260 m μ ; cpm, counts per minute; DNAase, deoxyribonuclease; RNAase, ribonuclease; γ , microgram; mc, millicurie; TCA, trichloroacetic acid.

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A GENETIC LOCUS IN *E. COLI* K12 THAT CONTROLS THE REACTIVATION OF UV-PHOTOPRODUCTS ASSOCIATED WITH THYMINE IN DNA

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A radiosensitive mutant *E. coli* B_{s-1} was discovered by Hill.^{1,2} It exhibits the same plating efficiency as the parental strain *E. coli* B for normal T1 bacteriophage, but a greatly reduced efficiency with ultraviolet (UV) irradiated T1 phage.³ The sensitive mutant apparently lacks some factor capable of reactivating certain photoproducts in the UV-irradiated DNA of T1 phage.

Sensitive mutants of *E. coli* K12 which show the same low efficiency for scoring UV-irradiated T1 phage were induced by nitrous acid treatment.⁴ This paper reports experiments on genetic crosses between a sensitive F⁻ strain and several resistant Hfr strains of K12. The inheritance of resistance to UV-irradiation among the progeny from the crosses was analyzed following methods similar to those previously reported,⁵ and a locus on the male chromosome was identified that can transmit UV resistance to the zygote. This locus will be referred to as *UV^R* or *UV^S* ac-

cording to whether the phenotype is resistant or sensitive to UV, as judged by the survival of irradiated T1 phage.

The substitution of thymidine by 5-bromodeoxyuridine (BUDR) in phage DNA inhibits various reactivating processes,^{6, 7} including the reactivation of UV-irradiated T1 phage that occurs in *E. coli* B, but is absent in B_{s-1}.⁸ Tests were therefore made to see if the *UV^R* locus controls this reactivation.

Materials and Methods.—Media: The bacteria were grown and mated in broth⁹ containing 1 per cent bactotryptone, 0.5 per cent yeast extract, 1 per cent NaCl, and 0.1 per cent glucose. Selective media¹⁰ contained the following minimal medium: 50 mM phosphate at pH 7, 0.4 mM MgSO₄, 8 mM (NH₄)₂SO₄, 30 μM Ca (NO₃)₂, 1 μM FeSO₄, and 1.5 per cent agar. This was supplemented by amino acids, vitamins, and a sugar as required to select for recombinants with particular characteristics. The concentrations used were as follows; 0.01 per cent DL threonine, 0.01 per cent DL leucine, 10⁻⁵ thiamine, 0.02 per cent L arginine, 0.01 per cent DL histidine, 0.04 per cent DL proline. Glucose, xylose, or arabinose was added at 0.2 per cent as an energy source. The nutrient agar plates contained 1 per cent agar, 1 per cent tryptone, 0.8 per cent NaCl, and 0.2 per cent glucose.

Strains: The characteristics of various strains of *E. coli* K12 obtained from E. A. Adelberg are given in Table 1. The UV-sensitive strain, formerly called *UV_s^S* (ref. 4) but now renamed AB1886, is a nitrous acid-induced mutant derived from AB1157. A thymineless strain, *E. coli* B T⁻ W4516, was from H. S. Kaplan and *E. coli* B_{s-1} was obtained from R. F. Hill.

TABLE 1
CHARACTERISTICS OF THE *E. coli* K12 STRAINS USED

Number	Auxotrophic Characters					Energy	Source	Utilization	
	TL	<i>pro</i>	<i>his</i>	<i>met</i>	<i>thi</i>			<i>arg</i>	<i>gal</i>
3000	+	+	+	+	+	+	+	+	+
AB312	—	+	+	+	—	+	+	+	+
AB313	—	+	+	+	—	+	+	+	+
AB451	—	+	+	+	—	+	+	+	+
AB1157	—	—	—	+	—	—	—	—	—
AB1886	—	—	—	+	—	—	—	—	—

Number	T1	Phage Growth		Drug resist. Sm	Injection order	Sex	UV resist.	Isolated by
		T6	λ					
3000	S	S	S	S	<i>pyr, T, ara</i>	Hfr	R	
AB312	S	S	S	R	<i>Sm, mtl, met</i>	Hfr	R	Taylor
AB313	S	S	(λ)	R	<i>mtl, Sm, his</i>	Hfr	R	Taylor
AB451	S	S	S	R	<i>pro, L, ara</i>	Hfr	R	Taylor
AB1157	S	R	S	R		F ⁻	R	S. Burns
AB1886	S	R	S	R		F ⁻	S	These authors

The following abbreviations are used: Sm^R, streptomycin-resistant; *ara*, arabinose; T1^S, sensitive to T1 phage; *xyl*, xylose; F⁻ female mating type; *mtl*, manitol; TL, threonine and leucine; *arg*, arginine; *pro*, proline; *thi*, thiamine; *lac*, lactose; T6^R, resistant to phage T6; *gal*, galactose; *pyr*, pyrimidine; λ^S, sensitive to λ phage; *met*, methionine; *his*, histidine; Hfr, high frequency of recombination.

Time of entry experiments and detection of the *UV^R* allele: The order in which certain genetic markers on the chromosome of the Hfr strains enter the female was determined by the interrupted mating method.¹¹⁻¹³ 10⁸ male and 5 × 10⁸ female bacteria per ml were mixed in glucose broth at 37°C and sampled at intervals. After killing the sensitive males with T6 phage, aliquots were spread on selective media and incubated until the colonies were visible. As a primary selective procedure for *UV^R* recombinants, other aliquots were spread on nutrient agar and incubated for 3 hr to allow time for phenotypic expression. They were then respread, exposed to 100 ergs/mm² of UV light, and incubated for 24 hr. The time of entry of the *UV^R* allele was determined from the time at which the number of survivors rose steeply above the initial level. When mating was interrupted too early for transmission of the *UV^R* allele to recombinants, the number of survivors was small and they were all *UV^S*. When longer periods were allowed for mating, the survivors were nearly all *UV^R*. Recombinants were tested for other characters by inoculating patches of each on plates which were then replica plated onto other selective media. The presence of the

UV^R allele in the patches was determined by replica plating onto nutrient agar spread with 10^7 T1 phage that had been irradiated with 400 ergs/mm² of UV light. The UV^S patches grow and may show occasional plaques, but the UV^R patches are killed by the irradiated phage.

Growth and assay of bacteria and bacteriophage: Bacteria for survival studies were grown overnight on nutrient agar and suspended in phosphate buffer saline. After irradiation, they were plated, and incubated overnight at 37°C. Bacteriophage in 0.5 per cent NaCl 0.8 per cent agar, seeded with bacteria, were plated on nutrient agar and incubated at 37°C overnight.

Preparation of T1 phage containing 5-bromodeoxyuridine: *E. coli* B T⁻ was grown in 3XD (ref. 14) medium containing 1 µg/ml thymidine and 10 µg/ml bromodeoxyuridine. 2×10^7 phage were added to the suspension when there were 2×10^8 cells/ml, and aeration and incubation at 37°C continued for 3 hr. A dense fraction of the BUDR-labeled phage was selected from a CsCl density gradient.⁸

Irradiation and photoreactivation: The source of UV light was a low-pressure mercury germicidal lamp. Suspensions of bacteria or phage were stirred magnetically and irradiated in phosphate buffer, pH 7. The suspensions were more than 90 per cent transparent to the 2,537 Å light which was measured with a General Electric germicidal UV meter. Phage to be photoreactivated were plated and then incubated at 37°C, 3 in. below four closely spaced 20-watt white fluorescent lamps. The X-irradiations were carried out at 250 kv 15 mA without added filtration. Ten ml of the suspension to be irradiated was placed in a 25 mm diameter cylindrical glass vessel surrounded by ice and bubbled with nitrogen or oxygen. The dose rate was measured with a Victoreen ionization chamber.

Results.—The survival of colony-forming ability as a function of the UV dose to various UV^R strains and to the UV^S mutant is shown in Figure 1A. The survival after X-irradiation of UV^R and UV^S strains is seen in Figure 2. The effects

FIG. 1.—The fraction of the cells retaining the ability to form colonies on nutrient agar is plotted on a logarithmic scale as a function of the dose at 2,537 Å. 1A shows the survival curves of the strains used in mating experiments. The inset graph shows the low dose portion of the survival curve of the UV^S strain AB1886 with an expanded abscissa scale and the ordinate displaced one unit downwards. 1B shows the survival curves of two of the progeny isolated as single colonies from the pro^+ , ara^+ , arg^- selection from Hfr AB451 $\times$ AB1886.

FIG. 2.—The fraction of cells of strain AB1157 and the UV^S strain AB1886 that retain the ability to form colonies on nutrient agar is plotted on a logarithmic scale as a function of the X-ray dose delivered in the presence of oxygen or under anoxia.

FIG. 3.—The fraction of T1 bacteriophage that form plaques is plotted on a logarithmic scale as a function of the dose of ultraviolet light at 2,537 Å. 3A: The phage were plated on the UV^S strain AB1886 and the UV^R strain AB1157 and were incubated with and without photoreactivating light. 3B and 3C: Phage from the experiment of Figure 3A were plated on the parent strains and on progeny from Hfr AB451 $\times$ AB1886, isolated from arg^+ selection after a 40-min mating period.

FIG. 4.—The time sequence of transfer of genetic markers. The number of colonies per ml is plotted against the time at which the sample was taken and mating was interrupted by adding T6 phage before plating on selective media. The procedure used to demonstrate transfer of the UV^R allele is described under *Methods*.

of the UV^R allele in the host bacterium on the survival of plaque-forming ability of UV-irradiated T1 phage, both in the dark and when photo-reactivated, are shown in Figure 3A. Figure 3B shows the survival of T1 after 350 ergs/ mm^2 with other hosts. Figure 4 shows the time of transfer of genetic markers in the cross AB451 $\times$ AB1886 and that UV^R enters between the arg and ara loci. The order of the markers was confirmed by analysis of unselected markers as seen in Table 2. Progeny in the zygote patches from the cross with Hfr AB451 that are arg^+ and ara^- must have a switch between these markers. Such a switch is illustrated by recombinants 1 and 2 in Figure 5. The same is true of the progeny for the cross with Hfr AB312 that are ara^+ and arg^- , illustrated by recombinants 3 and 4 in Figure 5. From the numbers of recombinants shown in Table 3, the distance from the arg to the UV^R locus is calculated to be 0.37 ± 0.1 ($2 \times$ standard deviation) of the distance between the arg and ara loci. It was found that UV^R is not an early marker in strains 3,000 or AB313.

TABLE 2
FREQUENCY OF UNSELECTED MARKERS IN AB451 $\times$ AB1886

Selected marker	Mating time (min)	Number of recombinants tested	Per Cent Positive for Unselected Marker					
			<i>pro</i> ⁺	<i>ara</i> ⁺	<i>UV</i> ^R	<i>arg</i> ⁺	<i>xyl</i> ⁺	<i>gal</i> ⁺
<i>pro</i> ⁺	10	96	100	2	0	0	0	0
<i>ara</i> ⁺	15	96	61	100	0	0	0	0
<i>UV</i> ^R	25	84	45	49	100	3.4	0	0
<i>arg</i> ⁺	40	85	60	65	59	100	2.5	0

FIG. 5.—The top line shows a section of the genetic map of *E. coli* K12, as deduced from time of entry experiments.¹¹⁻¹³ The arrowhead on the Hfr chromosome shows the first part to enter the zygote. The *arg*⁺, *ara*⁻ patches from cross AB451 × AB1886 include progeny of the structure 1 and 2, while the progeny from the *arg*⁻, *ara*⁺ patches from AB312 × AB1886 must include recombinants of the structure 3 and 4 (see Table 3).

FIG. 6.—The fraction of T1 phage that form plaques is plotted on a logarithmic scale as a function of the dose of ultraviolet light at 2,537 Å. Both normal T1 phage and T1 phage that contain BUDR in partial substitution for thymidine were plated on the *UV*^R strain AB1157 and the *UV*^S strain AB1886 and incubated overnight at 37°C.

Figure 1*B* shows survival curves obtained with single colony isolates of *UV*^R and *UV*^S progeny from the 30-min *ara*⁺, *arg*⁻ selection from AB312 × AB1886. These recombinants contain *pro*⁺ and *ara*⁺ from the Hfr chromosome but differ at the *UV*^R locus. The survival curves are similar to those of the parental strains shown in Figure 1*A*. A number of single-colony isolates from the 40-min *arg*⁺ selection from AB451 × AB1886 were used for plating T1 phage that had been exposed to 350 ergs/mm² of UV. Comparison of these results in Figure 3*C* with those in Figure 3*A* and 3*B* shows that the progeny are either *UV*^R or *UV*^S. Intermediate levels of gene action were not observed.

The effect of UV irradiation on normal and BUDR-containing phage when plated on the *UV*^R and *UV*^S strains is shown in Figure 6. Only a relatively small fraction of photoproducts in the BUDR-containing T1 phage DNA that were lethal in the *UV*^S strain were reactivated in the *UV*^R strain.

Discussion.—The mating experiments show that the wild type allele *UV*^R is carried on the Hfr chromosome and can be detected if the F⁻ mating partner is

TABLE 3
FREQUENCY OF SWITCHES BETWEEN *UV*^R AND NEAREST MARKERS IN PROGENY FROM AB1886 MATED WITH Hfr STRAINS AB312 AND AB451

Cross	Selected markers	Unselected		Number of Switches	
		<i>UV</i> ^R	<i>UV</i> ^S	<i>UV</i> ^R to <i>ara</i>	<i>arg</i> to <i>UV</i> ^R
AB312 × AB1886 Mated 50 min	<i>arg</i> ⁻ <i>ara</i> ⁺	13	33	33	13
AB451 × AB1886 Mated 50 min	<i>arg</i> ⁺ <i>ara</i> ⁻	34	27	34	27
		Totals		67	40

UV^s. The effects of UV-irradiation on the survival of colony-forming ability of *UV^R* and *UV^s* progeny are very like those on the parental *UV^R* and *UV^s* strains, as shown in Figure 1A and 1B. The survival curves of the *UV^s* cells are almost exponential, while those of the *UV^R* cells have a marked shoulder, indicating that at low doses, reactivation in *UV^R* cells is very effective. The reactivating process is evidently less efficient at higher doses. The survival of UV-irradiated T1 phage is likewise depressed if they adsorb to heavily irradiated cells.^{7, 8} These complementary observations may be explained by the hypothesis that the *UV^R* locus controls an enzyme system capable of reactivating without light certain photoproducts in the DNA of the bacterium or the T1 phage. After large UV doses to the bacterium, reactivation of phage or host DNA is depressed, possibly because the reactivating enzyme may bind to irradiated DNA, as occurs with the photoreactivating enzyme.¹⁵ Alternatively, the poor reactivation may reflect a failure to produce new enzyme.

About 74 per cent of UV photoproducts in the phage DNA that are lethal in the *UV^s* strain in the dark can be photoreactivated. About 83 per cent are *UV^R* reactivated in the resistant strain in the dark. This requires some overlap in the kind of photoproducts reactivated by these two processes. In view of this overlap, it may be pertinent that transforming DNA is rendered inactive by thymine dimers produced by UV¹⁶ and that they are the main products removed by photoreactivation.^{17, 18}

As seen in Figure 6, the sensitization of T1 bacteriophage resulting from the incorporation of BUDR into the phage DNA is largely due to a failure of *UV^R* reactivation. This suggests that *UV^R* reactivation operates on certain photoproducts associated with thymine in the DNA but that the corresponding photoproducts with BUDR are not reactivated.

The hypothesis that *E. coli* contains enzymes capable of reactivating certain UV-induced injuries in DNA has already been discussed in connection with the "mutation frequency decline" observed in *E. coli* following UV irradiation¹⁹ and the mechanism of host cell reactivation.²⁰

Summary.—Genetic crosses between a UV-sensitive F⁻ strain of *E. coli* K12 and several Hfr strains showed that a genetic locus situated between the arginine and arabinose loci on the male chromosome can confer UV resistance to the progeny of the zygotes. UV resistance was judged by the survival of colony-forming ability of the bacteria and from the survival of irradiated T1 phage with the bacteria as host. The properties of the *UV^R* phenotype suggest that the *UV^R* locus controls an enzyme system capable of reactivating certain photoproducts in DNA without light. UV-irradiated T1 phage is about 5 times more resistant when plated on *UV^R* cells or when photoreactivated in *UV^s* cells than it is when plated on *UV^s* cells in the dark. There is an overlap in the kinds of photoproducts reactivated, as either *UV^R* reactivation or photoreactivation can render harmless about 80 per cent of the UV photoproducts in the phage DNA that would be lethal in the *UV^s* strain without light. The substitution of 5-bromodeoxyuridine for thymidine in T1 phage DNA inhibits reactivation by the *UV^R* locus. This suggests that the *UV^R* reactivation acts on certain photoproducts associated with thymine in DNA.

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QUALITATIVE SURVEY OF RNA CODEWORDS*

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A degenerate genetic code was suggested a number of years ago by Gamow¹ and by Crick.² In such a code, an amino acid may be directed into protein by two or more codewords. Previous work demonstrated that C¹⁴-amino acids were directed into protein by synthetic polynucleotides in cell-free *E. coli* extracts,³ and that leucine incorporation was stimulated by either poly UG,† UC, or UA.⁴⁻⁶ Thus the code was shown to be degenerate with respect to leucine.⁴⁻⁶

Initially, all of the codewords found contained U. However, assuming a triplet code, the proportion of U compared with other nucleotides in codewords seemed unusually high, for natural template RNA, such as viral RNA, did not contain such a preponderance of U. To resolve this paradox, a more degenerate code was proposed with both non-U and U containing codewords.⁵ An alternative hypothesis was advanced by Roberts, who suggested a doublet code, for in such a code the proportions of nucleotides would be within the range found in viral RNA.^{7, 8}

The existence of non-U codewords was suggested when poly AC preparations were found to direct small amounts of proline and threonine into protein.^{9, 22} Recently, in a careful study, Bretscher and Grunberg-Manago clearly demonstrated coding by non-U words.¹⁰ Several poly AC preparations were reported to code well for proline, threonine, histidine, and glutamine. This work indicated that other