ANALYSIS OF THE ROLE OF RECOMBINATION AND REPAIR IN MUTAGENESIS OF *ESCHERICHIA COLI* BY UV IRRADIATION

TAKESI KATO*, ROBERT H. ROTHMAN† AND ALVIN J. CLARK

Department of Molecular Biology, University of California, Berkeley, California 94720

Manuscript received September 15, 1976
Revised copy received June 6, 1977

ABSTRACT

Multiple mutant strains have been tested for their mimicry of the UV-mutagenesis deficiency of a *recA* single mutant. Revertants to histidine prototrophy and clear plaque mutants of lambda were scored to determine capacity for UV-mutagenesis. Nearly normal capacity was shown by a *uvr*+ *recB*− *recF*− strain, which shows almost no *recA*-dependent recombination, by *uvr*− *recB*+ *recF*− strains, which show almost no *recA*-dependent repair and by a *uvrA*− *recB*− *recF*− strain, which shows neither *recA*-dependent recombination nor repair. Since the *uvr* mutants can be assumed to show additionally no excision repair, these results may mean that UV-mutagenesis occurs during processes other than recombination and repair. Alternative hypotheses are discussed. The slight difference in mutagenic capacity was traced to the *recF* single mutation, which blocks the production of unmixed bursts of clear-plaque lambda mutants. Since this accounts for only about 10% of the mutations leading to clear-plaque mutants, it is suggested that there is more than one UV-mutagenic process.

THE mutagenic effects of ultraviolet radiation on *E. coli* are well known (*e.g.*, WITKIN 1969a). In the three decades that this mutagenic action has been studied, three main questions have come to be formulated. First, what enzymes catalyze the mutational steps, what are their properties, and what are their substrates? Second, what regulatory mechanisms control the concentrations and activities of the mutational enzymes and what biochemical entities mediate this control? Finally, do the mutational enzymes act during replication, recombination, repair, transcription or a combination of these processes?

To answer these questions, a variety of techniques has been used. One technique involves determining the effects on UV mutagenesis of mutations blocking some process of DNA metabolism. Accordingly, it has been found that *recA* mutations, which block post-replication repair (SMITH and MEUN 1970) and most genetic recombination (CLARK and MARGULIES 1965; LOW 1968; GUYER and CLARK 1976), also block UV mutagenesis (MIURA and TOMIZAWA 1968; WITKIN 1969b). In addition, *recA* mutations prevent UV induction of lambda (BROOKS and CLARK 1967; HERTMAN and LURIA 1967; ROBERTS and ROBERTS

* Present address: Department of Fundamental Radiology, Osaka University Medical School, Osaka 530, Japan.
† Present address: Department of Biology, Brookhaven National Laboratory, Upton, L.I., New York 11973.

1975) and protein X (GUDAS and PARDEE 1975). These findings have been put together with others and the concept has developed that one or more mutagenic enzymes are induced by UV irradiation (DEFAIS *et al.* 1971; WITKIN 1975; RADMAN 1975). These enzymes are thought to produce mutations by participating in either repair or replication (WITKIN 1975). Post-replication repair, one form of which involves recombination between sister chromosomes that have been incompletely replicated because of UV-produced pyrimidine dimers (RUPP and HOWARD-FLANDERS 1968; WITKIN 1969a), and excision repair have both been implicated in the production of UV-induced mutations (WITKIN 1969a and NISHIOKA and DOUDNEY 1969, respectively). More recent experiments indicate that UV-induced mutation can occur in the absence of UV-produced lesions in the DNA that is undergoing mutation. ICHIKAWA-RYO and KONDO (1975) have shown, for example, that UV irradiation of bacterial cells increases the frequency of λc mutants produced following infection with unirradiated lambda.

Recombination, as well as repair and replication, has been suggested to involve intermediates acted upon by one or more UV-induced mutagenic enzymes (WITKIN 1967). This suggestion was based on the absence of UV-induced mutation of *lex*$^-$ mutants (WITKIN 1967; MOUNT, LOW and EDMISTON 1972). Since *lex*$^-$ mutants are recombination proficient (HOWARD-FLANDERS and THERIOT 1966; MOUNT, LOW and EDMISTON 1972), it was proposed that UV-induced mutations might be produced by a minor pathway of recombination dependent on *lexA*$^+$ (WITKIN 1969a). Recent studies (A. J. CLARK, A. TEMPLIN, H. NAGAISHI and K. MCLEOD, unpublished results) indicate that *lexA* mutations block the RecF pathway of genetic recombination in *E. coli*. Since the RecF pathway is responsible for only about 1% of *recA*-dependent recombination in wild-type (*i.e.*, *recB*$^+$ *recC*$^+$ *sbcB*$^+$) cells (HORII and CLARK 1973; CLARK 1973), this finding opens the possibility that the mutagenic enzyme or enzymes find their substrates uniquely in the RecF pathway of recombination. We have tested that possibility by examining spontaneous and UV mutagenesis in strains carrying a *recF* mutation blocking the RecF pathway. In addition we have examined the effects on mutagenesis of blocking both the RecBC pathway and the RecF pathway of recombination.

MATERIALS AND METHODS

Bacterial and bacteriophage strains: Most of the bacterial strains used and their derivatives are listed in Table 1. In addition, JC158 (BACHMANN 1972) was used as a donor strain to determine recombination proficiency and deficiency of strains. It is an HfrH derivative and carries the following mutant alleles: *serA6*, *lacI22*, *thi-1*. F$^-$ strain NO483 was a gift of K. ONODERA. It carries unnumbered mutations in the *tolA*, and *strA* loci (ROLFE *et al.* 1973). The strains which carry mutations in both *recB* (*recB21*) and *recC* (*recC22*) will be referred to as *recB*$^-$ for convenience. All *recB*$^+$ strains were also *recC*$^+$. A host-range mutant of lambda was used for mutagenesis experiments in which clear-plaque mutants were to be detected.

General methods: The methods used for conjugation and transduction have been described previously (CLARK and MARGULIES 1965; WILLETTS, CLARK and LOW 1969).

Media: Lambda broth consists of 1% tryptone and 0.5% NaCl, adjusted to pH 7.0. L broth consists of 1% tryptone, 0.5% yeast extract and 1.0% NaCl adjusted to pH 7.0. For detection of lambda plaques, L broth made 1.5% in Difco Bacto-agar was used as bottom agar and L broth

TABLE 1

Partial genotypes and derivations of bacterial strains*

Strain	*uvr*	*recA*	*recB*	*recC*	*recF*	*thyA*	*ilv*	*arg*	*metA*	Derivation† or reference
JC5422	+	+	+	+	+	*325*	+	*E3*	+	BACHMANN 1972
JC3839	+	+	+	+	+	*325*	*325*	*E3*	+	EMS treatment of JC5422
JC3842	+	+	+	+	+	+	*325*	*E3*	+	JC3839+P1·JC5519; Thy+ selection
JC3844	+	+	*21*	*22*	+	+	*325*	*E3*	+	JC3839+P1·JC5519; Thy+ selection
JC3872	+	+	+	+	+	+	+	*E3*	+	JC3842+P1·JC9239; Ilv+ selection
JC3875	+	+	+	+	*143*	+	+	*E3*	+	JC3842+P1·JC9239; Ilv+ selection
JC3878	+	+	*21*	*22*	+	+	+	*E3*	+	JC3844+P1·JC9239; Ilv+ selection
JC3881	+	+	*21*	*22*	*143*	+	+	*E3*	+	JC3844+P1·JC9239; Ilv+ selection
AB2463	+	*13*	+	+	+	+	+	*E3*	+	BACHMANN 1972
AB1927	+	+	+	+	+	+	+	*H1*	*28*	B. BACHMANN, personal communication
JC3910	+	+	+	+	+	*325*	*325*	+	*28*	JC3839+P1·AB1927; Arg+ selection
JC3911	*A6*	+	+	+	+	*325*	*325*	+	+	JC3910+P1·AB1886; Met+ selection
JC8945	*A6*	+	+	+	+	+	*325*	+	+	JC3911+P1·JC5519; Thy+ selection
JC8946	*A6*	+	*21*	*22*	+	+	*325*	+	+	JC3911+P1·JC5519; Thy+ selection
JC3912	*A6*	+	+	+	+	+	+	+	+	JC8945+P1·JC9239; Ilv+ selection
JC3913	*A6*	+	+	+	*143*	+	+	+	+	JC8945+P1·JC9239; Ilv+ selection
JC3914	*A6*	+	*21*	*22*	+	+	+	+	+	JC8946+P1·JC9239; Ilv+ selection
JC3915	*A6*	+	*21*	*22*	*143*	+	+	+	+	JC8946+P1·JC9239; Ilv+ selection
AB2480	*A6*	*13*	+	+	+	+	+	+	+	HOWARD-FLANDERS, THERIOT and STEDEFORD 1969
JC3833	*B301*	+	+	+	+	*325*	+	*E3*	+	Spontaneous Chl^R of JC5422, also Bio$^-$ Phr$^-$ Pgi$^-$
JC3836	*B301*	+	+	+	+	*325*	*328*	*E3*	+	EMS treatment of JC3833
JC3846	*B301*	+	+	+	+	+	*328*	*E3*	+	JC3836+P1·JC5519; Thy+ selection
JC3848	*B301*	+	*21*	*22*	+	+	*328*	*E3*	+	JC3836+P1·JC5519; Thy+ selection
JC3890	*B301*	+	+	+	+	+	+	*E3*	+	JC3846P1·JC9239; Ilv+ selection
JC3893	*B301*	+	+	+	*143*	+	+	*E3*	+	JC3846+P1·JC9239; Ilv+ selection
JC3896	*B301*	+	*21*	*22*	+	+	+	*E3*	+	JC3848+P1·JC9239; Ilv+ selection
JC3899	*B301*	+	*21*	*22*	*143*	+	+	*E3*	+	JC3848+P1·JC9239; Ilv+ selection

* AB1927 is the only strain in the table not derived from AB1157. It is an Hfr with the point of origin and direction of transfer of AB312 (Low 1972) and carries *purF1*, *xyl-7* and possibly *supE44* as other markers. All strains except one carry the following mutant alleles in addition to those listed: *thr-1*, *leu-6*, *proA2*, *his-4*, *thi-1*, *lacY1*, *galK2*, *ara-14*, *xyl-5*, *mtl-1*, *tsx-33*, *strA31*, *supE44*; the exceptional strain AB2480 carries *thr*+, *leu*+, *his*+, *arg*+ and *proA2 thi-1 lacY1 galK2 tsx-33 strA31*. We have not determined whether AB2480 carries the *ara xyl*, *mtl*, and *sup* alleles of AB1157. All gene symbols are defined by BACHMANN, LOW and TAYLOR (1976). The letters and numbers in the table are mutant alleles and symbols stand for the wild-type alleles.

† In the course of these derivations, several recombination-deficient strains were used as recipients for transduction. Very few transductants were found but, after several repetitions, enough were tested to result in the detection of the desired genotypes. *recB*$^-$ *recC*$^-$ *recF*$^-$ strains are more UV sensitive than *recB*$^-$ *recC*$^-$ *recF*+ strains. *RecB*+ *recC*+ *recF*$^-$ strains are more sensitive than *recB*+ *recC*+ *recF*+ strains. The following abbreviations are used: Thy+, thymine independence; Ilv+, isoleucine and valine independence; Arg+, arginine independence; Met+ methionine independence; Chl^R, chlorate resistance; Bio$^-$, biotin dependence; Phr$^-$, photoreactivation deficiency; and Pgi$^-$, phosphoglucoisomerase deficiency.

made 0.65% in agar was used as top agar. The formula for Davis minimal medium hsa been described (DAVIS and MINGIOLI 1950). It was solidified with 2% Difco Bacto-agar. Minimal medium lacking histidine (MM) was Davis minimal medium supplemented with 100 μg/ml each of threonine, proline, arginine and leucine and 0.5 μg/ml of thiamine. Semi-enriched medium (SEM) was MM medium to each liter of which 0.4 ml of a 10% solution of casamino acids had been added. The formula for 56 salts medium has been previously presented (WILLETTS, CLARK and Low 1969). Thymine when required was added to a final concentration of 10 μg/ml.

Spontaneous rate of His⁻ to His+ mutations: The rate of spontaneous mutation was determined by the SEM-plate method described by KONDO *et al.* (1970). Samples (0.1 ml) of starved overnight cultures at the concentration of 2×10^9, 2×10^8 and 2×10^7 cells/ml were plated on SEM and MM (Davis minimal medium supplemented with the required growth factors except histidine). After 68 hr incubation, His+ mutant colonies on SEM and MM plates were counted. The mutation rate μ was calculated by the formula $\mu = (M_{SEM} - M_{MM})/(N_t - N_0)$ where M_{SEM} and M_{MM} are the average numbers of His+ mutants scored on SEM and MM plates, respectively, and N_t and N_0 are the average numbers of parental His⁻ cells on SEM, at the end and the beginning of incubation, respectively. The standard deviation of mutation rate was calculated by multiplying μ by the following factor:

$$\sqrt{\left(\frac{m}{M_{SEM} - M_{MM}}\right)^2 + \left(\frac{n}{N_t - N_0}\right)^2}$$

where m is the standard deviation of $M_{SEM} - M_{MM}$ and n is the standard deviation of $N_t - N_0$. The total number of parental cells on SEM plates was determined by punching out two pieces of medium, each about 1.1 cm diameter (0.95 cm^2), from the part of an SEM plate where no visible His+ colonies had grown, suspending the cells in 10 ml of buffer by shaking vigorously, and counting the number of recovered cells with a light microscope and a bacterial counting chamber. The total number of parental His⁻ cells on the plate was then determined by multiplying (ratio of the total surface area of the SEM plate [56.5 cm^2] to the surface area removed) × (number of cells recovered from the plugs of agar).

UV irradiation: Samples of about 2×10^8 cells/ml in phosphate buffer (half-strength 56 medium) were irradiated in a petri dish at 75 cm by the light of two G.E. 15-W germicidal lamps. UV dose was measured either with a Thoshiba UV meter, which was calibrated against the standard carbon filament lamp (U.S. National Bureau of Standards) using an Eppley thermopile coupled with a DC amplifier (Beckman Model 14), or with a standard phage T4 survival curve (W. HARM, personal communication). The low dose rates used for UV-sensitive strains were attained by placing a diaphragm between lamps and cell suspension or using a motor-driven rotating sector in combination with the diaphragm.

Detection of UV mutagenesis: The method for detecting UV mutagenesis was basically that described by GREEN, ROTHWELL and BRIDGES (1972). Cultures were grown overnight with aeration at 37° in L broth. The cells were harvested by centrifugation, washed twice with, and resuspended in the original volume of half-strength minimal salts buffer 56 (*i.e.*, 56/2). They were then incubated without aeration at 37° for 1 hr, following which they were again harvested by centrifugation and resuspended in the original volume of 56/2. A sample of the suspension was diluted ten-fold to a cell concentration of about 2×10^8 cells/ml. Appropriate volumes of this cell suspension were irradiated with UV light. In most cases, samples were withdrawn for plating after each incremental exposure of the cells to UV radiation. Consecutive exposures were given to one suspension to increase the dose. For highly sensitive cells, separate samples were irradiated for different lengths of time. The cells from each sample were harvested by centrifugation and were resuspended in a small volume of 56/2 medium so that the concentration of surviving cells would by 10^7 to 10^8/ml.

In all cases, samples were plated on SEM medium, both to count the survivors and to determine the number of His+ revertants. This was possible because SEM medium contains a small amount of histidine by virture of the casamino acid supplement used. At high dilutions, used for counting survivors, the few His⁻ cells on the plate are capable of multiplying sufficiently to

produce visible colonies. At the low dilutions used for detecting His+ revertants, the histidine is exhausted by the very large numbers of His⁻ cells present before any one cell can produce a visible colony. Thus, only the His+ clones will be seen and counted. Plates were incubated at 37° for about three days before the visible colonies were counted. The frequency of induced mutations (number per 10^7 survivors) was obtained by subtracting the average number of spontaneous His+ mutants per plate (M_0, the His+ revertants found with unirradiated cells) from the average number per plate found after a given dose (M_{uv}), divided the difference by the average number of survivors plated (N_s) and multiplying by 10^7: $F_m = 10^7(M_{uv} - M_0)/N_s$.

Quantitative and qualitative significance of mutagenesis results: Several factors affect the quantitative accuracy of UV mutagenesis results. To begin with, there are three artifacts that stem from the different densities of cells plated (*i.e.*, number of cells per plate) to measure the number of survivors and the number of revertants to prototrophy of a given strain. The first was detected with the *lon*⁻ strain *E. coli* B (WITKIN 1967). Filamentation was observed when cells of this strain were UV irradiated and plated at cell densities less than 2×10^7 per plate. At higher densities (required for detection of Trp+ revertants), filamentation was inhibited. Since filamentation is inversely correlated with viability, the result was an overestimation of the number of mutants per survivor. Although this cell density artifact is not operative in *lon*+ *E.coli* K12 strains (WITKIN 1976), the effects of a *recF* mutation were not known. To test the influence of *recF143* on filamentation in a *lon*+ *E.coli* K12 background, we performed an experiment suggested by E. WITKIN. We examined by light microscopy (640 times magnification), the structure of microcolonies following exposure to UV irradiation of 10^6 cells of four strains spread separately on L plates and incubated at 37° for 3 hr. We found that *uvrA*+ *recF*⁻ and *uvrA*⁻ *recF*⁻ cells formed fewer filaments after 4 J/m² and 0.4 J/m² of irradiation, respectively, than did their *recF*+ counterparts. Thus we assume that errors due to the first cell density artifact are minimal.

The second and third artifacts relate to the amount of amino acid enrichment (per cell plated) included in the medium selection for His+ revertants (GREEN, ROTHWELL and BRIDGES 1972). To avoid these we tested the influence of cell density and amino acid concentration on mutant frequencies observed when AB1157 is UV irradiated with 15 J/m². With an enrichment of 0.4 ml of a 10% solution of casamino acids per liter of selective medium, from 38 to 42 His+ revertants per 10^7 survivors were observed in the density range from 3×10^6 to 1×10^8 cells per plate. Similar independence of UV-induced mutant frequency on cell density was observed with the *uvrA*⁻ strain JC3912 at the same amino acid concentration. By testing different levels of casamino acid supplementation, we found that 0.4 ml of a 10% solution added per liter of selective medium was the minimum level of amino acid enrichment required to produce the maximum number of revertants when 2×10^7 cells of AB1157 (irradiated with 30 J/m² of UV-radiation) were plated on each plate.

There is also a question about whether UV-induced mutant frequencies are more significantly to be considered a function of fluence or of surviving cell fraction (SMITH 1976). Following convention, we have interpreted our mutagenesis data as a function of fluence.

A factor which influences the apparent spontaneous mutation rate is the low viability of *recB*⁻ *recC*⁻ strains. Inviable cells of these strains, although unable to form colonies, nonetheless go through two to three generations before ceasing to multiply (CAPALDO, RAMSEY and BARBOUR 1974). These cells, although unable to form His+ revertants following spontaneous mutation, will inflate the denominator (*i.e.*, the number of bacterial divisions) and will lead to an underestimation of spontaneous reversion rates. A study of this underestimation and derivation of an appropriate compensating factor is underway. At present we think that $1/f(2\text{-}f)$ is an appropriate factor, where f is the fraction of viable cells. Thus when $f = 0.2$ (as for the *uvrA*⁻ *recB*⁻ *recF*⁻ strain, which showed the lowest viability of the *uvrA*⁻ strains we used), the corrected mutation rate would be about 2.7 times the one calculated.

Assay for survival and mutation of phage lambda: The frequencies of infective centers producing mixed bursts of wild type and clear-plaque mutant phage were measured by the method described by ICHIKAWA-RYO and KONDO (1975). In essence, infected cells are plated on the indicator AB1886 to measure the total number of infective centers and on the indicater NO483 to

detect infective centers producing one or more clear-plaque mutants. AB1886 is used because it is excision-repair deficient (*uvrA6*) and unable to repair many of the lesions in unadsorbed UV-irradiated lambda phage particles which have not been inactivated by the treatment with anti-λ serum which precedes the plating step. NO483 is a special strain, characterized by ROLFE *et al.* (1973), and is lysogenized by λ*c*+ phages with 100% efficiency. Hence, only λ*c* mutants will multiply and produce plaques. This technique basically differs from that of WEIGLE (1953) only in the use of NO483 to detect the infective centers, which produce clear-plaque mutants. For the WEIGLE method, both clear and turbid plaque-forming infective centers were determined by plating on AB1886. Only about 15% as many clear plaques are seen on AB1886 as on NO483. The remaining 85%, although containing λ*c* phage, appear turbid because of the large number of λ*c*+ phage produced in the original infective center.

In detail, the experiments were performed as follows. Bacteria were grown overnight in Lambda broth at 37°. A sample was diluted ten-fold in the same medium, and the suspension was incubated with aeration for 60 min at 37°. The cells were then harvested by centrifugation and resuspended in the same volume of 56/2 medium containing 0.01 M $MgSO_4$. A sample was irradiated with UV light. To this irradiated sample, or to the unirradiated remainder, a suspension of pre-irradiated *c*+ phage was added to a multiplicity of about 0.1 phage per cell. After 15 min of incubation at 37° to permit infection, anti-lambda serum was added to a K value of 1. Incubation was continued for an additional 10 min to allow inactivation of unadsorbed phage. Samples of appropriate dilutions were plated with indicator bacteria on fresh day-old L-broth agar plates. On each plate, 0.1 ml of an L-broth culture of AB1886 (aerated overnight at 37°) was used as the indicator to determine the titer of infective centers and the titer of infective centers producing unmixed bursts of clear-plaque mutants. On each plate, 0.2 ml of a two-fold concentrated L-broth culture of NO483 (aerated overnight at 30°) was used as an indicator of the infective centers which produced at least one clear-plaque mutant.

RESULTS

Sensitivities to UV-irradiation and recombination proficiencies of uvr+ *strains*

The UV sensitivities of the excision-repair-proficient strains are shown in Part (a) of Figure 1. *recB* and *recF* single mutations each reduce the survival of *E. coli*. The double mutant is much more sensitive than either single mutant; analysis of the graphs by the method of BRENDEL and HAYNES (1973) shows that the two mutations act synergistically in producing their effects. This has given rise to the hypothesis that there are two partially overlapping pathways of repair in which the *recB* and *recF* genes act separately (ROTHMAN, KATO and CLARK 1975). Meanwhile, it should be noted that, although it is very sensitive to UV radiation, the *uvr*+ *recB*− *recF*− strain is not as sensitive as a *uvr*+ *recA* mutant. Presumably there is some repair capacity in a *uvr*+ background that is blocked by the *recA* mutation, but is not blocked by the *recB* and *recF* mutations together.

This parallels the small amount of recombination found in the *recB*− *recF*− strain and not found in the *recA* mutant. The recombination-deficiency indices of both strains are presented in Part A of Table 2. Both *recA*− and *recB*− *recF*− strains produce about 10^{-4} as many transconjugants as the related *recA*+ *recB*+ *recF*+ strain from a conjugational cross with an Hfr strain. Analysis of the transconjugant progeny of Hfr × *recA*− crosses was carried out originally by Low (1968) and more recently by GUYER and CLARK (1976). Essentially all (*i.e.*, 99%) of these progeny are formed by repliconation rather than by recom-

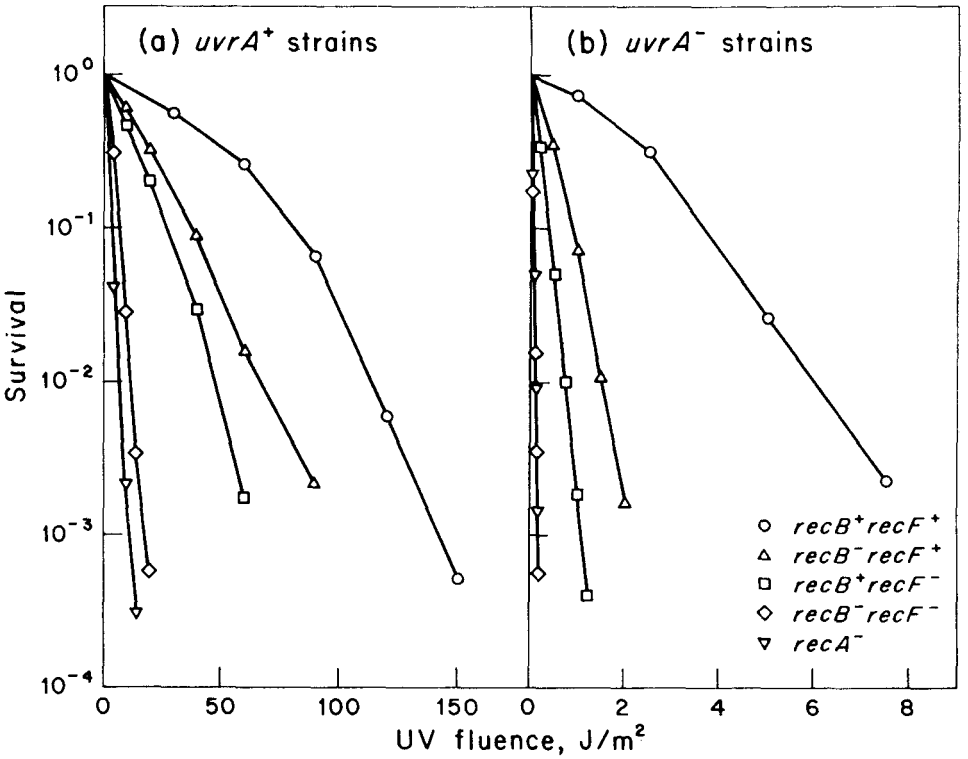

FIGURE 1.—Survival of syngenic *rec*⁻ strains after various fluences of UV radiation. An overnight culture of 2×10^6 cells/ml starved in 56/2 buffer was irradiated with various fluences of UV radiation. After appropriate dilution, 0.1 ml samples of the irradiated cells were plated on SEM and incubated for 48 hr at 37° (a) *uvrA*+ strains and (b), *uvrA*⁻ strains. Symbols: ○, (a) JC3872 and (b) JC3912; □, (a) JC3875 and (b) JC3913; △, (a) JC3878 and (b) JC3914; ◇, (a) JC3881 and (b) JC3915; ▽ (a) AB2463 and (b) AB2480.

bination, and inherit donor genes on F′ plasmids. Analysis of the transconjugant progeny of Hfr and F⁻ *recB*⁻ *recF*⁻ strains shows that the few that are formed inherit donor genes in a stable fashion and that no F genes are detectable (A. J. CLARK, M. S. GUYER, L. MARGOSSIAN and R. A. SKURRAY, unpublished results). Presumably the donor genes are not inherited by repliconation of F′ plasmids, but are formed by residual, presumably *recA*-dependent recombination. In experiments involving recombination following transduction and transformation, the deficiency of *recB*⁻ *recF*⁻ strains has been found to approximate that of *recA*⁻ strains (T. KATO, A. TEMPLIN and A. J. CLARK, unpublished results). On this basis, we are assuming that most *recA*-dependent recombination has been blocked by a combination of *recB* and *recF* mutations.

Production of His⁺ *revertants in* uvr⁺ *strains*

The rates of spontaneous production of His⁺ revertants were determined for *recB*⁺ *recF*⁺, *recB*⁻ *recF*⁺, *recB*⁺ *recF*⁻ and *recB*⁻ *recF*⁻ derivatives of *uvr*⁺ genotype. The rates in units of mutations per 10^9 cells per division were 4.1 ± 1.9,

TABLE 2

Characteristics of uvrA+ *and* uvrA- *strains*

Strain	Genotype *recA*	*recB*	*recF*	*uvrA*	Recombination deficiency index*	UV sensitivity† D_{37} (J/m²)
Part A: *uvrA*+ *uvrB*+ strains						
JC3872	+	+	+	+	1.0	48
JC3875	+	+	—	+	1.1	12
JC3878	+	—	+	+	2.3×10^2	17
JC3881	+	—	—	+	8.5×10^3	4
AB2463	—	+	+	+	1.0×10^4	2
Part B: *uvrA*- *uvrB*+ strains						
JC3912	+	+	+	—	1.0	2.2
JC3913	+	+	—	—	1.1	0.2
JC3914	+	—	+	—	2.0×10^2	0.5
JC3915	+	—	—	—	7.6×10^3	0.02
AB2480	—	+	+	—	N.T.	0.02

* A deficiency index is determined by dividing the frequency at which Thr+ Leu+ [Sm^R] progeny are produced (in a cross of JC158 with a Rec+ recipient) by the frequency at which those progeny are produced in a cross of the same donor with a Rec- recipient. N.T. stands for not tested.

† D_{37} is the fluence at which there is 37% survival. The D_{37} values were deduced from the survival curves measured on SEM (Figure 1).

2.3 ± 1.6, 11.0 ± 5.4 and 4.6 ± 1.0, respectively. We conclude that in a *uvr*+ background, the *recB* and *recF* mutations do not significantly affect the spontaneous reversion rate to His+.

The results of UV mutagenesis experiments in which His+ revertants are detected are shown in Figure 2. The results were obtained from several separate experiments and have been analyzed by statistical methods. The line best fitting the data was determined by linear regression and is shown in Figure 2. The slopes of the lines are listed in Table 3, together with their 95% confidence

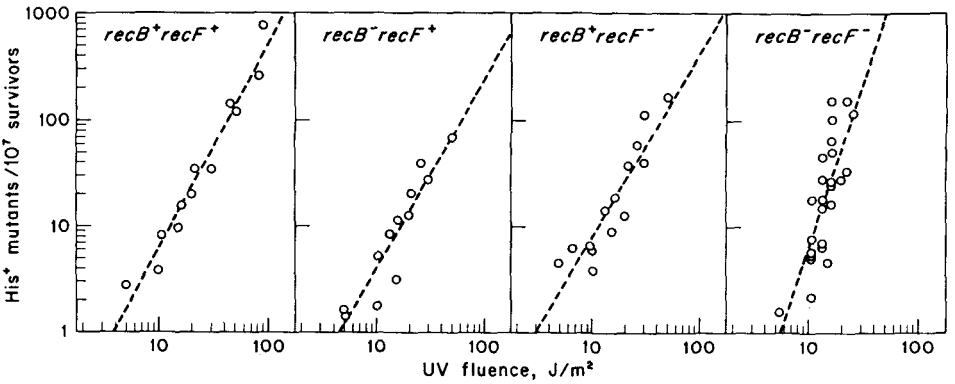

FIGURE 2.—Dependence of His+ revertant frequency on UV fluence: *uvrA*+ *uvrB*+ strains (JC3872, JC3875, JC3878 and JC3881). The results of several experiments are shown for each strain. The dashed lines represent the best straight line determined by linear regression analysis.

TABLE 3

Parameters of the best straight lines relating His+ mutants produced to UV fluence in uvrA+ uvrB+ *strains*

Strains						
uvr	*recB*	*recF*	Slope*	$Sy{\cdot}x$†	r‡	Calculated fluence (J/m²) to obtain 50 His+ per 10^7 survivors
+	+	+	1.93 ± 0.29	0.17	0.98	29
+	—	+	1.78 ± 0.46	0.21	0.93	40
+	+	—	1.73 ± 0.48	0.22	0.92	28
+	—	—	3.12 ± 1.04	0.35	0.94	19
—	+	+	1.72 ± 0.17	0.09	0.99	0.84
—	—	+	2.02 ± 0.26	0.10	0.98	1.3
—	+	—	1.63 ± 0.28	0.17	0.96	0.47
—	—	—	0.64 ± 0.98	0.15	0.64	0.18

* The 95% confidence interval for the slope has been calculated by multiplying the standard error of the slope (S_b) by $t_{0.05}$ obtained from a standard t table.

† $S_{y \cdot x}$ is an estimate of the standard deviation of the values of Y (*i.e.*, the $\log_{10}$ of the number of mutants per 10^7 survivors) for a given X (*i.e.*, the $\log_{10}$ of dose). It is calculated as the deviation of the differences between observed number of mutants and the number calculated from the best lines fitting all the points.

‡ r, the correlation coefficient, is the ratio of two standard deviations. The numerator is the standard deviation of the calculated values of Y at each X while the denominator is the standard deviation of the observed values of Y at each X. If the ratio is near 1.0, then an increase in mutants per 10^7 survivors is well correlated with an increase in fluence. If the ratio is close to zero then an increase in fluence will not result in an increase in mutants per 10^7 survivors.

intervals. The slope of the log of the mutant frequency *vs.* the log of fluence is about 2. Thus, a 10-fold increase in the fluence of UV light applied to the *recB*+ *recC*+ strain will increase by about 100-fold the number of His+ mutants per survivor. This is typical for UV radiation (WITKIN and GEORGE 1973). Neither *recB* nor *recF* mutations affect this relationship, within experimental error. The combination of *recB* and *recF* mutations, which blocks nearly all *recA*-dependent recombination, does not block the mutagenic effect of UV radiation. In fact it appears that the effectiveness of UV radiation is amplified by these mutations because the slope of the curve in Figure 2 is at least 3.0, indicating a thousand-fold increase in the number of mutants for a ten-fold increase in fluence.

The main point to be gained from these experiments is that while the *recB recF* double mutant lacks nearly all *recA*-dependent recombination, it is nonetheless highly UV mutable. This point is emphasized by the results in Table 4, which were obtained by testing *recB*⁻ *recF*⁻ and *recA*⁻ strains for their UV mutability. We found no increase in the numbers of His+ revertants per 10^7 survivors with increasing fluence given to the *recA* mutant whereas we found a substantial increase with the *recB recF* double-mutant strain.

The influence of uvrA *and* uvrB *mutations on mutagenesis in* recB *and* recF *mutants*

uvrA and *uvrB* mutations block excision repair of pyrimidine dimers formed in DNA during UV irradiation (HOWARD-FLANDERS 1968). Possibly as a result

TABLE 4

Yield of His+ revertants following UV irradiation of uvr+ recA- *and* uvr+ recB- recF- *strains*

Strain	UV fluence J/m²	Concentration factor*	Survivors per ml	Average number His+ mutants/plate†	UV-induced mutant frequency (per 10^7 survivors)
	0	1	1.8×10^8	9.2	—
JC3881	108	5	1.1×10^8	15	5.3
recB- recF-	136	10	4.5×10^7	26	37
	162	20	4.2×10^7	60	120
	0	1	3.6×10^8	3	—
AB2463	54	2	4.2×10^7	<1	<2
recA-	108	10	7.2×10^7	<1	<1
	136	20	4.0×10^7	<1	<2
	162	20	1.2×10^7	<1	<6

* Cells were irradiated at 2×10^8/ml and were concentrated before plating by centrifugation and resuspension in an amount of medium equal to the original volume divided by the concentration factor.

† 0.1 ml of the concentrated suspension of irradiated cells was spread on each plate.

of this, *uvrA* and *uvrB* mutations also decrease dramatically the fluences of UV required to produce mutagenesis, W-reactivation and induction of lambda phage and protein X (RADMAN 1975). In order to determine the effect of *recB* and *recF* mutations on the occurrence of His+ reversions, we constructed derivatives of *uvrA-* and *uvrB-* strains which were *recB- recF+*, *recB+ recF-* and *recB- recF-*. Spontaneous reversion rates were, respectively: 3.5 ± 1.8, 15.8 ± 4.2 and 3.7 ± 2.3 mutations per 10^9 cells per division. The *recB+ recF-* strain showed a small but significant increase in reversion rate from the *recB+ recF+* strain (5.3 ± 1.3 mutations per 10^9 cells per division), indicating a slight mutagenic effect of the *recF* mutation in the *uvrA-* genetic background.

The sensitivities of these strains to UV light are shown in Figure 1B. By using the analysis of BRENDEL and HAYNES (1973), it can be seen that the *recB* and *recF* genes interact synergistically to produce the resistance of the *recB+ recF+* strain. In the *uvr+* background, the combination of *recB* and *recF* mutations mimics the *recA* mutation in reducing recovery of UV irradiated cells. Consistent with this, it has been found that *uvrA-* or *uvB-* derivatives of *recB- recF-* strains show no detectable postreplication repair of UV damage to their DNA (R. H. ROTHMAN and A. J. CLARK, unpublished results). Repair has not been detected with either *uvrA- recB+ recF-* or *uvrB- recB+ recF-* strains (GANESAN and SEAWELL 1975; R. H. ROTHMAN and A. J. CLARK, unpublished results).

Since the recombination deficiency of the *uvrB- recB- recF-* strain also seems almost to equal that of the *recA-* strain (Table 2B), we are in a position to determine whether UV mutagenesis can occur in the virtual absence of recombination and repair. The results are shown in Figure 3. The data for both *uvrA-* and *uvrB-* derivatives, with each combination of *rec* alleles, are plotted together. The best line fitting the points has been calculated by linear regression analysis, and the parameters of these lines are shown in Table 3. In three cases there is an excellent

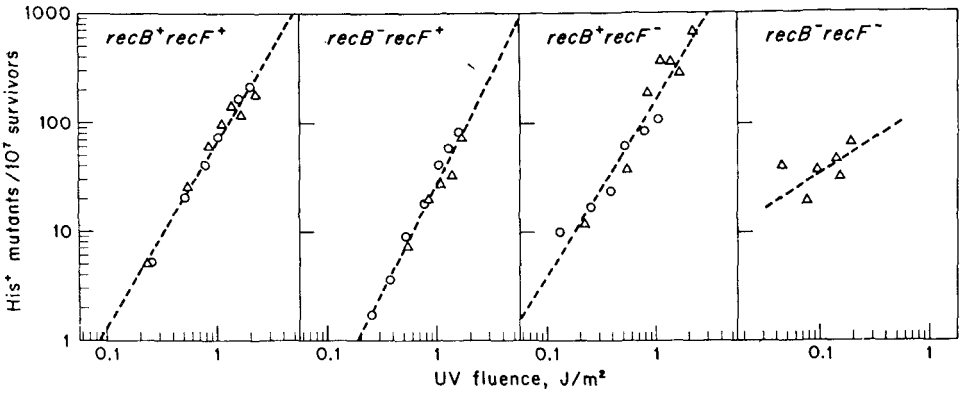

FIGURE 3.—Dependence of His+ revertant frequency on UV fluence. ○, *uvrA⁻ uvrB+* strains (JC3912, JC3913, JC3914, JC3915); △, *uvrA+ uvrB⁻* strains (JC3890, JC3893, JC3896, JC3899). See legend of Figure 2 for further information.

positive correlation between increase in UV fluence applied to the cells and increase in the number of induced His+ revertants; these are the *recB+ recF+*, *recB⁻ recF+* and *recB+ recF⁻* strains. In each, the slope of the best line is 2 or nearly so; thus, no change in slope was produced by the addition of the *uvr* mutation. The *uvr* mutation has, however, lowered the calculated fluences required to produce 50 His+ revertants per 10^7 survivors to about one-thirtieth or one-sixtieth of that calculated for the respective *uvr+* strains. The important point is that neither the *recB* nor the *recF* single mutations markedly affect the fluence reduction for a given amount of mutagenesis that is characteristic of *uvr⁻* strains.

The effects of combined *recB*, *recF* and *uvr* mutations are less clear. The data we have obtained are plotted in Figure 3, and the parameters are listed in Table 3. There is a weak positive correlation between increase in fluence and increase in the number of His+ revertants in excess over those produced spontaneously. There is also considerable scatter in the points. These two features are probably explained by the extreme UV sensitivity of *uvrB⁻ recB⁻ recF⁻* strains. It takes less than three dimers per genome to kill a cell of this genotype (ROTHMAN, KATO and CLARK 1975; R. H. ROTHMAN and A. J. CLARK, unpublished results). Thus, His+ revertants produced at high fluences would be killed, while at low fluences, consonant with measurable survival, so few additional His+ revertants would be formed that the error in determining their number would be great. As a result, we prefer not to draw any conclusions about the UV mutability of the *uvrB⁻ recB⁻ recF⁻* strain with these data, although they are consistent with a normal mutability.

Mutagenesis of phage lambda in uvr+ *and* uvr⁻ *strains*

The effectiveness of UV radiation as a mutagen can be measured by looking for clear-plaque mutants of phage lambda as well as by looking for His+ revertants. We irradiated lambda phage and infected unirradiated or UV-irradiated

TABLE 5

Direct mutagenesis of lambda phage as detected by determining the percent of infective centers which produce unmixed bursts of clear-plaque mutants when the host has been UV-irradiated

Strain	*uvr*	*recA*	*recB*	*recF*	UV fluence to phage (J/m²)	UV fluences (J/m²) to host 0	5	100
JC3872	+	+	+	+	100	0.041	—	0.21
JC3878	+	+	*21*	+	100	0.035	—	0.20
JC3875	+	+	+	*143*	100	0.033	—	0.036
JC3881	+	+	*21*	*143*	100	0.032	—	0.038
AB2463	+	*13*	+	+	100	0.015	—	0.019
JC3912	*A6*	+	+	+	30	0.045	0.29	—
JC3914	*A6*	+	*21*	+	30	0.027	0.24	—
JC3913	*A6*	+	+	*143*	30	0.041	0.039	—
JC3915	*A6*	+	*21*	*143*	30	0.022	0.035	—
AB2480	*A6*	*13*	+	+	30	0.015	0.019	—

cells. The clear-plaque mutants were detected either by the method of WEIGLE (1953) or by that of ICHIKAWA-RYO and KONDO (1975). WEIGLE's method detects the small fraction of mutants that result from unmixed bursts, while ICHIKAWA-RYO and KONDO's method detects the mutants that occur in mixed as well as unmixed bursts.

Table 5 shows a five-fold increase in the number of unmixed bursts of clear-plaque mutants produced by irradiating the *uvr*$^+$ *recB*$^+$ *recF*$^+$ strain with 100 J/m². A single *recB* mutation has no effect on this UV-produced increase, while a single *recF* mutation blocks it completely. The UV-induced increase in unmixed bursts is also blocked in the *uvr*$^+$ *recB*$^-$ *recF*$^-$ mutant and in the *recA* single mutant. The results of the second measurement of lambda mutagenesis can be seen in Figure 4A. The *uvr*$^+$ *recB*$^+$ *recF*$^+$ strain exhibits an approximately seven-fold increase in the percent of infective centers producing at least one λ*c* mutant as a result of UV irradiation of 100 J/m² to both phage and cells. The *recF* single mutant exhibits an even greater increase. Based on the *recF*$^-$ inhibition of induced mutation observed with the WEIGLE test this seems unexpected. However, the frequency of infective centers producing at least one λ*c* mutant, detected by N0483, is several-fold higher than the frequency producing unmixed bursts detected by the WEIGLE method. Thus the *recF* block in the mutagenesis leading to unmixed bursts cannot be seen when using N0483 because the reduction in the number of clear plaques is negligible. Not even a combination of *recB* and *recF* mutations can block the stimulation of the production of λ*c* mutants. By contrast, a *recA* mutant shows only a two to three-fold stimulation over the same UV dose range.

Experiments on UV mutagenesis of lambda were also performed with the *uvrA*$^-$ strains. The data for the production of unmixed bursts of λ*c* mutants are shown in Table 5, and for the production of λ*c* mutants in mixed bursts in Figure 4B. As was the case with *uvrA*$^+$ strains, the *recF* mutation mimicked the

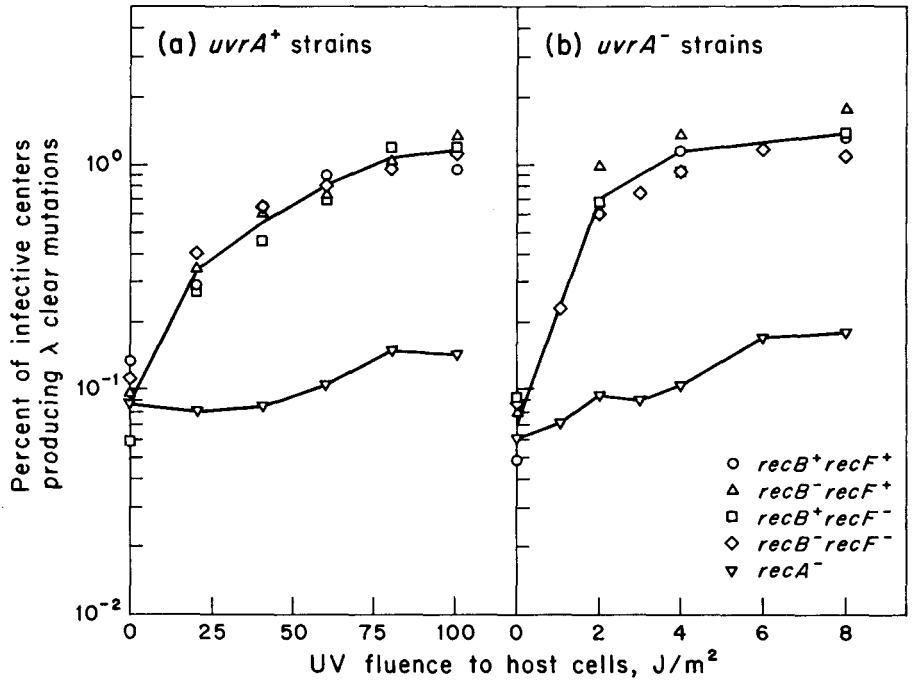

FIGURE 4.—Frequencies of induced λ*c* mutations plotted against UV fluence to host cells. Host cells carrying various *rec* and *uvr* alleles were prepared as described in the text, irradiated with the indicated UV fluences, and subsequently infected with λ*c*+ phages irradiated with UV fluences of 30 and 100 J/m² for *uvrA*⁻ and *uvrA*+ strains, respectively. After inactivation of unadsorbed phages by antiserum, the infective centers were assayed for clear-plaque mutants and survivors with indicator strains NO483 and AB1886, respectively, as described in the text. Mutant frequencies are ratios of clear-plaque titers on NO483 to total titers on AB1886. Each point is the average of three experiments. (a) *uvrA*+ strains; (b) *uvrA*⁻ strains. Symbols: ○, (a) JC3872 and (b) JC3912; □, (a) JC3875 and (b) JC3913; △, (a) JC3878 and (b) JC3914; ◇, (a) JC3881 and (b) JC3915; ▽, (a) AB2463 and (b) AB2480.

recA mutation in blocking the UV-induced production of unmixed bursts of λ*c*⁺ phage. There was no such effect of the *recF* mutation on the production of λ*c* mutants in mixed bursts in the *uvrA*⁻ strains. More importantly, the *uvrA*⁻ *recB*⁻ *recF*⁻ strain showed as much increase in the frequency of λ*c* mutants in mixed bursts with increase in UV fluence to the cells as did the wild-type and single-mutant strains.

DISCUSSION

There are three major questions concerning UV mutagenesis: (1) what enzyme or enzymes produce the mutated base sequences; (2) how are these enzymes regulated, with respect to both their synthesis and their activities, and (3) in which realm of DNA metabolism does each of the mutagenic enzymes act, *i.e.*, do the substrates for the enzymes occur at stages of replication, repair, recombination, transcription or some other realm. In this study the third question

is the main focus, although the results lead to some hypotheses concerning the other two as well.

Mainly we have been interested in determining whether or not the mutagenic enzymes act in the realms of *recA*-dependent recombination and *recA*-dependent repair of UV-induced damage. In order to do this, we have determined the frequency of UV-induced mutations in a *uvr*$^+$ *recB*$^-$ *recF*$^-$ strain, which lacks the ability to perform *recA*-dependent recombination, and in *uvr*$^-$ *recB*$^-$ *recF*$^-$ strains, which for the most part lack both *recA*-dependent repair and recombination abilities. We found the *uvr*$^+$ *recB*$^-$ *recF*$^-$ strain to be highly UV mutable when we assayed induced His$^+$ revertants; in fact, increments of UV radiation were more efficient in producing increments in the number of mutants in this strain than in the wild type. This greater efficiency may result either from a basic change in the underlying mechanisms of UV mutagenesis or from a difference in the relative growth rate of mutants and surviving nonmutants (ECKHARDT and HAYNES 1977). Nevertheless, it appears that the mutagenic enzymes can act effectively in the absence of most *recA*-dependent recombination. The *uvr*$^-$ *recB*$^+$ *recF*$^-$ strains were normally UV mutable, thereby apparently indicating that the mutagenic enzymes can act effectively in the absence of *recA*-dependent post-replication repair. The *uvr*$^-$ *recB*$^-$ *recF*$^-$ strain was so sensitive to the lethal action of UV light that it was impossible to determine accurately the numbers of His$^+$ revertants included. The data were consistent with UV mutability, but were not conclusive.

As a result we turned to experiments in which UV-induced mutations were detected to phage lambda as clear-plaque mutants. We used two different techniques so as to determine both the percent of infective centers producing an unmixed burst of clear-plaque mutants and the percent of infective centers producing at least one clear-plaque mutant. With regard to the frequency of infective centers producing at least one clear-plaque mutant (Figure 4), the *uvr*$^-$ *recB*$^+$ *recF*$^-$ and *uvr*$^-$ *recB*$^-$ *recF*$^-$ strains were similar, both when unirradiated and when UV-irradiated cells were used. UV-produced stimulation of the frequency of *unmixed* bursts of λc mutants was greatly reduced in the triple mutant, however. This reduction was not characteristic of the combination of the three mutations because it was also a characteristic of the *recF* single mutant. It should be noted that *unirradiated cells* of the *recF* single mutant or the *uvr*$^-$ *recB*$^-$ *recF*$^-$ triple mutant showed the same ability to produce unmixed bursts of λc mutants as the wild-type strain; only the UV stimulation of this ability is affected by the *recF* mutation. Thus, the *uvr*$^-$ *recB*$^-$ *recF*$^-$ strain shows normal *uvr*$^-$ levels of mutagenesis in three of the four tests applied. It is therefore tempting to conclude that the combination of *uvr*, *recB* and *recF* mutations is without major effect on the UV-mutagenic processes of *E. coli*.

This conclusion would not be justified, however, because lambda phage is not necessarily a neutral indicator of UV mutagenesis. It may carry genes and determine products that affect UV mutagenesis by either substituting for inactive host products or by passing the blocked steps. Lambda carries the *redX* gene, for

instance, which determines an exonuclease that we have called *Lam*ExoVIII in accord with a convention in the nomenclature of deoxyribonucleases (MILLER and CLARK 1976). This enzyme is functionally similar to the host exonuclease VIII (*Eco*ExoVIII), which is found in *sbcA* mutants of *E. coli*. *Eco*ExoVIII is thought to participate in *recA*-dependent recombination and repair by the RecE pathway (GILLEN and CLARK 1974). It therefore seems likely that *redX*$^+$ lambda-infected cells carry out *recA*-dependent recombination and repair processes alternative to those blocked by the *uvr*, *recB* and *recF* mutations. This has been found both for the repair of X-ray damage (RUPP, LEVINE and TRGOVCEVIC 1975) and UV damage (A. G. BRAUN, personal communication) when appropriate strains lysogenic for λ*cI857* derivatives are transiently induced by pulse-heating at 42°. Consequently, we cannot yet conclude that UV mutagenesis is independent of most *recA*-dependent recombination and repair.

There is another difficulty, deeper than that already discussed, in drawing firm conclusions about the realm of DNA metabolism in which mutagenic enzymes act. The *uvr*, *recB* and *recF* mutations may not act simply to block pathways in which the mutagenic enzymes might act. They may also affect the regulation of the enzymes by affecting either their synthesis or their activity. In fact, all three mutations are known to have regulatory effects. *uvr* mutations dramatically lower the UV fluence required to induce lambda (RADMAN 1975), protein X (D. SPENCER and W. GINOZA, personal communication) and W-reactivation (RADMAN and DEVORET 1971). A *recB* mutation blocks nalidixic acid-induction of protein X (D. SPENCER and W. GINOZA, personal communication), although it does not block UV induction of the same protein (D. SPENCER and W. GINOZA, personal communication), lambda (BROOKS and CLARK 1967) and W-reactivation (KERR and HART 1972). A *recF* mutation does not affect nalidixic acid induction of protein X, but it does block its induction by UV radiation (D. SPENCER and W. GINOZA, personal communication). *recF* mutations also reduce the inducibility of lambda (CLARK 1973; ROBERTS and ROBERTS 1975) and W-reactivation (R. H. ROTHMAN and A. J. CLARK, unpublished results), although induction is not completely blocked. Accordingly the data we have obtained may be interpreted as showing effects of *uvr*, *recB* and *recF* mutations on the regulation of the mutagenic enzyme or enzymes and not on the realm of DNA metabolism in which they find their substrates. Suppose, for example, that the mutagenic enzyme or enzymes actually act on replicational intermediates and not on recombinational or repair intermediates. Then the *recA* dependency of UV mutagenicity would stem from the necessity of the *recA*$^+$ product for mutagenic enzyme synthesis or activation. The inability of the *uvr*, *recB* and *recF* mutations to prevent the UV-stimulated increase in the number of infective centers producing λ*c* mutants would indicate *recA* specificity of the regulation of the mutagenic enzyme or enzymes. That there are at least two mutagenic enzymes would be indicated by the *recF* block in the UV stimulation of the number of infective centers producing unmixed bursts of λ*c* mutants without affecting the other mutational process of lambda. The *recF* block might also be

regulatory in nature, since *recF* mutation is known to block UV induction of protein X (D. SPENCER and W. GINOZA, personal communication). Perhaps protein X is responsible for the production of unmixed bursts of λ*c* mutants.

The authors are grateful to A. TEMPLIN for her assistance and to K. SMITH and E. WITKIN for their very constructive comments on the manuscript. ROBERT H. ROTHMAN was a predoctoral trainee supported by Public Health Service Predoctoral Training Grant 5T01-GM00367-14. This research was supported by Public Health Service Research Grant AI 05371 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

BACHMANN, B. J., 1972 Pedigrees of some mutant strains of *Escherichia coli* K12. Bacteriol. Rev. **36:** 525–557.

BACHMANN, B. J., K. B. LOW and A. L. TAYLOR, 1976 Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40:** 116–167.

BRENDEL, M. and R. H. HAYNES, 1973 Interactions among genes controlling sensitivity to radiation and alkylation in yeast. Molec. Gen. Genet. **125:** 197–216.

BROOKS, K. and A. J. CLARK, 1967 Behavior of λ bacteriophage in a recombination deficient strain of *Escherichia coli*. J. Virol. **1:** 283–293.

CAPALDO, F. N., G. RAMSEY and S. D. BARBOUR, 1974 Analysis of the growth of recombination-deficient strains of *Escherichia coli* K-12. J. Bacteriol. **118:** 242–249.

CLARK, A. J., 1973 Recombination deficient mutants of *E.coli* and other bacteria. Ann. Rev. Genet. **7:** 67–86.

CLARK, A. J., and A. D. MARGULIES, 1965 Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. Proc. Nat. Acad. Sci. U.S. **53:** 451–459.

DAVIS, B. D. and E. S. MINGIOLI, 1950 Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol **60:** 17–28.

DEFAIS, M., P. FAUQUET, M. RADMAN and M. ERRERA, 1971 Ultraviolet reactivation and ultraviolet mutagenesis of λ in different genetic systems. Virology **43:** 495–503.

ECKHARDT, R. and R. H. HAYNES, 1977 Kinetics of mutation induction by ultraviolet light in excision deficient yeast. Genetics **85:** 225–247.

GANESAN, A. K. and P. C. SEAWELL, 1975 The effect of *lexA* and *recF* mutations on post-replication repair and DNA synthesis in *Escherichia coli* K12. Molec. Gen. Genet. **141:** 189–205.

GILLEN, J. R. and A. J. CLARK, 1974 pp. 123–136. In: *Mechanisms in recombination*. Edited by R. F. GRELL. Plenum Press. New York.

GREEN, M. H. L., M. A. ROTHWELL and B. A. BRIDGES, 1972 Mutation to prototrophy in *Escherichia coli* K-12: effect of broth on UV-induced mutation in strain AB1157 and four excision-deficient mutants. Mutation Res. **16:** 225–234.

GUDAS, L. J. and A. B. PARDEE, 1975 Model for regulation of *Escherichia coli* DNA repair functions. Proc. Nat. Acad. Sci. U.S. **72:** 2330–2334.

GUYER, M. S. and A. J. CLARK, 1976 Cis-dominant transfer-deficient mutants of the F sex factor of *Escherichia coli* K12. J. Bacteriol. **125:** 233–247.

HERTMAN, I. and S. E. LURIA, 1967 Transduction studies on the role of a *rec*+ gene in the ultraviolet induction of prophage lambda. J. Mol. Biol. **23:** 117–133.

HORII, Z. I. and A. J. CLARK, 1973 Genetic analysis of the RecF pathway of genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. J. Mol. Biol. **80:** 327–344.

HOWARD-FLANDERS, P., 1968 DNA repair. Ann. Rev. Biochem. **37**: 175–200.

HOWARD-FLANDERS, P. and L. THERIOT, 1966 Mutants of *Escherichia coli* K12 defective in DNA repair and in genetic recombination. Genetics **53**: 1137–1150.

HOWARD-FLANDERS, P., L. THERIOT and J. B. STEDEFORD, 1969 Some properties of excision-defective recombination-deficient mutants of *Escherichia coli* K12. J. Bacteriol. **97**: 1134–1141.

ICHIKAWA-RYO, H. and S. KONDO, 1975 Indirect mutagenesis in phage lambda by ultraviolet preirradiation of host bacteria. J. Mol. Biol. **97**: 77–92.

KERR, T. L. and M. C. R. HART, 1972 Effects of the *rec* and *exr* mutations of *Escherichia coli* on UV reactivation of bacteriophage Lambda damaged by different agents. Mutation Res. **15**: 247–258.

KONDO, S., H. ICHIKAWA, K. IWO and T. KATO, 1970 Base-change mutagenesis and prophage induction in strains of *Escherichia coli* with different DNA repair capacities. Genetics **66**: 187–217.

LOW, B., 1968 Formation of merodiploids in matings with a class of Rec⁻ recipient strains of *Escherichia coli* K12. Proc. Nat. Acad. Sci. U.S. **60**: 160–167. ——, 1972 *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**: 587–607.

MILLER, R. V. and A. J. CLARK, 1976 Purification and properties of two deoxyribonucleases of *Pseudomanas aeruginosa* strain PAO. J. Bacteriol. **127**: 794–802.

MIURA, A. and J. TOMIZAWA, 1968 Studies on radiation-sensitive mutants of *E. coli* III: participation of the Rec system in induction of mutation by ultra-violet irradiation. Molec. Gen. Genet. **103**: 1–10.

MOUNT, D. W., K. B. LOW and S. J. EDMISTON, 1972 Dominant mutations in *Escherichia coli* K12 (*lex*) which affect radiation sensitivity and frequency of ultra-violet-induced mutations. J. Bacteriol. **112**: 886–893.

NISHIOKA, H. and C. O. DOUDNEY, 1969 Different modes of loss of photoreversibility of mutation and lethal damage in ultraviolet-light resistant and sensitive bacteria. Mutation Res. **8**: 215–228.

RADMAN, M., 1975 pp. 355–367. In: *Molecular mechanisms for repair of DNA*. Edited by P. C. HANAWALT and R. B. SETLOW. Plenum Press. New York.

RADMAN, M. and R. DEVORET, 1971 UV-reactivation of bacteriophage λ in excision repair-deficient hosts: independence of *red* functions and attachment regions. Virology **43**: 504–506.

ROBERTS, J. W. and C. W. ROBERTS, 1975 Proteolytic cleavage of bacteriophage lambda repressor in induction. Proc. Nat. Acad. Sci. U.S. **72**: 147–151.

ROLFE, R., J. SCHELL, A. BECKER, J. HEIP, K. ONODERA and E. SCHELL-FREDERICK, 1973 A colicin-tolerant mutant of *Escherichia coli* with reduced levels of cyclic AMP and a strong bias towards λ lysogeny. Mol. Gen. Genet. **120**: 1–16.

ROTHMAN, R. H., T. KATO and A. J. CLARK, 1975 pp. 283–291. In: *Molecular mechanisms in the repair of DNA*. Edited by P. C. HANAWALT and R. B. SETLOW. Plenum Press. New York.

RUPP, W. D. and P. HOWARD-FLANDERS, 1968 Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultra-violet irradiation. J. Mol. Biol. **31**: 291–304.

RUPP, W. D., A. D. LEVINE and Z. TRGOVCEVIC, 1975 pp. 307–312. In: *Molecular mechanisms for the repair of DNA*. Edited by P. C. HANAWALT and R. B. SETLOW. Plenum Press. New York.

SMITH, K. C., 1976 Ultraviolet radiation-induced mutability of *uvrD3* strains of *Escherichia coli* B/r and K-12: a problem in analyzing mutagenesis data. Photochem. and Photbiol. **24**: 433–437.

SMITH, K. C. and D. H. C. MEUN, 1970 Repair of radiation-induced damage in *Escherichia coli* I. effect of *rec* mutations on post-replication repair of damage due to ultraviolet radiation. J. Mol. Biol. **51**: 459–472.

WEIGLE, J. J., 1953 Induction of mutation in a bacterial virus. Proc. Nat. Acad. Sci. U.S. **39**: 628–636.

WILLETTS, N. S., A. J. CLARK and B. LOW, 1969 Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. **97**: 244–249.

WITKIN, E. M., 1967 Mutation-proof and mutation-prone modes of survival in derivatives of *Escherichia coli* B differing in sensitivity to ultraviolet light. pp. 17–55. In: *Recovery and repair mechanisms in radiobiology*. Brookhaven Symposium in Biology **20**. ——, 1969a Ultraviolet-induced mutation and DNA repair. Ann. Rev. Genet. **3**: 525–552. ——, 1969b The mutability toward ultraviolet light of recombination-deficient strains of *Escherichia coli*. Mutation. Res. **8**: 9–14. ——, 1975 Relationships among repair, mutagenesis and survival: overview. pp. 347–353. In: *Molecular mechanisms for repair of DNA*. Edited by P. C. HANAWALT and R. B. SETLOW. Plenum Press, New York. ——, 1976 Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. **40**: 869–907.

WITKIN, E. M. and D. L. GEORGE, 1973 Ultraviolet mutagenesis in *polA* and *uvrA polA* derivatives of *Escherichia coli* B/r: evidence for an inducible error-prone repair system. Genetics (suppl.) **73**: 91–108.

Corresponding editor: J. W. DRAKE