LOCUS FOR RADIATION RESISTANCE IN *ESCHERICHIA COLI* STRAIN B/r[1]

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STRAIN B of *Escherichia coli* is sensitive to ultraviolet radiation (UV) and becomes filamentous (Fil) when irradiated with small doses of UV. Its sensitivity to UV is dependent on postirradiation treatment, so that it is less sensitive when plated on minimal than on complex medium (SDR) (ROBERTS and ALDOUS 1949), and when incubated at 42°C than at 37°C (HR) (ANDERSON 1951). The gene responsible for these properties is transducible with *proC* (DONCH and GREENBERG 1968). When transduced into a K-12 strain the gene produces a mucoid phenotype, suggesting that it may be the same as the *lon* gene described by HOWARD-FLANDERS, SIMSON and THERIOT (1964) or *capR* mutants of MARKOVITZ (MARKOVITZ 1964; MARKOVITZ and ROSENBAUM 1965; MARKOVITZ and BAKER 1967). Strain B is not mucoid presumably because of a mutation elsewhere on the genome which interferes with the synthesis of mucoid polysaccharide.

UV resistant mutants of strain B have been isolated, the best characterized of these being strain B/r (WITKIN 1946, 1947). Not only is this mutant UV resistant, it no longer produces filaments on UV irradiation and is not subject to SDR or HR. In these respects it resembles most wild-type strains of *E. coli*. The presumption, therefore, is that B/r is a revertant to wild type at the *lon* locus of strain B. However, it is possible that it is not a revertant at the *lon* locus but a mutation elsewhere on the genome.

In this report we will show that when B/r was used as a donor to transduce *proC*$^+$ to a *proC* UV resistant K-12 derivative, mucoid UV sensitive transductants occurred at a frequency of 3%. When used to transduce *proC*$^+$, *lac*$^+$ or *purE*$^+$ into a polyauxotrophic *lon* K-12 strain neither UV resistant nor nonmucoid transductants were observed. Strain B/r appears to be *lon* and its mutation to resistance is at another cistron probably between *lac* and *ara*.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are shown in Table 1.

Phage: P1*kc* was supplied to us by C. YANOFSKY and from this we isolated a virulent mutant, P1 *vir*, which overcomes immunity of P1 lysogenic strains and produces large, clear plaques.

P1 *vir* has a low efficiency of plating on strain B. Therefore, it was grown by the overlay method described in ADAMS (1959) on strain B and a single plaque was picked and replated on strain B. After two cycles of plating on strain B a high titer preparation for strain B was obtained,

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TABLE 1

Survey of strains

	Mating type	Relevant markers	Source
E. coli B	F-	"wild type" Fil+	R. HILL
E. coli B/r (CSH)	F-	"wild type" Fil-	E. WITKIN
HB45	F-	*met pro(A* or *B) thr leu ara gal lac mal xyl ton+ tsx- str-* Fil-	H. BOYER
HB33†	Hfr	Fil-	H. BOYER
AB261††	Hfr	*met* Fil-	E. ADELBERG
AB1899	F-	*proB lac tsx- str-* Fil+ Mu+	P. HOWARD-FLANDERS
χ478	F-	*proC purE lacZ tsx- str-* Fil-	R. CURTISS III
PAM 7	F-	*ara gal lac xyl ton- tsx- str-* Fil+	J. GREENBERG
PAM 42	F-	*purE lacZ tsx+ str-* Fil+ Mu+	Tranduction
PAM 43	F-	*proC lacZ tsx- str-* Fil+ Mu+	Tranduction
PAM 2328	F-	*proC lacZ tsx- str-* Fil+ Mu+	Tranduction
W1485	F+	wild	C. YANOFSKY

Abbreviations conform where possible to the suggestions of DEMEREC, ADELBERG, CLARK and HARTMAN (1966). Fil+ means form long filaments after UV irradiation. Mu+ means that colonies produce large amounts of mucoid material at 37°C on minimal glucose medium. For *ton*, *tsx* and *str*, — means resistance, + means sensitivity.

† Order of transmission: O—*try*—*lac*—*pro* . . . (BOYER 1966).

†† Order of transmission: O—*proB*—*thr*—*leu* . . . *lac* sex factor (DEWITT and ADELBERG 1962).

and this grew efficiently on K-12 strains. This strain was used in all experiments so we shall refer to it as P1. P1 was grown on donor strains for at least two cycles and harvested as described in ADAMS (1959).

Media: The minimal medium used for selection in transduction experiments was Davis Minimal (DM) Broth (Difco) to which Noble Agar (Difco) was added at a final concentration of 2%, glucose (or lactose in Lac+ selections) at a concentration of 0.5%, and streptomycin, to prevent contamination, at 200 μg/ml; amino acids were used at a concentration of 50 μg/ml, vitamin B_1 at a concentration of 0.17 μg/ml. The purine requirement was satisfied interchangeably by adenine, adenosine, guanine or guanosine at a concentration of 5 μg/ml.

Complete (DK) broth consisted of tryptone 10 g, NaCl 5 g, yeast extract 5 g and glucose 1 g per liter of deionized water. Viable counts, survival curves and filament formation studies were done on this medium, without glucose, solidified with 1.5% Bacto Agar (Difco).

Transductions: Transductions were performed using bacteria grown overnight with aeration in DK broth supplemented with 2.5×10^{-3} M $CaCl_2$, diluted 1:10 into fresh broth of the same composition and incubated for 2 hrs (about 8×10^8 cells/ml) with aeration. All incubations were at 37°C. At this time P1 was added to give a multiplicity of infection of 0.05–0.1. Adsorption was allowed to proceed without shaking for 30 min, after which the adsorption mixture was centrifuged at approximately 5,000 rpm for 15 min. The supernatant was decanted and used to determine the percent of phages adsorbed. The pellet was resuspended in water, appropriately diluted and 0.1 ml amounts spread onto selective media.

As controls P1 lysates were spotted on DK agar to test for bacterial sterility, and to test for reversions an aliquot of recipient cells was treated as experimental cells but without P1. Bacterial survival was determined by plating on complete medium appropriate dilutions of cells before adding P1 and also at the end of the adsorption period. All selection plates were incubated from 2–5 days. Transductants and recombinants were purified at least once on selective medium.

Filament formation: Filaments were induced by first growing cells overnight with shaking at 37°C in DM Broth plus supplements required by the strain. After overnight growth the cultures

were diluted in supplemented DM broth, 1:50 or 1:100, depending upon growth rate, and incubated with shaking until a titer of approximately 1 to 4×10^8 cells per ml was reached. 1.0 ml amounts were placed in 60 mm Petri plates and exposed to 75 erg/mm^2 of UV from a Westinghouse germicidal lamp producing 15.4 ergs/mm^2/sec at the distance of 51.5 cm. Appropriate dilutions were spotted onto ringed slides (Perma-Slides, Progressive Laboratory Specialties, Inc.) set in a 150 mm Petri plate. Slides were held above the bottom of the Petri plate by applicator sticks. A moist filter paper on the bottom of the Petri plate prevented drying of the spots. The entire assembly was covered by the Petri cover and incubated for 2 hrs. The slides were then examined under 100 × magnification for the presence of elongated cells. Under these conditions filamentous cells were at least four to five times normal cell length.

Alternatively, diluted, irradiated (75 ergs/mm^2) cells were spotted onto DK agar, incubated for 3 hrs, and then examined under 100 × magnification. Under these conditions filamentous cells were from 10 to 50 times normal cell length.

Initial classification of filamentous strains produced by transduction was done coincidentally with testing for UV sensitivity. The rapid streak method described by GREENBERG (1964) was used. UV irradiated streak plates (1078 ergs/mm^2) were incubated overnight and filament formation was determined by microscopic examination of areas within the streaks. Under these conditions filamentous cells were three to four times normal cell length.

Recombination: Recombination experiments were performed by conventional methods and, as done in our laboratory, have been described (GREENBERG 1964).

Ultraviolet survival curves: Ultraviolet survival curves were performed by the methods described in GREENBERG (1967) with the exception that log cultures were used instead of stationary cultures.

RESULTS AND DISCUSSION

Sexual recombination: Using the fertile B/r derivative HB33 (BOYER 1966) as donor and PAM 7 (GREENBERG 1964) as recipient and selecting for Lac$^+$, Ara$^+$ and Xyl$^+$ and counterselecting against the donor with streptomycin, the results show (Table 2) that UV resistance was transferred to PAM 7 at high frequency with markers close to *lac*$^+$. Transductions described below excluded the radiation resistance locus from the region between *purE* and *lac*. The recombination data suggested that the locus of radiation resistance, *sul**, was to the right of the *ara* region (Figure 1). Analysis of frequency of quadruple crossovers (Table 3) shows that the least frequent classes were compatible with the order *ara–ton–sul–lac*.

Evidence for this order is also strengthend by the results obtained using AB261 as a donor and HB45 an auxotrophic derivative of B/r (BOYER 1966) as a recipi-

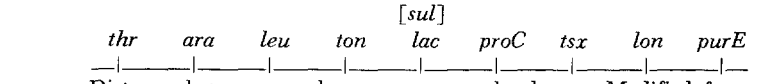
TABLE 2

Frequency of donor markers among recombinants of crosses between HB33 (Hfr) × PAM 7 lac ara xyl ton tsx str *F*$^-$

Recombinants selected	Number examined	Frequency (percent) donor alleles					
		UVR	*lac*$^+$	*ton*$^+$	*tsx*$^+$	*ara*$^+$	*xyl*$^+$
lac$^+$ *str*^{r}	50	84	. .	86	94	80	16
ara$^+$ *str*^{r}	50	64	98	96	86	. .	22
xyl$^+$ *str*^{r}	50	54	52	60	32	58	. .

UVR means resistant to ultraviolet radiation.

* We shall call the gene involved in UV resistance of strain B/r *sul* (suppressor for *lon*), since it is nonallelic to the *lon* gene of B or AB1899.



Distance between markers not accurately drawn. Modified from TAYLOR-THOMAN (1964).

FIGURE 1.—Schematic partial map of genome of *E. coli.*

ent. When such a cross was performed the conjugation was allowed to proceed for 30 min to ensure only limited transfer of chromosomal material which was further proved by the fact that none of *pro(A* or *B) str*$^+$ recombinants had received markers beyond the *metB* locus. Furthermore approximately 4% of the recombinants were UV sensitive. Figure 4 shows the UV survival curve of one such recombinant, PAM 2613. This shows that AB261, taken to represent K-12 strains, is *sul*$^+$ and that the *sul*$^+$ gene lies between the point of entry of AB261 to the left of *lac* and to the right of the *metB*. Previous evidence given in the first paragraph located *sul*$^+$ to the right of the *ara*$^+$ region. Therefore *sul*$^+$ would be between *lac*$^+$ and *ara*$^+$.

Transduction of UV sensitivity from B/r to χ478: P1 · B/r was used to transduce *proC*$^+$, *lac*$^+$, and *purE*$^+$ to χ478 and unselected markers were analyzed (Table 4). 3% of the 1445 *proC*$^+$ transductants were mucoid but none of 1023 *lac*$^+$ and only one of 1680 *purE*$^+$ transductions were mucoid. All of 43 mucoid *proC*$^+$ transductants examined were UV sensitive, when tested by the rapid method. Definitive UV survival curves of four of these were done (Figure 2). For comparison the survival curves of strains B, B/r and χ478 are shown. The UV

TABLE 3

Frequency of uv ara ton lac tsx *phenotype among recombinants between HB33 (Hfr* × *PAM 7* lac ara xyl ton tsx str^r *selected for* xyl+ str^r

Possible order of markers:	*ara*	*ton*	*uv*	*ton*	*ara*	*uv*	*ara*	*uv*	*ton*
Quadruple crossover type	+	—	—*	+	—	—	+	+	+
		and			and			and	
	—	+	+	—	+	+	—	—	—
		2 (4%)			3 (6%)			13 (26%)	
Possible order of markers:	*ara*	*ton*	*lac*	*ton*	*ara*	*lac*	*ton*	*lac*	*ara*
Quadruple crossover type	+	—	+	+	—	+	+	—	+
		and			and			and	
	—	+	—	—	+	—	—	+	—
		1 (2%)			4 (8%)			7 (14%)	
Possible order of markers:	*uv*	*lac*	*tsx*	*uv*	*tsx*	*lac*	*tsx*	*uv*	*lac*
Quadruple crossover type	—	—	+	—	—	+	+	+	+
		and			and			and	
	+	+	—	+	+	—	—	—	—
		0 (0%)			3 (6%)			7 (14%)	

* + means resistant to UV and phage, ability to ferment sugar. — means resistant to UV and phage, inability to ferment sugar.

TABLE 4

Linkage relationships among lacZ+ proC+ purE+ *and genes controlling UV sensitivity and mucoidy*

Selected marker	Number observed	Number tested	Frequency of donor markers (percent)					
			lacZ+	*proC+*	*tsx+*	UV	Mu	*purE+*
lacZ+	1023	300	100	100	5	0	0	0
proC+	1402	...	6	100	12	3	3	0
nonmucoid	...	300	6	100	9	0	0	0
mucoid	43	43	0	100	100	100	100	0
purE+	1680	...	0	0	0	0.09	0.09	100
nonmucoid	...	300	0	0	0	0	0	100
mucoid	1	1	0	0	0	100	100	100

P1 donor was B/r and the recipient was χ478.
UV means sensitive to ultraviolet radiation.
Mu means mucoid, spreading colonies when grown at 37°C on minimal medium.

sensitive phenotypes resulting from transduction into χ478 from B and B/r were indistinguishable, both being slightly less sensitive than B itself. The UV sensitive transductants from B/r to χ478 became filamentous following UV irradiation. It is apparent from these results that strain B/r has a gene, cotransducible with

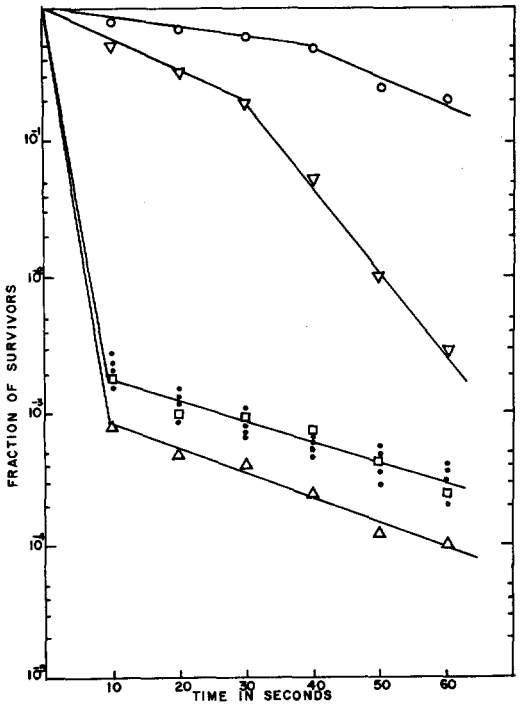


FIGURE 2.—Survival following UV irradiation of *E. coli* strain χ478 ○, B/r ▽, B △, PAM 43 □, and four mucoid, *proC+* transductants of χ478 by P1·B/r ●.

proC$^+$, producing a phenotype which is UV sensitive, filament-inducible, and mucoid. The frequency of cotransduction of the UV sensitivity gene of strain B/r and *proC*$^+$ is somewhat less (3%) than our previous experience with the cotransduction of the UV sensitivity gene of strain B and *proC*$^+$ (7%) (DONCH and GREENBERG 1968).

Transduction of UV resistance: When P1 · W1485 was used to transduce *proC*$^+$ into PAM 2328 and PAM 43, 20% and 15%, respectively, of the transductants were nonmucoid and UV resistant. Survivals of these strains following UV are shown in Figure 3 together with those of the donor strain W1485. We concluded from these experiments that W1485 has a locus for UV resistance, a *lon*$^+$ allele, cotransducible with *proC*$^+$.

On the other hand, when P1 · B/r was used to transduce *proC*$^+$ into PAM 43 all of the 1241 transductants were mucoid and none were UV resistant. When *lac*$^+$ or *purE*$^+$ transductants of PAM 42 were selected, none of 802 and 987 transductants, respectively, were nonmucoid. The gene for UV resistance in strain B/r was not cotransducible with *proC*$^+$, *lac*$^+$ or *purE*$^+$.

We conclude from the data that strain B/r still has the *lon* gene of parental strain B. This gene is transducible with *proC* into a K-12 strain which then is phenotypically mucoid, UV sensitive and filament-inducible. The mutation in-

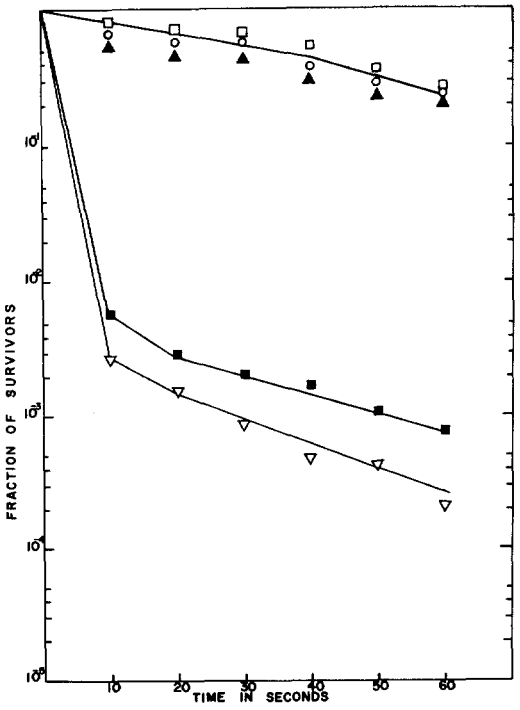


FIGURE 3.—Survival following UV irradiation of *E. coli* strain W1485 ▲, PAM 2328 ■, PAM 43 ▽, and two nonmucoid UV resistant *proC*$^+$ transductants of PAM 2328 and PAM 43, PAM 3713 □, and PAM 1528 ○, respectively.

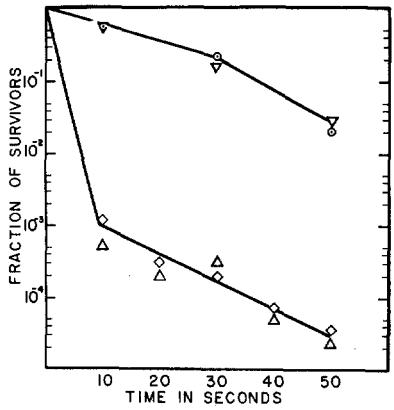


FIGURE 4.—Survival following UV irradiation of *E. coli* strain AB 261 ⊙, HB 45 ▽, B △, and PAM 2613 ◇.

volved in radiation resistance of B/r is at a locus between *lac* and *ara*, probably between *lac* and *ton*. Evidence derived from crosses between AB261 and HB45 strongly suggests this location of the *sul* locus and furthermore demonstrates the effect of the *sul*$^+$ locus on the B *lon* gene. The allele in strain B/r responsible for UV resistance suppresses the induction of filaments by UV. Its effect on mucoidy is unknown, since we have not transferred it into a strain capable of synthesizing mucoid polysaccharide. A cross between the Hfr strain HB33 and AB1899 should tell us whether it is possible to transfer the *sul* gene to a K-12 *lon* strain. We assume that the allele in K-12 is the same or similar to that in strain B. Also not yet known is whether all radiation resistant mutants of strain B are mutant at the *sul* locus or whether some are mutant at the *lon* locus.

SUMMARY

The gene responsible for UV resistance of strain B/r of *Escherichia coli* was investigated. A gene (*lon*) was cotransduced with *proC* from strain B/r to strain K-12 which produced a UV sensitive, mucoid, filament-inducible phenotype. UV resistance could not be cotransduced with *proC* (or *lac* or *purE*) from strain B/r to a K-12 *lon* strain. The gene for UV resistance in strain B/r is not an allele of *lon* but is located on the genome between *lac* and *ton*.

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