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Isolation and Genetic Analysis of Amber *uvrA* and *uvrB* Mutants

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Genetic properties of amber *uvrA* and *uvrB* mutants of *Escherichia coli* K-12 are described. The isolation of three amber *uvrA* and two amber *uvrB* mutants indicates that the products of these genes are proteins.

The genes *uvrA*, *-B*, and *-C* are involved in releasing pyrimidine dimers from ultraviolet (UV)-irradiated deoxyribonucleic acid. Recently it has been reported that the *uvrA* and *uvrB* genes affect the activity of an endonuclease specific for UV-irradiated deoxyribonucleic acid (1, 16) and that in the *uvrC* mutant strain the number of nicks formed by incising UV-irradiated deoxyribonucleic acid is increased slowly with time after irradiation (7). It is, however, not known yet whether the products of the *uvr* genes are protein or ribonucleic acid, although Kondo et al. (8) suggested that the product of the *uvrB* gene was ribonucleic acid, or a protein whose synthesis is hardly inhibited by chloramphenicol. To answer this question, we have tried to isolate amber mutations in the *uvr* genes.

The methods for isolating UV-sensitive mutants were essentially the same as those described by Howard-Flanders and Theriot (5). To obtain a *uvr* amber mutation, a derivative of strain KN250 carrying a temperature-sensitive amber suppressor, *sup-126* (11), and amber *trp* and *tyr* mutations was used. A log-phase culture was treated with 0.5% ethyl methane sulfonate for 1 h at 37 C and washed to remove ethyl methane sulfonate. The cells were resuspended in fresh medium, divided into many tubes, cultured to stationary phase, and then plated on NBT (nutrient broth + 50 μg of thymine per ml) agar plates together with about 10^9 T3 or λ phages irradiated with 1,000 ergs of UV light per mm^2. Colonies from the survivors were selected, purified successively by single colony isolation, and tested for sensitivity to UV by irradiating with appropriate doses and incubating at both 30 and 42 C. The ability to reactivate UV-irradiated bacteriophage was also examined. Out of 133 UV-sensitive strains isolated, 94 mutants showed the Hcr$^-$ phenotype. Among these Hcr$^-$ mutants, 54 independent mutants were classified into *uvrA*, *-B*, and *-C* groups by examining UV sensitivity of heterodiploid strains constructed by transferring F112 (9), F450 (2), and F4102 (10) carrying the *uvrA*$^+$, *-B*$^+$, and *-C*$^+$ genes, respectively. The *uvrD* mutation (12) was identified by examining the Hcr phenotype, X-ray sensitivity, and the UV sensitivity of Arg$^+$ recombinants after conjugation with Hfr J4 (6) for 30 min. Results are shown in Fig. 1. In these *uvr*$^-$ strains three *uvrA (uvr-24, uvr-43,* and *uvr-72)* and two *uvrB* mutations *(uvr-46* and *uvr-59)*, shown as underlined numbers in Fig. 1, were concluded to be amber mutations by two different types of experiments (Table 1).

First, upon transferring plasmid KHF6 carrying *supD*$^-$ into these mutants they became UV^R, Trp$^+$, and Tyr$^+$ simultaneously, whereas upon transferring plasmid KHF17 not carrying *supD*$^-$ they remained as they were.

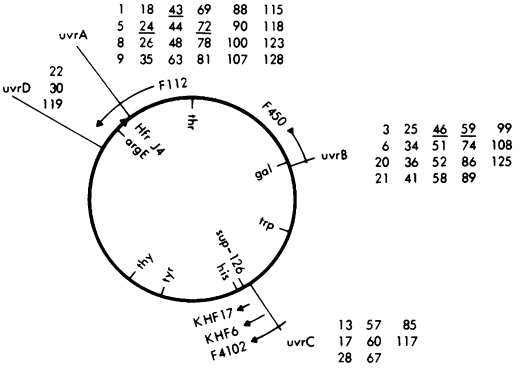

FIG. 1. *Location of uvr mutations on the linkage map of Escherichia coli K-12 (15). Isolation and genetic mapping of uvr mutations were described in the text. Underlined numbers represent amber uvr mutations. The arrows represent origin and direction of transfer of F-prime factors and an Hfr strain used in the experiments. The approximate extent of the chromosomal segments carried by the various F-prime factors are indicated by their length and position.*

Secondly, when the spontaneous back mutants from Trp amber to Trp prototroph or from Tyr amber to Tyr prototroph were isolated, they also showed the UV^RTyr^+ or UV^RTrp^+ phenotype, respectively, an indication that the three phenomena, from Trp and Tyr amber to Trp and Tyr prototroph and from UV^S to UV^R, were reversed by a single mutation, namely, an amber suppressor mutation. Note that under these conditions the spontaneous reversion frequency ranged from 10^{-6} to 10^{-8}.

The mutation sites of these amber *uvr*$^-$ strains were analyzed by complementation and by P1 transduction. The F-prime factor, F112 carrying *uvrA*$^+$ or *uvrA6* (13), was transferred to strains EA512 *(uvr-24)*, EA1803 *(uvr-43)*, and EA3601 *(uvr-72)*. The result with strain EA3601 is shown in Fig. 2 (a and b). The mero-

TABLE 1. *Characterization of UV-sensitive amber mutants*

Strain no.	*uvr*	Hcr	KHF17 transferred			KHF6 transferred			Trp$^+$ back mutants			Tyr$^+$ back mutants		
			UV	Trp	Tyr	UV	Trp	Tyr	Frequency	UV^R Tyr^+	UV^S Tyr^-[a]	Frequency	UV^R Trp^+	UV^S Trp^-[a]
EA512	A24	−	S	−	−	R	+	+	2.1×10^{-7}	58	5	2.7×10^{-7}	55	8
EA1803	A43	−	S	−	−	R	+	+	9.3×10^{-9}	7	0	9.3×10^{-9}	8	0
EA3601	A72	−	S	−	−	R	+	+	3.0×10^{-7}	27 (Tyr^-)	0	—[b]		
EA2401	B46	−	S	−	−	R	+	+	3.1×10^{-7}	61	0	2.9×10^{-7}	64	0
EA3103	B59	−	S	−	−	R	+	+	1.5×10^{-6}	59	0	1.7×10^{-6}	64	0

[a] The back mutants UV^STyr^- and UV^STrp^- seem to be the true backs of amber *trp* and amber *tyr*, respectively.
[b] Since EA3601 had acquired another mutation besides amber in *tyr*, Tyr^+ back mutants could not be obtained.

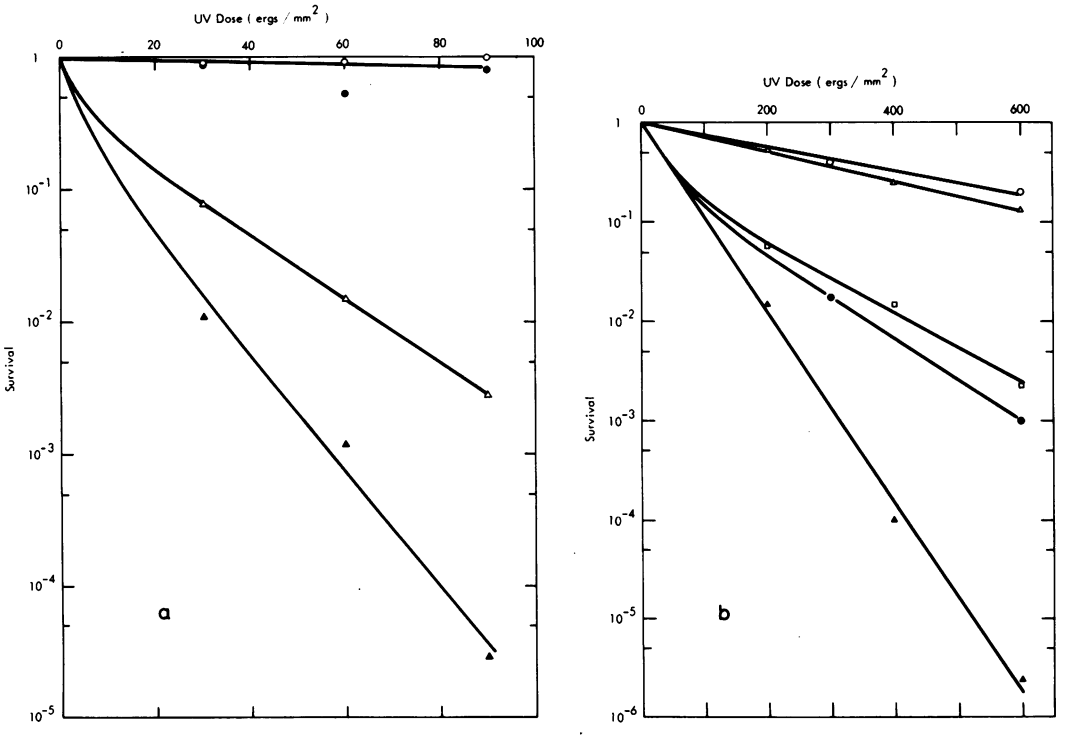

FIG. 2. *(a) UV survival curves of merodiploid strains F112 (uvr$^+$)/EA3601 (○, ●) and F112 (uvrA6)/EA3601 (△, ▲) at 30 (open symbols) and 42 C (closed symbols). Overnight cultures in a minimal medium were diluted into M9 buffer to a density of about 10^7 cells/ml, irradiated with various doses of UV, and plated on NBT agar at 30 and 42 C. The fraction surviving was determined from the number of visible colonies after incubation overnight at 42 C or for 2 days at 30 C. (b) Host-cell reactivation of λ_{vir} by F112 (uvr$^+$)/EA3601 (○), F112 (uvrA6)/EA3601 (●), EA3601 (□), a wild-type strain (△), and AB1886 (▲).*

diploid strain carrying F112 (*uvrA*$^+$) became as resistant as a wild-type strain and showed a host-cell reactivation of UV-irradiated bacteriophage λ, whereas the merodiploid carrying F112 (*uvrA6*) remained as sensitive as a parent strain and showed the Hcr$^-$ phenotype. Similar results were also obtained for the other two amber mutants. This information demonstrates that the mutation occurs in the *uvrA* gene in these three amber mutants. When the *sup*$^+$ derivatives of strains EA2401 (*uvr-46*) and EA3103 (*uvr-59*) as well as strain AB1885 (4) were tested for the Hcr of λ and λ *b2 bio*$^+$*uvrB*$^+$, they reactivated much more UV λ *b2 bio*$^+$*uvrB*$^+$ than λ bacteriophage (Fig. 3). Then the mutation site *uvr-59* was mapped by P1 transduction (Table 2); the genes *uvr-59* and *uvrB5* of strain AB1885 were linked to *gal* at frequencies of 38 and 31%, respectively. Hence, mutations *uvr-59* and *uvr-46* are concluded to be *uvrB* mutations.

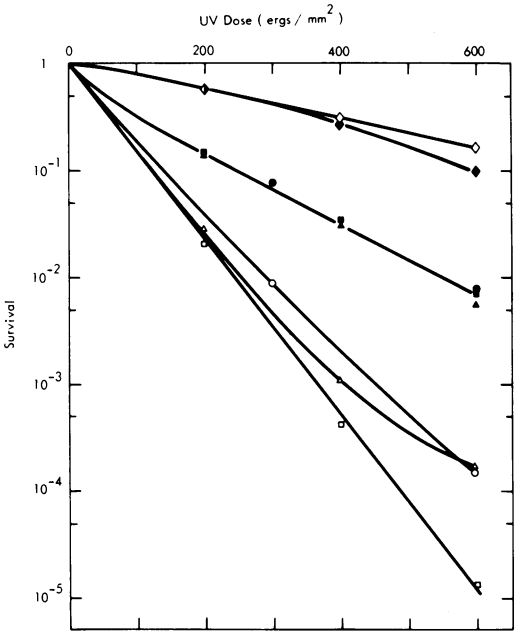

FIG. 3. *Host-cell reactivation of* λ_{vir} *(open symbols) and* λ*b2 bio*$^+$*uvrB*$^+$ *(closed symbols) by various uvrB mutants. Symbols: Wild-type strain (◇, ◆); E54 (su*$^-$*uvrB46) (△, ▲); E95 (su*$^-$*uvrB59) (○, ●); and AB1885 (uvrB5) (□, ■).*

The effect of temperature-sensitive suppressor *sup-126* on the UV sensitivity and on a host-cell reactivation of amber *uvr*$^-$ mutants was also examined (Table 3). All these amber mutants showed a higher survival after UV irradiation on agar plates at 30 C than after that at 42 C and partially reactivated UV-irradiated bacteriophage λ when in the presence of *sup-126*, whereas in substituting *sup-126* with *supD*$^+$ by conjugation they became all extremely sensitive to UV even at 30 C and showed the Hcr$^-$ phenotype.

Previously we have reported the isolation of a temperature-sensitive *uvrA* mutant (13, 14). This information and the isolation of amber *uvrA* mutants presented in this communication demonstrate that the product of the *uvrA* gene is a protein. Similarly, the isolation of amber *uvrB* mutants also indicates that the product of the *uvrB* gene is a protein.

In view of the fact that only three out of 24 *uvrA* mutants and only two out of 19 *uvrB* mutants carried the amber lesion, the eight *uvrC* and three *uvrD* mutants examined might

TABLE 2. *Co-transduction of uvrB with gal by P1kc phage*

Donor	Recipient	No. of Gal$^+$ transductants scored	Frequency of unselected marker (%)	
			UR^R	UV^S
W3876 (*gal*$^+$ *uvrB*$^+$)	E50 (*gal*$^-$ *uvr-59*)	111	38	62
W3876 (*gal*$^+$ *uvrB*$^+$)	AB1885 (*gal*$^-$ *uvrB5*)	94	31	69

TABLE 3. *Effect of a temperature-sensitive amber suppressor sup-126 on UV survival and on Hcr of* λ *phage*

Mutations	Survival after irradiation with 90 ergs/mm^2				Survival of λ irradiated with 600 ergs/mm^2	
	sup-126		*su*$^-$			
	30 C	42 C	30 C	42 C	*sup-126*	*su*$^-$
uvrA24	4.0×10^{-4}	1.2×10^{-5}	3.8×10^{-5}	2.7×10^{-4}	6.7×10^{-4}	7.8×10^{-5}
uvrA43	2.1×10^{-2}	8.2×10^{-5}	7.5×10^{-6}	1.4×10^{-4}	4.8×10^{-3}	1.1×10^{-5}
uvrA72	8.5×10^{-5}	1.0×10^{-5}	8.6×10^{-6}	1.5×10^{-5}	2.3×10^{-3}	3.0×10^{-5}
uvrB46	3.0×10^{-2}	4.6×10^{-4}	6.8×10^{-4}	1.5×10^{-4}	2.3×10^{-2}	1.7×10^{-4}
uvrB59	2.0×10^{-2}	4.8×10^{-3}	5.5×10^{-5}	2.8×10^{-4}	1.6×10^{-2}	1.3×10^{-4}

be not a large enough group to obtain an amber mutation.

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