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The dominance of *exrB* over *exrB*$^+$ in heterodiploids of *Escherichia coli**

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SUMMARY

To test whether *exrB*, a mutation in *Escherichia coli* B making this strain sensitive to ultraviolet radiation and unable to divide normally, is dominant to *exrB*$^+$, merodiploids were constructed by crossing Hfr *exrB metA*$^+$ *his* × F$^-$ *malB metA thr proA recA*. *metA*$^+$ recombinants were fertile (F′) and sensitive to UV. One of these, F′ *exrB proA*, was crossed with an F$^-$ *metA malB thr*, selecting for *metA*$^+$ *proA*$^+$. All such progeny acquiring *malB*$^+$ were UV-sensitive, fertile and segregated *met mal* UV-resistant progeny. They were, therefore, meriodiploids, F′ *exrB*/*exrB*$^+$. *exrB* is dominant to *exrB*$^+$, in which respect it resembles *exrA* (*lex*).

1. INTRODUCTION

The sensitivity to UV and γ radiation of PAM 26, a mutant of *Escherichia coli* strain B ,was attributed to the mutant gene *exrB*, which is co-transducible with *malB* (Greenberg, Berends, Donch & Green, 1974). PAM 26 exhibits an abnormality in cell division such that it forms filaments spontaneously. In contrast, *exrA*, which is also cotransducible with *malB* (Donch & Greenberg, 1968*b*) and is associated with sensitivity to radiation, does not filament spontaneously, and in fact represses filamentation in *lon* strains even after irradiation (Donch, Green & Greenberg, 1968). Despite this difference in phenotypic expression, it is not certain whether *exrB* is an allele of *exrA*, though the high frequency of wild-type recombinants in crosses between them suggests it is not (Greenberg *et al.* 1974). While awaiting a genetic resolution of this problem, we have been looking for phenotypic differences between *exrA* and *exrB* strains other than their different effect on cell division. We have found that *exrA* is dominant over *exrA*$^+$ in *exrA*/*exrA*$^+$ heterodiploids (Donch & Greenberg, submitted) as is *lex* (Mount, Low & Edminston, 1972) with which *exrA* is probably isogenic (Donch & Greenberg, 1974). In this report we shall show that *exrB* is also dominant when on the episome of *exrB*/*exrB*$^+$ heterodiploids.

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2. METHODS

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

Phage. P1 *vir* and the methods for transductions have been described (Donch & Greenberg, 1968*a*).

Media. Complete and minimal media and diluents used have been described (Donch & Greenberg, 1968*b*).

Mating techniques. Matings were done as previously described (Donch & Greenberg, 1968*b*).

UV survival curves. Preliminary estimates of UV sensitivity were obtained by the rapid spotting method (Donch & Greenberg, 1968*b*). Definitive UV survival curves were done as previously described (Donch & Greenberg, 1968*a*). Colony counts were done on an automatic colony counter made to the design of Ingels, Daughters & Burzio (1968).

Fertility test. The test for fertility was performed by spotting broth cultures on minimal selection medium spread with a suitable F^- recipient. Plates were incubated for 48–72 h. A fertile strain yielded from 10 colonies to almost confluent growth within the spotted area. A control plate without recipient was used.

Construction of F-primes. The procedure was to perform sexual crosses between an Hfr donor which was *exrB* and a *recA* recipient, the 'recombinants' being mostly F′ (Low, 1968); *mal*$^+$ was introduced by transduction into PAM 26 from B251, conserving *exrB*. This *malB*$^+$ *exrB* strain in turn was used to transduce *malB*$^+$ into MPE (Hfr *malB his*), selecting a UV-sensitive (*exrB*) transductant (PAM 2611). To obtain F-primes in the region between *metA* and *thr*, JC5088 (Hfr *recA the ilv*) was first crossed with PAM 5764 (*his metA thr leu proA str*^{r}), selecting *his*$^+$ *ilv*$^+$ *str*^{r} recombinants, one of which was PAM 5788 (*recA metA thr leu proA str*^{r}). PAM 2611 was crossed with PAM 5788 selecting *met*$^+$ *his*$^+$. The few 'recombinants' which were observed were purified and tested for fertility by spotting on minimal agar, selective for *met*$^+$ *thr*$^+$ *his*$^+$, spread with strain PAM 5739 (*metA thr*). Those which were fertile were assumed to be F′, able to transfer an episome covering the region between *metA* and *thr*. One of these is F′ PAM 26.

3. RESULTS

The results of a cross between F′ PAM 26 × PAM 5739 are shown in Table 2. Selection was made for Met$^+$ Pro$^+$ and the frequency of unselected donor markers determined in the F-ductants. Seventy per cent of the Met$^+$ acquired *malB*$^+$, and all of these were UV-sensitive; most of these Mal$^+$ UV-sensitive Pro$^+$ F-ductants also acquired *thr*$^+$. Thirty per cent of the Met$^+$ Pro$^+$ F-ductants acquired only the *metA*$^+$ marker and all of these were UV-resistant. Recipients which acquired donor genetic material sufficiently large to incorporate the *malB*$^+$ marker always acquired *exrB* but not necessarily *thr*$^+$. This is consistent with previous experience showing *exrB* to be closely linked to *malB* with the order of markers *metA malB exrB thr* (Greenberg *et al.* 1974).

No differences in UV sensitivity could be detected by the rapid spot method for any of the Mal$^+$ UV-sensitive sexductants. A definitive UV survival curve of one of these sexductants (PAM 2639) is shown in Fig. 1 together with that of parental strain PAM 5739 and PAM 5725, a haploid *exrB* transductant of PAM 5764. The F-ductant was as sensitive to UV as PAM 57245.

Table 1. *Bacterial strains used*

Strain	Relevant markers	Source
PAM 26	F$^-$ *exrB malB*	This laboratory
B251	F$^-$ *malB*$^+$	W. Arber
AB1911	F$^-$ *metA purD*	E. Adelberg
AB1157	F$^-$ *arg thr leu proA his str*^{r}	E. Adelberg
PAM 5764	F$^-$ *metA thr leu proA his str*^{r}	P1.AB1911 × AB1157
JC5088	Hfr *redcA thr ilv*	A. J. Clark
PAM 5788	F$^-$ *recA metA thr leu proA str*^{r}	JC5088 × PAM 5764
MPE1	Hfr *malB his*	M. Schwartz
PAM 5739	F$^-$ *metA malB thr*	MPE1 × PAM 5764
PAM 27	F$^-$ *exrB mal*$^+$	P1.B251 × PAM 26
PAM 2611	Hfr *exrB his*	P1.PAM 27 × MPE1
PAM 2639	F′ *exrB/exrB*$^+$ *thr*	This paper
F′ PAM 26	F′ *exrB/recA proA*	This paper
PAM 5725	F$^-$ *exrB malB*$^+$	P1.PAM 27 × PAM 5764
PAM 5740	F$^-$ *metA malB*	From PAM 5764

Table 2. *Phenotypes produced in the cross F′ PAM 26* metA$^+$ malB$^+$ thr$^+$ proA exrB × *PAM 5739* metA malB thr proA$^+$ exrB$^+$, *selecting* met$^+$ pro$^+$

		Frequency (%) of phenotypic classes			
		Mal$^+$		Mal	
No. of Met$^+$ F-ductants examined		UV^s	UV^r	UV^s	UV^r
50	Thr$^+$	56	0	0	0
	Thr	14	0	0	30

UV^s, sensitive to ultraviolet radiation. UV^r, resistant to ultraviolet radiation.

To determine whether the UV-sensitive F-ductants were recombinants or heterodiploids we examined the progeny of two representative strains for segregation. After growth in non-selective JN broth, cultures were plated at appropriate dilutions on EMB maltose agar and irradiated with 200 ergs/mm^2 UV light. Many survivors were Mal, and all of these which were examined were Met and not fertile. This indicates that, when UV-sensitive were F-ductants grown under conditions which exerted no selective pressure for either Met$^+$ or Mal$^+$, the cultures were enriched for UV-resistant segregants indistinguishable from the recipient parent. When maintained in selective minimal medium no segregation was observed.

To determine if the F-ductants were fertile diploids, five of the strains which were UV-sensitive, Mal$^+$ and Thr were tested for fertility using PAM 5740 (*metA exrB*$^+$) as recipient. The selection was for Met$^+$ Thr$^+$. Clones able to grow on

minimal medium were observed in all spots. One of the presumably fertile, UV-sensitive, Mal+ Thr F-ductants was used as a donor in a cross with PAM 5740 with selection for Met+ Thr+. All of the 50 F-ductants examined were Mal+ and as sensitive to UV as the donor. Therefore the UV-sensitive (*exrB*) Mal+ and Thr F-ductants of the original cross, F′ PAM 26 × PAM 5739, were fertile, and the

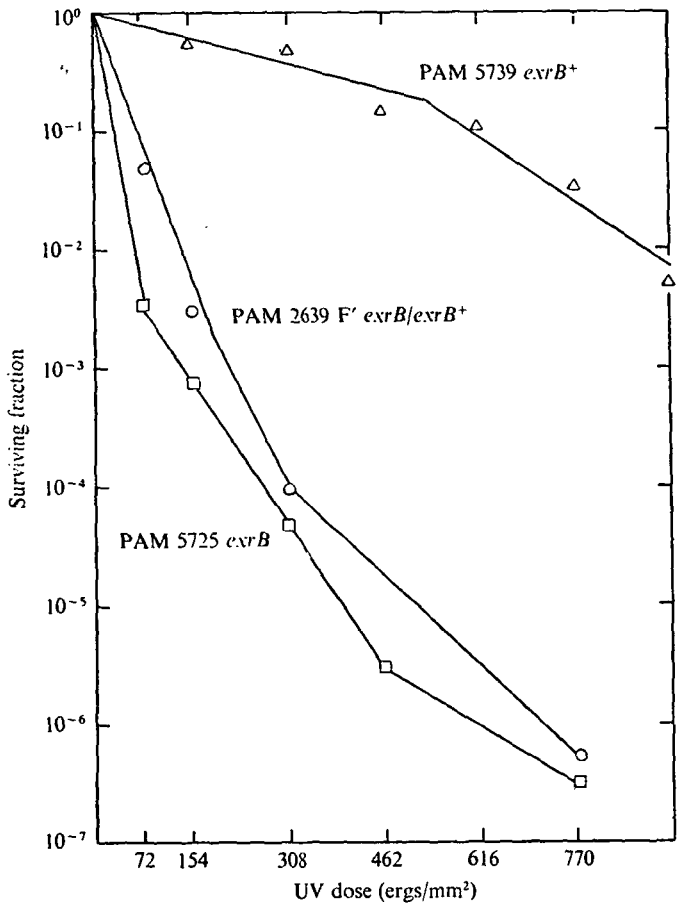

Fig. 1. Survival after UV of heterodiploid and haploid *exrB* strains of *E. coli*.

F-ductants obtained, when these in turn were used as donors in a cross with a UV-resistant recipient, were also UV-sensitive. Thus *exrB* on the episome of heterodiploid, *exrB/exrB*+ strains, is dominant.

4. DISCUSSION

The results demonstrate that *exrB* is dominant over *exrB*+ with regard to colony-forming-ability following UV. In this respect it resembles *exrA* (Donch & Greenberg, submitted for publication) and *lex* (Mount, Low & Edminston, 1972),

both of which are genes associated with UV sensitivity and closely linked to *malB*. Because *exrA*, *exrB* and *lex* are dominant over their wild-type alleles, demonstration of complementation among them would be impossible; *exrA* and *lex* behave identically with respect to all phenotypic expressions for which they have been measured and it has not been possible to demonstrate wild-type recombinants in crosses between strains containing them (Donch & Greenberg, 1974). This has led us to conclude that *lex* and *exrA*, independently isolated in two different strains of *E. coli*, B and K12, respectively, are isogenic.

The decision as to the relationship between *exrA* (*lex*) and *exrB* is more difficult. The precise mechanism of action of the products of $exrA^+$ and $exrB^+$ are not known. Furthermore, though the dominance of *exrB* and *exrA* in the episome of heterodiploids suggests that both mutant genes are involved in the production of a diffusible product, this has not been identified in either case. While the phenotypes associated with *exrA* and *exrB* are similar in many respects, such as sensitivity to radiation of wavelengths equal to or shorter than UV, sensitivity to monofunctional alkylating agents, reduced and delayed induction of prophages, degradation of DNA following UV, and failure of UV to induce mutations, they differ in one important way. Whereas *exrA* inhibits the formation of filaments even in irradiated *lon* strains, filamentation occurs spontaneously in *exrB lon* strains. This suggests that both genes are involved, though with different effects, in the control of cell division, and does not preclude the possibility that they are mutants of the same cistron.

Another difference between the two genes is that whereas *lex* and *exrA* strains are heat-stable (Mount, Walker & Kosel, 1973 and unpublished observations), *exrB* strains are unable to divide at an elevated temperature (Donch & Greenberg, submitted for publication). In fact, even $exrB\ lon^+$ strains form filaments at 42 °C, at which temperature the synthesis of DNA is inhibited. In these respects *exrB* strains resemble *ts dnaB* strains, the *dnaB* cistron also being closely linked to *malB* (Weschler & Gross, 1971); *ts dnaB* mutants are not associated with sensitivity to UV, though one, FA22, is associated with sensitivity to X-rays (Fangman & Novick, 1968). It might appear that, based on similarities of phenotypes, *exrB* may be isocistronic with *dnaB*, but preliminary evidence derived from complementation between PAM 26 (*exrB*) and FA21 (*dnaB*) suggests that these two mutations are in different cistrons.

Returning to phenotypic differences between *exrA* and *exrB* strains, neither the temperature sensitivity associated with *exrB* nor the different effect of the two mutations on cell division precludes the possibility that they are mutants of the same cistron. What suggests that they might be mutants of different cistrons is the observation that wild-type recombinants occurred at a relatively high frequency in crosses between strains containing each of them (Greenberg *et al.* 1974). Since this evidence is not conclusive, several possible genetic models are conceivable. In one the mutants, *exrA* (*lex*) and *exrB*, are mutations of one cistron with different phenotypic expressions, depending on the altered gene product. In another model these are mutants of different cistrons, forming an operon concerned

with DNA synthesis and cell division, and which is involved in some way with repair of damaged DNA.

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