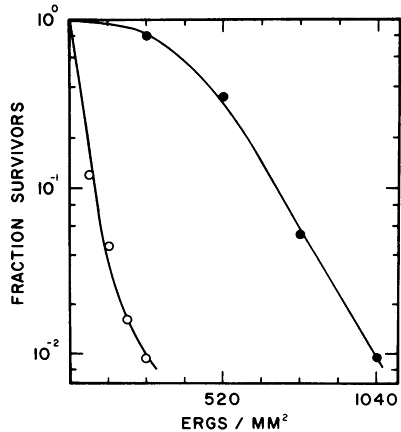

FIG. 2. *Ultraviolet sensitivity of AX83,* ○ (*ras*$^-$); *AX100,* ●, *was ras*$^+$.

growth medium, cells were elongated during subsequent incubation, but not to a greater extent than those of the wild-type given the same dose. Strain AX83 does not appear to have a defect specific for septum formation.

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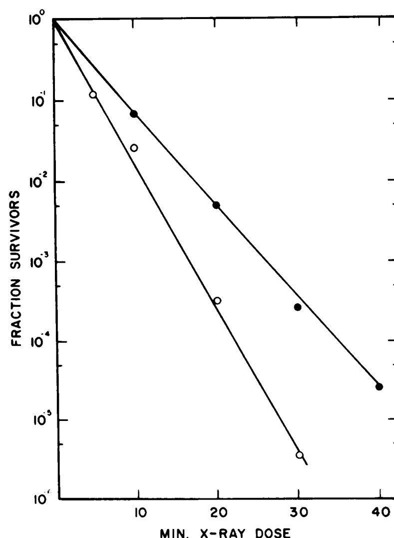

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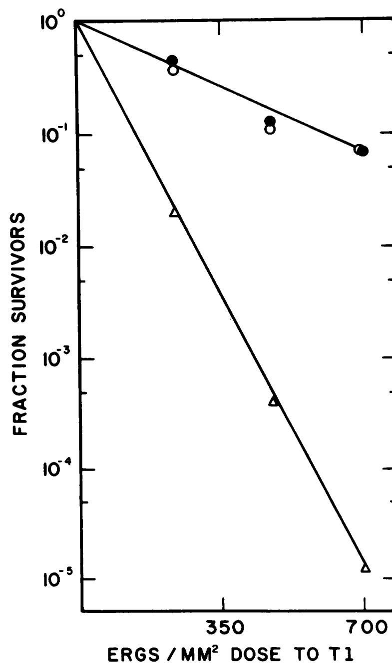

FIG. 4. *Host cell reactivation in AX83,* ○ (*ras*$^-$)*; AX100,* ● (*ras*$^+$)*; AB1886,* △ (*uvrA*$^-$)*. AB1885* (*uvrB*$^-$) *and AB1884* (*uvrC*$^-$) *gave results identical to AB1886.*

the conjugation experiment in which the *ras*$^+$ *lon*$^+$ Hfr W1895 was mated with the *thr*$^-$ *leu*$^-$ *ras*$^-$ *lon*$^-$ AX82. A 25-min period for mating was used. Of the *thr*$^+$ *leu*$^+$ *str-r* recombinants, 73% (181 of 249) were *lon*$^+$ and 27% (68 of 249) were *lon*$^-$, as determined by observation of colony morphology for mucoidness. Of the *lon*$^+$ recombinants, 96% (74 of 77) were *ras*$^+$. Of the *lon*$^-$ recombinants, 89% (57 of 64) were *ras*$^-$.

A second *ras*$^+$ *lon*$^+$ Hfr, 3000U118, was mated with the *ras*$^-$ *lon*$^-$ AX82. After 25 min of mating, *thr*$^+$ *leu*$^+$ *str-r* recombinants were selected. Of the

TABLE 3. *Genetic recombination in ras*$^-$ *strains*[a]

Recipient strain	Input recipient cells per ml	Recombinants per ml	
		W1895[b]	AX1000[b]
2e01c	1.2×10^8	6.4×10^3	
AX82	1.1×10^8	5.4×10^3	
AX46	1.3×10^8		4.3×10^4
AX81	1.1×10^8		4.4×10^4

[a] AX81 and AX82 were *ras*$^-$; 2e01c and AX46 were wild-type with respect to radiation sensitivity.

[b] For the W1895 donor, input of cells per milliliter was 1.1×10^7, mating time was 20 min, and selection was *thr*$^+$ *leu*$^+$ *str-r*; for the AX1000 donor, input of cells per milliliter was 1.1×10^7, mating time was 15 min, and selection was *his*$^+$ *thy*$^+$.

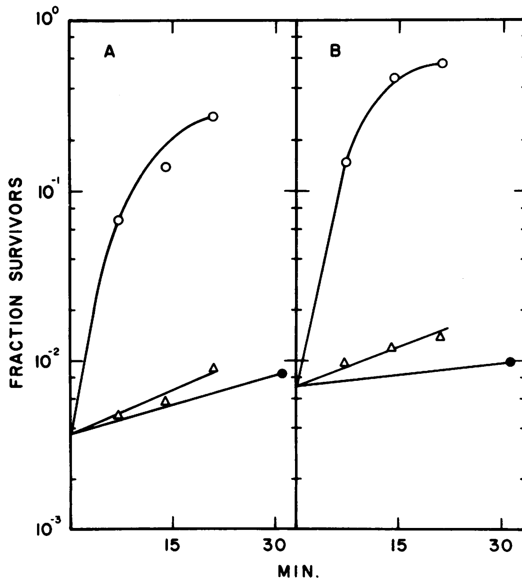

FIG. 5. *Photoreactivation of AX83. A, AX14 (lon*$^-$*); B, AX83 (ras*$^-$*). Illumination at 35 C (○); Illumination at 4 C (△); liquid holding recovery at 35 C (●). Min refers to period of illumination or to period of liquid holding.*

TABLE 4. *Induction of mutations to val-r and thyR*$^-$ *by UV light in ras*$^+$ *and ras*$^-$ *strains*

Strain	UV dose (ergs/mm^2; incident)	Survival	Induced mutations per plate[a]		Induced mutations per 10^7 survivors	
			val-r	*thyR*$^-$	*val-r*	*thyR*$^-$
		%				
AX83	60	15	6[b]		3[b]	
(*ras*$^-$	120	6	18[b]		16[b]	
val-s)	120	6	246[c]		22[c]	
AX100	60	90	0		<0.08	
(*ras*$^+$	500	13	4		20	
val-s)						
AX84	60	11		42		230
(*ras*$^-$	300	0.5		6		660
thy$^-$	300	0.5		71[b]		760[b]
thyR$^+$)						
AX103	60	84		0		<0.06
(*ras*$^+$	300	58		0		<0.1
thy$^-$	500	14		31		120
thyR$^+$)						

[a] Average of 10 plates.

[b] Sample concentrated 10-fold before plating.

[c] Sample concentrated 100-fold before plating.

recombinants, 10% (69 of 660) were *lon*$^+$ and 90% (591 of 660) were *lon*$^-$. Of the *lon*$^+$ recombinants, 83% (40 of 48) were *ras*$^+$. Of the *lon*$^-$ recombinants, 98% (63 of 64) were *ras*$^-$.

In both crosses, *lon* and *ras* segregated together in 83% to 98% of the recombinants.

To confirm the map position and to test dominance, diploids were prepared by mating AX83 with the F′ strain W3747. W3747 harbors F'_{13}, an episome that extends from *lac* to *purE*. Merogenotes, identified by their sensitivity to the male-specific bacteriophage, f2, were UV-resistant. Thus, *ras*$^+$ was dominant over *ras*$^-$ and *ras*$^+$ is carried on F'_{13}. (The dominance was not complete, since the merogenotes were not fully as UV-resistant as wild-type).

Thus, *ras* is located between *lac* and *purE* and probably is close to *lon*.

DISCUSSION

The mutation in strain AX83 has been located between *lac* and *purE*, and probably it is close to *lon*. Previously described UV-sensitivity markers on this segment of the *E. coli* chromosome are *lon* of K-12 strains (17) and *fil* of *E. coli* B strains (9). Both *lon* and *fil*, which probably are equivalent (7, 8, 21, 22), specifically involve septum formation after UV (1, 42). The *lon* locus certainly is distinct from *ras*, since recombination occurred between the two markers. Witkin (45) demonstrated that the *fil*$^+$ mutation has no effect on UV-induced mutation frequency. The *ras*$^-$

AX83 had greatly increased UV-induced mutation frequency. UV doses too low to cause mutation of the wild-type strain produced numerous mutants of *ras*$^-$ strains.

Greenberg (10) described a UV-sensitive *E. coli* K-12 strain, PAM401, which has a mutation between *lac* and *purE*. However, PAM401 now is known to differ from wild-type by at least three mutations, one of which is *lon* and another of which is a suppressor of *lon* (Greenberg, *personal communication*).

The *ras* locus is presumed to be involved in pyrimidine dimer repair, since direct photoreactivation reversed the UV effect on *ras*$^-$ AX83. Photoreactivating enzyme, in the presence of light of about 405-nm wavelength and by a strongly temperature dependent process, monomerizes pyrimidine dimers (20, 38, 41, 46).

The *uvr*$^-$ (excision repair defective) and *ras*$^-$ strains have some common properties. In addition to increased UV sensitivity, both are only slightly more X-ray-sensitive than is wild-type *E. coli*. The *uvr*$^-$ strains are about 1.3 times more X-ray-sensitive than wild-type (16); the *ras*$^-$ AX83 increased in X-ray-sensitivity by a factor of 1.5. Strains defective in excision repair are much more susceptible to UV mutagenesis than is the wild-type (12, 44). Similarly, the *ras*$^-$ AX83 formed UV-induced mutants after UV doses too low to cause mutation in the wild-type.

The *ras* gene might participate in the excision-repair system. If so, it participates at a step after pyrimidine dimer excision, since the *ras*$^-$ AX83 excises pyrimidine dimers after UV (*unpublished observation*). A difference between *uvr*$^-$ and *ras*$^-$ strains is in ability to reactivate UV-irradiated bacteriophages T1 and λ (host cell reactivation). The *uvr*$^-$ strains are incapable of host cell reactivation (16). The *ras*$^-$ AX83 reactivated irradiated T1 and λ as well as did the wild-type. Possibly the excision repair enzymes involved in steps after dimer excision (i.e., repair replication and rejoining) are host- and bacteriophage-specific.

Strains defective in the *exr* (45) or in the *rec* (28) repair systems do not form UV-induced mutations. Both *exr*$^-$ and *rec*$^-$ strains are more X-ray-sensitive than wild-type by factors of three to eight times (13, 18).

A second system of recovery from pyrimidine dimers involves the formation of gaps in DNA strands synthesized opposite the dimers (35). The *ras* locus could be involved in this process.

ACKNOWLEDGMENTS

This investigation was initiated in the laboratory of A. B. Pardee, whom I thank for advice. Discussions with Max M. Burger, E. L. Powers, and Jack L. Myers are gratefully acknowledged. Etheria Robinson and Nawal A. Shafiq provided capable technical assistance.

This investigation was supported by Public Health Service grants AI-04409 (to A. B. Pardee) and AI-08286 from the National Institute of Allergy and Infectious Diseases, by National Cancer Institute Fellowship 1-F2-CA-29, 413-01, and by grants from the Research Corporation and the University of Texas Biomedical Science Support Program.

Addendum in Proof

While this manuscript was in press, Witkin (Mutation Res. 8:9, 1969) demonstrated that a *recA*$^-$ strain formed no detectable UV-induced mutations and that a *recC*$^-$ strain formed UV-induced mutations with reduced frequency.

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