JOURNAL OF BACTERIOLOGY, Mar. 1969, p. 1129-1133
Vol. 97, No. 3
Copyright © 1969 American Society for Microbiology
Printed in U.S.A.

Dark Recovery Processes in *Escherichia coli* Irradiated with Ultraviolet Light

II. Effect of *uvr* Genes on Liquid Holding Recovery

ANN K. GANESAN AND KENDRIC C. SMITH

Department of Radiology, Stanford University School of Medicine, Stanford, California 94305

Received for publication 1 November 1968

The *uvr* mutations of *Escherichia coli* K-12 decrease the ability of cells to survive ultraviolet light (UV), to excise pyrimidine dimers from their deoxyribonucleic acid and to reactivate bacteriophage exposed to UV. The *rec* mutations decrease the ability of the cells to survive UV and to undergo genetic recombination. Certain *rec* mutations, including *recA1*, *rec-12*, *recA13*, and *rec-56*, are necessary for the expression of liquid-holding recovery (LHR), observed as an increase in colony-forming ability when irradiated cells are held in buffer in the dark. These *rec* mutations appear to act indirectly to permit the detection of LHR rather than to affect its occurrence directly. We have tested the effect of *uvr* markers on LHR in cells containing one of these *rec* mutations. Recombinants containing *rec-56* together with a *uvr* marker were constructed and tested for LHR. None of the 39 recombinants examined, carrying *uvrA6*, *uvrB5*, or *uvrC34*, showed LHR. Three *rec*$^-$ *uvr*$^-$ strains were also tested for photoreactivation. In all three, photoreactivation was observed, indicating that they contained detectable amounts of pyrimidine dimers. Our results are consistent with the idea that *uvr* mutations inactivate LHR, and suggest that LHR reflects excision-dependent repair of pyrimidine dimers.

Liquid holding recovery (LHR) is one type of dark recovery that occurs in *Escherichia coli* B after ultraviolet (UV) irradiation. It is characterized by an increase in the number of colony-forming units when the irradiated cells are held in buffer and plated at intervals on a complex medium such as nutrient agar (3, 10, 11, 17). Derivatives of *E. coli* K-12 show little or no recovery under such conditions unless they carry certain *rec* mutations (7). These mutations also have other phenotypic consequences, including decreases in resistance to UV radiation and in the capacity to undergo genetic recombination (4, 5, 13, 15).

Harm presented evidence suggesting that genes controlling host-cell reactivation (HCR) of phage might affect LHR (9, 18). Castellani et al. (2) and Jagger et al. (16) showed indirectly that LHR may reflect the repair of pyrimidine dimers. In *E. coli* K-12, HCR and the excision of pyrimidine dimers are both controlled by genes at three loci, *uvrA*, *uvrB*, and *uvrC* (1, 14). We therefore wished to determine the effect of mutations in these genes on LHR.

Because LHR cannot be detected readily in *rec*$^+$ derivatives of K-12 (7), it was necessary to examine bacteria which contained both a *rec* marker permitting the detection of LHR and the *uvr* mutation to be tested. Since *rec-56* appeared to be suitable for this purpose (7), an Hfr strain was used to transfer this marker into *uvr*$^-$ recipient strains. The resulting *rec*$^-$ *uvr*$^-$ recombinants were tested for LHR.

Although a strain containing *recA13* and a *uvrA* mutation had previously been studied and found to lack LHR (7, 12), we felt that the response of a single strain did not provide sufficient evidence for a relationship between LHR and the *uvr* genes. We therefore examined recombinants which carried *uvrB* or *uvrC* mutations and several more strains carrying the *uvrA* marker.

MATERIALS AND METHODS

Most of the materials and methods used have been described previously (7).

Bacterial strains. Table 1 lists the bacterial strains employed. We are grateful to John Folls for JC5088, to A. J. Clark for KL-16, to R. P. Boyce for AB2480, AB2497, AB2498, AB2499, and AB2500, and to E. Lederberg and J. Lederberg for W2252 and W4099.

TABLE 1. *Escherichia coli derivatives used*[a]

Designation	Mating type	Relevant genotype	Phenotype	Reference
W2252	Hfr_1 (Cavalli)		λ^s Met	22
W4099	Hfr_6		λ^s	—[b]
AB2480	F⁻	*recA13 uvrA6*	Pro Gal Str^r λ^s	13
AB2497	F⁻		Leu Arg His Pro Thr Thy Thi Lac Ara Gal Mtl Xyl Str^r $T6^r$ λ^s	14
AB2498	F⁻	*uvrC34*	Leu Arg His Pro Thr Thy Thi Lac Ara Gal Mtl Xyl Str^r $T6^r$ λ^s	14
AB2499	F⁻	*uvrB5*	Leu Arg His Pro Thr Thy Thi Lac Ara Gal Mtl Xyl Str^r $T6^r$ λ^s	14
AB2500	F⁻	*uvrA6*	Leu Arg His Pro Thr Thy Thi Lac Ara Gal Mtl Xyl Str^r $T6^r$ λ^s	14
JC5088	Hfr	*rec-56*	Thr Ilv Thi Spm^r	4
SR57	F⁻	*rec-56 uvrC34*	His Thr Leu Thi Arg Pro Lac Ara Gal Mtl Xyl $T6^r$ λ^s Str^r	
SR58	F⁻	*rec-56 uvrB5*	His Thr Leu Thi Arg Pro Lac Ara Gal Mtl Xyl $T6^r$ λ^s Str^r	
SR59	F⁻	*rec-56 uvrA6*	His Thr Leu Thi Arg Pro Lac Ara Gal Mtl Xyl $T6^r$ λ^s Str^r	
KL-16	Hfr		λ^s	4, 22

[a] Each genetic locus is designated by three lower case italicized letters (6, 22). All other symbols refer to phenotype. Arg, His, Ilv, Leu, Met, Pro, Thi, Thr, Thy, and Trp denote arginine, histidine, isoleucine and valine, leucine, methionine, proline, thiamine, threonine, thymine, and tryptophan, respectively (⁻ indicates a requirement; ⁺ no requirement); Ara, Gal, Lac, Mal, Mtl, and Xyl denote arabinose, galactose, lactose, maltose, mannitol, and xylose, respectively (⁻ indicates nonutilization; ⁺, utilization); T6, λ, Spm, Str, response to the phages T6 and λ, and to the antibiotics spectinomycin and streptomycin (^{r} indicates resistance; ^{s}, sensitivity); *rec* denotes genes affecting genetic recombination and UV sensitivity; *uvr* designates genes affecting host-cell reactivation and UV sensitivity.

[b] A. A. Richter, Ph.D. Thesis, Univ. of Wisconsin, Madison, 1959.

Figure 1 shows the origins and directions of chromosomal transfer of the Hfr strains, together with the locations of relevant markers.

LHR. Irradiated cells were held at 37 C in 67 mM sodium-potassium phosphate buffer, *p*H 7.0, and plated at intervals on yeast extract-agar (7).

Photoreactivation. Cells suspended in buffer (10^5 per ml) were exposed at room temperature (23 C) for 10 min to the light of two 15-w Westinghouse "Daylight" fluorescent bulbs (21). Portions (10 ml) of the cell suspensions were placed in the bottoms of 10-cm Pyrex petri dishes on a platform rotator, the surface of which was positioned 6 cm below the fluorescent bulbs. The tops of the petri dishes, inverted and filled with 10 ml of distilled water, were positioned over the bottoms to serve as filters.

RESULTS

Matings were made between JC5088 (an Hfr which injects *rec-56* as an early marker linked to *thyA*) and three *thyA⁻ uvr⁻* recipients, AB2498 (*uvrC34*), AB2499 (*uvrB5*), and AB2500 (*uvrA6*). Figure 1 indicates the linkage relationships of these markers. As a control, the same Hfr was also mated with a *thyA⁻ uvr⁺* recipient, AB2497.

From each mating Thy⁺ Ilf⁺ recombinants were selected by plating on minimal medium supplemented with threonine, leucine, histidine, proline, arginine, and thiamine. These were then tested for UV sensitivity, HCR, and genetic recombination. Recombinants which behaved as Rec⁻ HCR⁻ (and were therefore expected to be *rec-56 uvr⁻*) were tested for LHR. None of them showed significant LHR (Table 2). However, all of the Rec⁻ HCR⁺ recombinants from the control mating with AB2497 showed LHR (Table 2). Table 3 illustrates the amounts of LHR found in

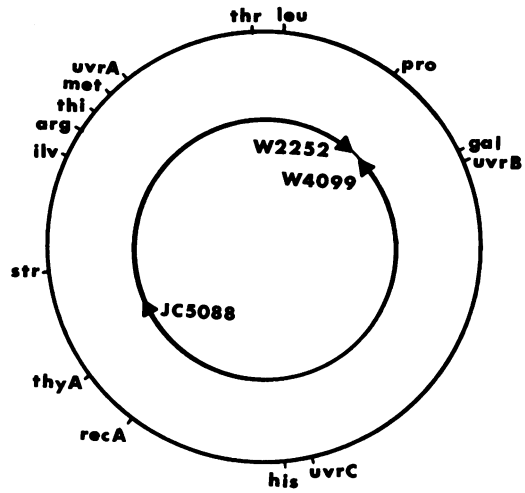

FIG. 1. *Diagram of the E. coli linkage map (4, 14, 22). Positions of various genetic markers are indicated on the outer circle. The arrowheads on the inner circle indicate the origin and direction of transfer of the Hfr strains used (4, 22; Richter, Ph.D. Thesis, Univ. of Wisconsin, Madison, 1959).*

representative Rec⁻ HCR⁻ recombinants compared to their parents. Those carrying *uvrA6* and *uvrB5* showed no recovery. The one containing *uvrC34* showed slight recovery but much less than its *uvr*⁺ *rec-56* parent.

If the *uvr*⁻ mutations were responsible for the failure of the *rec*⁻ *uvr*⁻ recombinants to undergo LHR, *rec*⁻ *uvr*⁺ progeny derived from them should show LHR. This expectation was tested by mating recombinants with *rec*⁺ *uvr*⁺ Hfr strains. In the first two series of matings, AB2480 (*recA13 uvrA6*) and SR59 (*rec-56 uvrA6*) were crossed with W2252. Selection was made for Pro⁺ Str^r progeny. In the third series, SR57 (*rec-56 uvrC34*) was crossed with W4099, and His⁺ Str^r progeny were selected. The recombinants obtained from each cross were tested for HCR and genetic recombination. Those which behaved as Rec⁻ HCR⁺ were tested for LHR. Although none of the parents of these matings showed LHR, all of their Rec⁻ HCR⁺ offspring did (Table 4). Matings were also made between SR58 (*rec-56 uvrB5*) and two *rec*⁺ *uvr*⁺ Hfr strains, W4099 and KL-16. However, since no Rec⁻ HCR⁺ progeny were found among 400 recombinants (Gal⁺ Str^r or Gal⁺ His⁺ Str^r) examined, none was tested for LHR.

The *rec-56 uvr*⁻ recombinants, like the *recA13 uvrA6* strain previously described (13), were more sensitive to UV than their *rec*⁻ *uvr*⁺ or *rec*⁺ *uvr*⁻ parents. Figure 2 shows the survival of three representative recombinants and their parent strains plated on yeast extract-agar immediately after exposure to UV.

Three *rec*⁻ *uvr*⁻ strains, AB2480, SR57, and SR58, were examined for photoreactivation as an indication of whether they contained detectable amounts of pyrimidine dimers (19). A 10-min exposure to visible light was used for photoreactivation because it yielded the largest amount of recovery from UV, although it caused up to a 50% decrease in the frequency of colony formers among *rec*⁻ *uvr*⁻ cells which had not been exposed to UV. As Table 5 shows, all three *rec*⁻ *uvr*⁻ derivatives could be photoreactivated not only immediately after exposure to UV but also after 20 hr of holding under conditions favoring LHR.

DISCUSSION

Derivatives of *E. coli* K-12 containing a mutation in any of the genes *uvrA*, *uvrB*, or *uvrC* showed little or no LHR, even though they also carried the marker *rec-56* to permit the expression of this type of recovery. The *rec-56 uvr*⁺ clones tested all showed LHR and were also more re-

TABLE 2. *LHR in progeny from matings between JC5088 and various recipient strains*

Recipient	Genotype of progeny	Phenotype of progeny		
		LHR⁺	LHR⁻	Total tested
AB2500	*rec-56 uvrA6*	0	7	7
AB2499	*rec-56 uvrB5*	0	21	21
AB2498	*rec-56 uvrC34*	0	11	11
AB2497	*rec-56 uvr*⁺	15	0	15

TABLE 4. *LHR in progeny obtained from crossing rec-56 uvr*⁻ *recombinants with rec*⁺ *uvr*⁺ *Hfr's*

Parental strains		Phenotype of *rec*⁻ *uvr*⁺ progeny		
Hfr (*rec*⁺ *uvr*⁺ LHR⁻)	F⁻ (*rec*⁻ *uvr*⁻ LHR⁻)	LHR⁻	LHR⁺	Total tested
W2252	SR59 (*rec-56 uvrA6*)	0	13	13
W2252	AB2480 (*rec-13 uvrA6*)	0	29	29
W4099	SR57 (*rec-56 uvrC34*)	0	29	29

TABLE 3. *LHR in representative rec-56 uvr*⁻ *recombinants compared to their parents*

Strain	Markers affecting UV sensitivity	UV dose (ergs/mm²)	Surviving fraction without holding in buffer	Relative survival[a]	
				4 hr in buffer	8 hr in buffer
JC5088	*rec-56 uvr*⁺	90	8.4×10^{-4}	35.9	87.2
AB2500	*rec*⁺ *uvrA6*	270	1.1×10^{-3}	1.2	0.9
SR59	*rec-56 uvrA6*	2.5	8.7×10^{-4}	1.1	1.2
AB2499	*rec*⁺ *uvrB5*	135	2.5×10^{-3}	0.3	0.3
SR58	*rec-56 uvrB5*	2.5	9.6×10^{-4}	0.8	0.8
AB2498	*rec*⁺ *uvrC34*	315	4.0×10^{-4}	0.7	1.0
SR57	*rec-56 uvrC34*	4	3.2×10^{-4}	2.6	4.2
AB2497	*rec*⁺ *uvr*⁺	2160	1.1×10^{-2}	0.6	0.3

[a] Relative survival is expressed as the ratio between the number of colony-forming units after holding in buffer and the number before holding in buffer.

TABLE 5. *Photoreaction in rec⁻ uvr⁻ derivatives of E. coli K-12*

Derivative	Markers affecting UV sensitivity	UV dose (ergs/mm²)	Surviving fraction without treatment	Relative survival[a]					
				Without treatment	After visible light	After 4 hr in buffer	After 4 hr in buffer + visible light	After 20 hr in buffer	After 20 hr in buffer + visible light
AB2480	*recA13 uvrA6*	2	5.4×10^{-3}	(1.0)	13	1.0	12	0.8	15
SR58	*rec-56 uvrB5*	2	5.5×10^{-3}	(1.0)	20	1.0	14	0.7	13
SR57	*rec-56 uvrC34*	2	6.5×10^{-3}	(1.0)	26	2.8	27	4.5	39

[a] Relative survival is expressed as the ratio between the surviving fraction after treatment (either exposure to visible light or holding in buffer or both) and the surviving fraction without treatment.

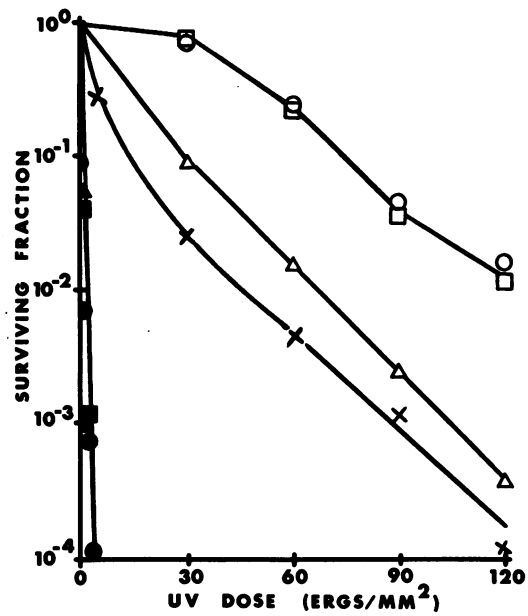

FIG. 2. *Survival of representative Rec⁻ Uvr⁻ recombinants and their parents exposed to various doses of UV and plated on yeast extract-agar. JC5088 (rec-56), ×; AB2498 (uvrC34), ○; AB2499 (uvrB5), △; AB2500 (uvrA6), □; SR57 (rec-56 uvrC34), ●; SR58 (rec-56 uvrB5), ▲; SR59 (rec-56 uvrA6), ■.*

sistant to UV than the *rec-56 uvr⁻* derivatives examined. These results indicate that the loss of the function incurred by *uvr* mutations also eliminates LHR.

Irradiated *rec⁻ uvr⁻* cells appeared to contain detectable amounts of potentially reparable pyrimidine dimers, as judged by the fact that they could be photoreactivated (Table 5). They retained the capacity to be photoreactivated and showed little or no increase in viability even after 20 hr under conditions favoring LHR in *rec⁻ uvr⁺* cells. Since *uvr* mutations impair the ability to excise pyrimidine dimers (1, 14), our data support the idea that LHR reflects excision-dependent repair of such lesions. The low levels of LHR observed in the *rec-56 uvrC34* recombinants are consistent with this interpretation, since mutants carrying *uvrC34* have been reported to retain a slight capacity for excision (12, 20).

Previously, we found that certain *rec⁻* mutations were necessary for the expression of LHR in *E. coli* K-12 (7). These mutations resulted in increased sensitivity to UV and also to the appearance of LHR which was not observed in the *rec⁺* cells. However, it was not clear whether the *rec* genes directly controlled LHR or whether their effect was indirect. The fact that *rec⁻ uvr⁻* strains are much more sensitive to UV than either *rec⁻ uvr⁺* or *rec⁺ uvr⁻* strains suggests that the *rec* genes determine different repair functions than the *uvr* genes. Since the data presented in this paper indicate that the *uvr* genes may directly control the process reflected in LHR, it seems likely that the *rec* mutations do not directly affect the occurrence of LHR but act indirectly to permit its detection. The *rec* genes may determine a repair system different from that reflected in LHR, and supplementary to it (8). When this system is damaged by mutation, the activity of the remaining system, the excision repair mechanism controlled by the *uvr* genes, becomes apparent as LHR.

ACKNOWLEDGMENTS

We are grateful for the able technical assistance of Doreen Challis and John Miller.

This investigation was supported by Public Health Service research grant CA-02896, research program project grant CA-10372, and research career development award CA-3709 (to K.C.S.) from the National Cancer Institute.

ADDENDUM IN PROOF

Harm [Mutation Res. 6:25 (1968)] has photoreactivated a *rec⁻ uvr⁻* strain (AB2480) after 120 hr of holding in buffer.

LITERATURE CITED

1. Boyce, R. P., and P. Howard-Flanders. 1964. Release of ultraviolet light induced thymine dimers from DNA in *E. coli* K-12. Proc. Natl. Acad. Sci. U.S. 51:293–300.
2. Castellani, A., J. Jagger, and R. B. Setlow. 1964. Overlap of photoreactivation and liquid holding recovery in *Escherichia coli* B. Science 143:1170–1171.
3. Charles, R. L., and L. N. Zimmerman. 1956. Dark reactivation

in ultraviolet irradiated *Escherichia coli*. J. Bacteriol. 71: 611–616.

4. Clark, A. J. 1967. The beginning of a genetic analysis of recombination proficiency. J. Cell. Physiol. Suppl. 1 70: 165–180.
5. Clark, A. J., and A. D. Margulies. 1965. Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S. 53:451–459.
6. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics 54:61–76.
7. Ganesan, A. K., and K. C. Smith. 1968. Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light. I. Effect of *rec*$^-$ mutations on liquid holding recovery. J. Bacteriol. 96:365–373.
8. Ganesan, A. K., and K. C. Smith. 1968. Recovery of recombination deficient mutants of *Escherichia coli* K-12 from ultraviolet irradiation. Cold Spring Harbon Symp. Quant. Biol. 33:000–000.
9. Harm, W. 1966. The role of host-cell repair in liquid-holding recovery of u.v.-irradiated *Escherichia coli*. Photochem. Photobiol. 5:747–760.
10. Hollaender, A., and W. Claus. 1937. An experimental study of the problem of mitogenetic radiation. Bull. Natl. Res. Council, Natl. Acad. Sci. U.S., Washington, D. C. 100:75–88.
11. Hollaender, A., and J. T. Curtis. 1935. Effect of sublethal doses of monochromatic ultraviolet radiation on bacteria in liquid suspensions. Proc. Soc. Exptl. Biol. Med. 33:61.
12. Howard-Flanders, P. 1968. DNA repair. Ann. Rev. Biochem. 37:175–200.
13. Howard-Flanders, P., and R. P. Boyce. 1966. DNA repair and genetic recombination: studies on mutants of *Escherichia coli* defective in these processes. Radiation Res. Suppl. 6:156–184.
14. Howard-Flanders, P., R. P. Boyce, and L. Theriot. 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics 53:1119–1136.
15. Howard-Flanders, P., and L. Theriot. 1966. Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics 53:1137–1150.
16. Jagger, J., W. C. Wise, and R. S. Stafford. 1964. Delay in growth and division induced by near ultraviolet radiation in *Escherichia coli* B and its role in photoprotection and liquid holding recovery. Photochem. Photobiol. 3:11–24.
17. Roberts, R. B., and E. Aldous. 1949. Recovery from ultraviolet irradiation in Escherichia coli. J. Bacteriol 57:363–375.
18. Rupert, C. S., and W. Harm. 1966. Reactivation after photobiological damage. Advan. Radiation Biol. 2:1–81.
19. Setlow, J. K. 1966. Photoreactivation. Radiation Res. Suppl. 6:141–155.
20. Setlow, R. B. 1968. Steps in the repair of DNA: fact and fancy. Brookhaven Symp. Biol. 20:1–12.
21. Smith, K. C., and M. E. O'Leary. 1967. Photoinduced DNA-protein crosslinks and bacterial killing: A correlation at low temperatures. Science 155:1024–1026.
22. Taylor, A. L., and C. H. Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. 31:3.32–353