A LOCUS THAT CONTROLS FILAMENT FORMATION AND SENSITIVITY TO RADIATION IN ESCHERICHIA COLI K-12

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SEVERAL strains of *Escherichia coli* K-12 are about as resistant to ultraviolet light (UV) or to X-irradiation as *E. coli* B/r (ADLER and COPELAND 1962; WITKIN, 1947). This paper describes mutants of strain K-12 F⁻ that are relatively sensitive to radiation. These mutants tend to grow in long forms either after irradiation or when grown in nutrient broth without aeration and they form mucoid colonies when grown on minimal agar. The long form radiation-sensitive strains of K-12 will be referred to as *lon* and the parental strain with the wild-type allele as *lon*⁺.

It will be shown that, as with *E. coli* B/r (HOLLAENDER and STAPLETON 1956; ALPER and GILLIES 1960), the radiosensitivity of the *lon* strains is markedly affected by postirradiation growth conditions. The greater sensitivity to UV of the *lon* strains is not due to a loss of the capacity to reactivate UV-induced photoproducts in deoxyribose nucleic acid (DNA). This is shown by the equality in the numbers of plaques formed when UV-irradiated T1 phage are plated on *lon* and *lon*⁺ cells. The results of experiments in which *lon* strains are mated with Hfr cells indicate that the site of the *lon*⁺ locus in the Hfr chromosome is between the *lac* and *gal* markers, near the *T6* locus.

MATERIALS AND METHODS

Bacterial strains: The parental strain AB1157 was an auxotropic F⁻ strain of K-12 obtained from E. A. ADELBERG. The first *lon* mutant was isolated from a culture of AB1157 that had been exposed to UV. This mutant is referred to as AB1899 and was selected for investigation because of its high sensitivity to UV as judged by the survival of colony forming ability. Subsequently, several similar mutants were isolated by exposing log phase cells of the same strain AB1157 to UV so that one in about 10^3 survived. After the survivors had grown overnight in broth or supplemented minimal medium, aliquots were spread on minimal agar supplemented so as to support the growth of this auxotrophic strain. After incubation for 24 hours, the colonies that formed a mucoid exudate were picked and streaked twice. Single colony isolates were made, which, when restreaked, formed large and small colonies. Fresh isolates were made from the small colonies which are referred to as AB1157 *lon-2*, *lon-3*, *lon-4* and *lon-5* in this paper. Table 1 shows the characteristics of the F⁻ strains together with those of the Hfr strains used in the crosses, which were also obtained from DR. ADELBERG.

Media: The bacteria were grown and mated in broth containing 1 percent Bacto-tryptone, 0.5 percent yeast extract, 1 percent NaCl. To this was added 0.1 percent glucose when glucose broth was required. YET nutrient agar plates contained this broth with 2.2 percent agar (LURIA and BURROUS, 1957). Selective agar contained the following minimal medium: 50 mM phosphate at pH 7, 0.4 mM $MgSO_4$, 8 mM $(NH_4)_2SO_4$, 30 μM $Ca(NO_3)_2$, 1 μM $FeSO_4$ and 1.5 percent

TABLE 1

Characteristics of the K-12 strains

Strain	Auxotrophic characters						Energy-source utilization					Phage growth			Streptomycin resistance	Sex	*uvr*	*lon*	Injection order
	thr-leu	*pro*	*his*	*met*	*thi*	*arg*	*lac*	*gal*	*ara*	*xyl*	*mtl*	T1	T6	λ					
AB2593000	+	+	+	+	—	+	+	+	+	+	+	S	S	S	S	Hfr	+	+	*pyr, thr, ara*
AB2574000	+	+	+	—	+	+	+	+	+	+	+	S	S	R	S	Hfr	+	+	*T6, lac, pro*
AB451	—	+	+	+	—	+	—	+	+	+	+	S	S	S	R	Hfr	+	+	*pro, leu, thr*
AB1157	—	—	—	+	—	—	—	—	—	—	—	S	R	S	R	F⁻	+	+	
AB1899*	—	—	—	+	—	—	—	—	—	—	—	S	R	S	R	F⁻	+	—	
AB1886	—	—	—	+	—	—	—	—	—	—	—	S	R	S	R	F⁻	—	+	

* AB1899 (which may also be referred to as AB1157 *lon*-1) has the same markers as AB1157 *lon*-2, AB1157 *lon*-3, AB1157 *lon*-4, and AB1157 *lon*-5.

agar (MONOD, COHEN-BAZIRE, and COHN 1951). This was supplemented by amino acids, vitamins and a sugar, as required to select for recombinants with particular characteristics. The concentrations were: 0.01 percent DL-threonine, 0.01 percent DL-leucine, 10^{-5} percent thiamine, 0.02 percent L-arginine, 0.01 percent DL-histidine, 0.04 percent DL-proline. Glucose, arabinose, lactose or galactose was added at 0.2 percent as an energy source. Streptomycin was added at 200 μg per ml when required. The nutrient agar plates contained 1 percent agar, 1 percent tryptone, 0.8 percent NaCl, and 0.2 percent glucose.

Time of entry experiments: The order in which certain genetic markers on the Hfr chromosome enter the F^- strains was determined by the interrupted mating method (WOLLMAN and JACOB 1955; HAYES 1957; ADELBERG and BURNS 1960; JACOB and WOLLMAN 1961). The crosses were prepared from log-phase cultures by mixing 5×10^7 Hfr and 10^8 F^- bacteria per ml in glucose broth and allowing them to stand in a 2mm deep layer at 37°C. Samples were taken at intervals. When using the Hfr strains AB259 and 4000, aliquots were diluted in streptomycin broth, agitated in a vortex mixer, spread on selective media that contained streptomycin and incubated until colonies were visible. When using the Hfr strain AB451, the samples were incubated 15 min with 10^{10} T6 phage per ml to kill the sensitive males, and then plated on selective media. As a primary selective procedure for radiation resistant recombinants from the crosses with the Hfr strains AB259 and 4000, aliquots were spread on YET agar containing streptomycin and incubated 3 hr to allow time for phenotypic expression. They were then respread with buffered saline, exposed to 75 ergs per mm^2 UV, and incubated 24–36 hr at 37°C. The approximate time of entry of the *lon*+ allele was determined from the time at which the numbers of survivors rose steeply above the initial level. Recombinants from the various primary selections were tested for other characters by inoculating patches of each onto streptomycin YET agar master plates which were incubated overnight and then replica plated onto other selective media. To test for the presence of the *lon*+ allele, the patches were printed on YET agar, exposed to 1200 ergs per mm^2 UV, and incubated overnight. Growth in the *lon*+ patches was confluent, while only isolated colonies grew in the patches printed with *lon* cells.

Growth and assay of bacteria for radiation studies: Bacteria for the experiments on survival after UV-irradiation were grown overnight on YET agar and were harvested, washed and then suspended in 0.02 M phosphate 0.13 M NaCl at pH 6.8. After irradiation they were plated on YET or minimal agar and incubated overnight at 37°C. Cells for the X-ray experiments were grown and plated on nutrient agar plates and were suspended in the same buffer.

Irradiations: The UV source was a 15 watt low pressure mercury germicidal lamp. Suspensions of bacteria in the buffer were placed in a glass dish and UV-irradiated from above while being stirred magnetically. The suspensions were more than 90 percent transparent to the light of 2537A, which was measured with a General Electric Germicidal Light Meter. 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 oxygen. The dose rate, measured with a Victoreen ionization chamber, was about 3,750 rads per minute.

RESULTS

Figure 1 shows the fraction of bacteria that retain the capacity for colony formation when plated on minimal or YET agar after UV-irradiation. The fraction of *lon*+ cells that survive when plated on the two media is about the same, while *lon* cells appear to be much more radiosensitive when plated on YET agar. To test which of the ingredients of the YET plates were required to increase the UV-radiosensitivity of the *lon* strains, stationary phase cells were exposed to UV and then plated on media of various compositions intermediate between minimal and enriched. The results in Table 2 show that it is principally the presence of casamino acids together with sodium chloride in the medium that causes the higher level of radiosensitivity.

TABLE 2

Fraction of stationary phase K-12 lon *cells (strain AB1899) that survive irradiation with 500 ergs per mm² UV when plated on various media*

Medium					
Minimal and glucose, threonine, leucine, proline, histidine, arginine and thiamine	0.17M NaCl	1 μg/ml adenine, 1 μg/ml thymine, 1 μg/ml cytosine and 1 μg/ml guanosine	0.25% casamino acids	0.5% yeast extract 1% tryptone	Fraction surviving
+	..	..	..	..	0.48
+	+	..	..	..	0.39
+	..	+	..	..	0.35
+	+	+	..	..	0.29
+	..	..	+	..	0.10
+	..	+	+	..	0.13
+	+	..	+	..	.017
+	+	+	+	..	.019
+	+	..	..	+	.008
..	+	..	..	+	.006

Figure 2 shows the time of transfer of genetic markers in the cross 4000 × AB1899. Samples were taken at intervals from the mating mixture, diluted, agitated in a vortex mixer and plated on streptomycin agar. It is seen that *lon*+ is an early marker in this Hfr strain and enters about 3 minutes before *lac*+. Sensitive and resistant colonies formed on YET agar by the zygotes were different in appearance, the sensitive *lon* colonies being smaller, more convex and glistening than those of resistant Hfr *lon*+ strain. The time of entry of the wild-type *lon*+ allele could therefore be determined from the time of sampling at which the colonies with morphology characteristic of the Hfr strain first appeared on the irradiated plates. As seen in Figure 2, this occurred at the same time that the total number of colonies began to increase.

The order in which the markers on the Hfr chromosome entered the zygote was also determined from an analysis of unselected markers in the *pro*+ recombinants from the cross AB259 × AB1899. The results given in Table 3 confirm that the *lon* locus is between the *lac* and *gal* markers. The position of the *lon* locus was determined more precisely by analyzing the frequency with which switches occur between this locus and the neighboring markers. For this purpose the unselected

TABLE 3

Analysis of unselected markers from K-12 Hfr pro+ lac+ T6s lon+ gal+ his+ *AB259* × *F*- pro- lac- T6r lon- gal- his- *AB1899*

Primary selection	Time (minutes)	Number tested	Percent of unselected markers positive			
			pro+	*lac*+	*lon*+	*gal*+
pro+	20 and 30	115	100	74	50	4.4
lac+	20	64	86	100	66	7.8
lon+	25	115	77	82	100	1.7

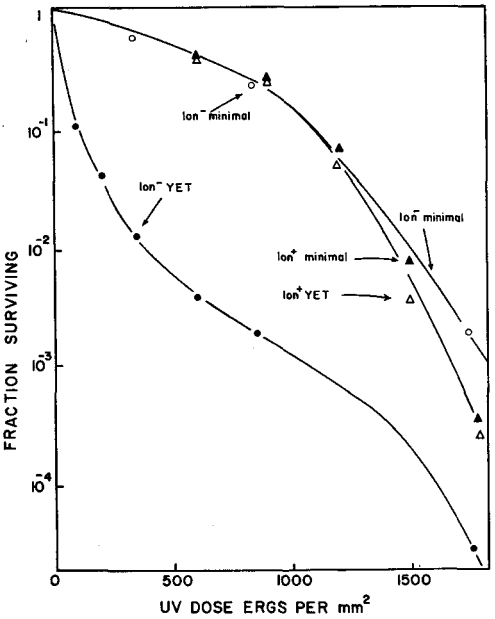

FIGURE 1.—The fraction of parental *lon*+ strain AB1157 and the long-form mutant *lon* AB1899 that survive ultraviolet irradiation when plated on minimal or on yeast extract tryptone (YET) agar. The bacteria were harvested from overnight cultures of YET agar, washed and suspended in buffered saline. After irradiation the cells were plated on the two media and incubated at least 24 hr at 37°C. A shoulder in the survival curve of the *lon* strain on minimal agar has been noted in many experiments.

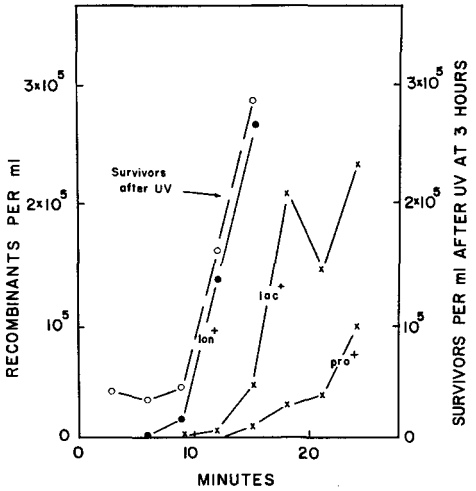

FIGURE 2.—The number of recombinants per ml from the cross K-12 Hfr *lon*+ AB4000 × F⁻ *lon* AB1899 as function of the time after mixing. Samples were taken at intervals, and were diluted, blended in a vortex mixer and plated on streptomycin agar. The entry of the *lac* and *pro* markers was determined from the number of colonies appearing on minimal agar plates supplemented to select for recombinants with *lac*+ and *pro*+. Other aliquots were spread on YET streptomycin agar and incubated for 3 hours. They were re-spread with buffered saline and exposed to 350 ergs/mm² UV. The total number of survivors per ml is shown by the dashed line, while the solid line marked *lon*+ shows the number of large flat colonies that were typical of the *lon*+ Hfr strain.

markers were analyzed in the *his*+ and *gal*+ selections from AB259 × AB1899. The frequencies with which switches occur between *lac* and *lon* or between *lon* and *gal* are shown in Table 4. Assuming that the observed frequency of switches is proportional to the distance between markers, it is calculated that the distance from *lac* to *lon* is 0.23 ± 0.10 (2 × standard deviation) of the distance between the *lac* and *gal* loci. Single colony isolates were made from zygote colonies and were grown on YET agar plates. The cells were harvested and exposed to various doses of UV.

As judged by the fraction of cells retaining the ability to form colonies, the

TABLE 4

Frequency of switches in his+ *and* gal+ *selections from Hfr* pro+ lac+ T6^s lon+ gal+ his+ *AB259* × *F*$^-$ pro$^-$ lac$^-$ T6^r lon$^-$ gal$^-$ his$^-$ *AB1899*

Primary selection	Number tested	Number of recombinants			
		lac+ *lon*$^-$	*lac*$^-$ *lon*+	*lon*+ *gal*$^-$	*lon*$^-$ *gal*+
his+	182	4	6	34	2
gal+	92	0	2	0	8
his+	36*	2	5	13	8
		19		65	
		lac+ *T6*^{r}	*lac*$^-$ *T6*^{s}	*T6*^{s} *gal*$^-$	*T6*^{r} *gal*+
his+	36*	1	6	17	5
		7		22	

* A group of 36 single colony isolates were selected from *pro*+ *gal*$^-$ or *pro*$^-$ *gal*+ patches and tested for *lac*+, *T6*^{r}, *lon*+ and *gal*+.

TABLE 5

Relation between colony type and resistance to radiation among progeny in zygote patches from Hfr pro+ lon+ his+ *AB259* × *F*$^-$ pro$^-$ lon$^-$ his$^-$ *AB1889*

Mating time (minutes)	Selection	Appearance of colony on minimal agar	Total number tested	Radiation resistant*	Radiation sensitive*
120	*his*+	mucoid	82	0	82
		nonmucoid	91	91	0
25	*pro*+	mucoid	57	0	57
		nonmucoid	56	56	0

* The sensitivity to radiation was generally judged by the patch test described under METHODS. Where the result was not clear-cut, single colony isolates were made and tested again. The fraction surviving after exposure to a UV dose of 350 ergs/mm^2 ranged from 70 percent to 90 percent for resistance cells, and 1 percent to 9 percent for sensitive cells, when plated on YET agar.

lon+ and *lon* progeny were very similar to the *lon*+ or *lon* parent strains, and no recombinants with intermediate properties were found.

Tests were then made to see if colony type and filament formation were always correlated with the increased sensitivity to UV in the progeny from AB259 × AB1899. An analysis was made of the recombinants in the *pro*+ and *his*+ selection which contained nearly equal numbers of *lon*+ and *lon*. In several cases where the progeny from a single zygote included both *lon*+ and *lon* characteristics, single colony isolates were made and tested. It is seen from Table 5 that the recombinants that formed mucoid colonies were always radiosensitive and no exception was detected in over 200 tests made. It appears that filament formation and the greater sensitivity to radiation are controlled by a very short length of the chromosome, and probably by the same locus.

Four UV-induced radiosensitive *lon* mutants of independent origin were isolated from mucoid colonies of AB1157 by the procedure already described. Each radiosensitive mutant in turn was crossed with the resistant Hfr strain 4000 and mating was interrupted at various times. In each case *lon*+, the wild-type allele

for radiation resistance in the Hfr chromosome, entered the zygote about 9 minutes after mating was started.

Figure 3 shows the fractions of stationary phase cells of these *lon* mutants that retain the capacity for colony formation after receiving various doses of UV. These independently isolated strains exhibit varying levels of sensitivity to ultraviolet irradiation. The position of the break in the survival curve is characteristic of each mutant.

To test the ability of *lon*$^-$ strains to reactivate UV-induced photoproducts in phage DNA, the bacteria were grown in YET glucose broth and used to seed the agar on which T1 phage that had been exposed to various doses of UV were plated. The *uvr*$^+$ strain AB1157 and the *uvr*$^-$ strain AB1886 were included in this experiment for comparison as they have been previously investigated (HOWARD-FLANDERS, BOYCE, SIMSON, and THERIOT 1962). (The locus in which these strains differ was formerly referred to as UV^R, but is being renamed *uvrA* to conform with the recommendations of the 1963 Microbial Genetics Bulletin.) The results presented in Figure 4 show that the survival of UV-irradiated T1 phage is about the same on *lon*$^+$ and *lon*$^-$ strains and is much higher than on the *uvr*$^-$ strain AB1886. Evidently, the reactivation of UV photoproducts in the phage DNA is not impaired in the *lon* mutants tested.

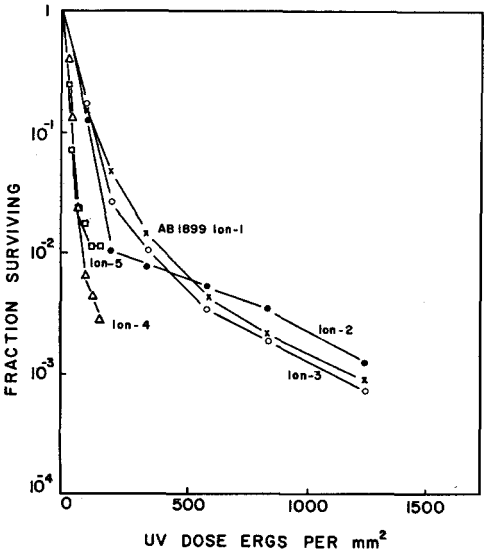

FIGURE 3.—The fraction of cells retaining the capacity to form colonies on YET agar after exposure to various doses of UV. AB1899 and the four other mutants, *lon-2*, *lon-3*, *lon-4* and *lon-5*, derived from AB1157 were grown overnight on YET agar.

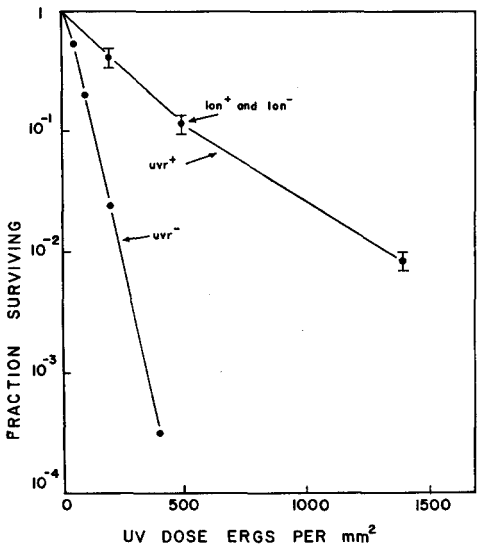

FIGURE 4.—The fraction of ultraviolet irradiated T1 bacteriophage that survive when plated on the *lon*$^+$ strain AB1157, and the *lon* mutants AB1899, *lon-2*, *lon-3*, *lon-4* and *lon-5*. Also shown is the fraction that survive when plated on the *uvr*$^-$ strain AB1886, which lacks the ability to reactivate UV-irradiated DNA.

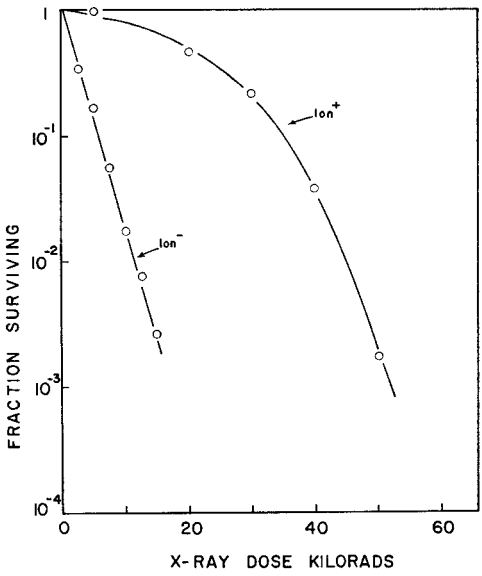

FIGURE 5.—The fraction of cells that retain the ability to form colonies when grown on nutrient agar after irradiation in oxygen with various doses of X rays. The *lon*+ strain AB1157 and the *lon* mutant AB1899 were harvested from overnight growth on nutrient agar.

As is shown in Figure 5, cells of the *lon*⁻ strain AB1899 are more sensitive than those of the *lon*+ strain AB1157 to the lethal effects of X rays. This experiment was carried out on cells harvested from the nutrient agar plates. We have confirmed the finding of ADLER (personal communication) that this difference is due to the greater radioresistance of *lon*+ cells grown on the glucose-containing nutrient agar. The survival of *lon*+ and *lon*⁻ cells after X-irradiation is almost identical when they are grown and plated on the YET agar.

DISCUSSION

The five independently isolated *lon*⁻ mutants of *E. coli* K-12 have similar properties. They tend to grow in long forms, they produce mucoid colonies on minimal agar, and they are abnormally sensitive to radiation. The similarity of all five suggests a causal relationship between filament formation, the tendency to form mucoid colonies and the higher radiosensitivity. But, this does not, of course, mean that all other mucoid mutants will be found to have these characteristics. The *lon*+ allele is situated on the Hfr chromosome between *lac* and *gal*, close to the *T6* locus. The position of the *lon* marker in strain AB1899 has been determined independently with concordant results in experiments that made use of the greater sensitivity to X rays of *lon* cells (ADLER and HARDIGREE 1964).

It is known from the analysis of the chemical composition of stable long-form strains of other bacteria, that they contain abnormal amounts of cell wall materials and fail to form rigid cell walls (WEIBULL 1958; PANOS, BARKULIS and HAYASHI 1959); EDWARDS and PANOS 1962). Both the mucoid character of the colonies formed on minimal media and the tendency to filament formation of the

lon strains suggests that there is an abnormality in the synthesis of cell wall precursors or in the control of cell volume, but the nature of the metabolic defect in the *lon* cells has not been determined. The relationship between filament formation and radiosensitivity has been discussed recently by VAN DE PUTTE, WESTENBROEK and RORSCH (1963). These authors give evidence that the *fil* locus may lie between the *gal* and *try* markers in strain B, some distance from the position between *gal* and *lac* assigned to the *lon* locus in this paper.

As regards sensitivity to radiation, K-12 *lon*$^+$ is similar to strains B/r, Bpr5 or B_{fil} (WITKIN 1947; ALPER and GILLIES 1960; RORSCH, EDELMAN, VAN DER KAMP 1962); K-12 *lon*$^-$ resembles strains B or B_{S-2}, while strains B_{S-1} and B_{syn-} (HILL and SIMSON 1961; ELLISON, FEINER and HILL 1960; RORSCH *et al.* 1963) resemble K-12 *uvr*$^-$ *lon*$^-$ (HOWARD-FLANDERS, SIMSON and THERIOT, in preparation). The strains K-12 *uvr*$^-$ (HOWARD-FLANDERS *et al.* 1962) and K-12 *hcr*$^-$ (HARM 1963) are more resistant as they carry the *lon*$^+$ allele of the wild-type K-12 strain.

There are two reasons for thinking that the greater sensitivity of the *lon* strains to UV is not due to a failure of the cell to reactivate DNA that contains UV photoproducts. This is shown first, by the similarity in the numbers of plaques formed by UV-irradiated T1 phage when plated on *lon*$^+$ and *lon* strains, and is in contrast to the results with sensitive mutants such as B_{S-1} (ELLISON, FEINER and HILL 1960) or K-12 *uvr*$^-$. Second, if the reactivation of photoproducts is needed for the *lon* allele to affect radiosensitivity, it would be expected that the *lon* allele would have no effect in nonreactivating cell lines such as K-12 *uvr*$^-$. It has been found, however, that the double mutant K-12 *lon*$^-$ *uvr*$^-$ is much more sensitive than the *lon*$^+$ *uvr*$^-$ strain, while these two strains show about the same numbers of plaques when used to plate UV-irradiated T1 phage (HOWARD-FLANDERS, SIMSON and THERIOT, in preparation). Evidently, the *lon* alleles can express in *uvr*$^-$ as well as in *uvr*$^+$ strains. The interpretation that appears to fit the data best is that *lon*$^-$ cells are less able than *lon*$^+$ cells to tolerate photoproducts in their DNA. This applies to the photoproducts that remain in the DNA after reactivation in the *uvr*$^+$ strain, as well as to the photoproducts remaining in the *uvr*$^-$ strain in which little reactivation is thought to occur.

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SUMMARY

Long form radiosensitive mutants were selected from among the progeny of survivors of an ultraviolet-irradiated parental strain of *E. coli* K-12 by their mucoid appearance on supplemented minimal agar. The parental strain was F$^-$ and carried various auxotrophic markers. Crosses with Hfr strains revealed that all these long-form cells carried a mutation at a locus designated *lon*, which is situated between *lac* and *gal*, close to T6. The parental *lon*$^+$ strain resembles B/r in

survival following X- or UV-irradiation, while *lon* mutants are relatively sensitive and are similar to strain B. The greater radiosensitivity of the *lon* mutants is not due to the loss of the capacity to reactivate UV-photoproducts in DNA because equal numbers of plaques are found when UV-irradiated T1 phage is plated on *lon* and *lon*$^+$ strains.

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