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Conditional Lethality of Deletions Which Include *uvrB* in Strains of *Escherichia coli* Lacking Deoxyribonucleic Acid Polymerase I

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Deletions of the *uvrB* gene were not obtained in *polA1* strains of *Escherichia coli* either by selecting for spontaneous deletions or by transduction from strains carrying such deletions. A strain forming a temperature-sensitive deoxyribonucleic acid polymerase I and carrying a deletion of the *uvrB* gene is inviable at the nonpermissive temperature.

DeLucia and Cairns (4) isolated a mutant (*polA1*) of *Escherichia coli* which is deficient in deoxyribonucleic acid (DNA) polymerase I activity in vitro. Although the mutant has increased sensitivity to ultraviolet light (UV), X rays, and methylmethanesulfonate (MMS), it grows almost normally (4, 5).

In this report, we describe deletions of the genes around the attachment site of lambda in a strain carrying the *polA1* mutation. Even though normally a deletion from *gal* through *chlA* is viable (1), no deletions including the *uvrB* gene were found in strains carrying the *polA1* mutation. *uvrB* deletions were selected in strain MM383 which carries *polA12* (11), a temperature-sensitive DNA polymerase I mutation, at the permissive temperature (30 C). The *polA12 uvrB*Δ strain was inviable at the nonpermissive temperature (42 C). At the nonpermissive temperature, there was rapid death in growing cultures, but not in nongrowing cultures.

MATERIALS AND METHODS

Bacterial strains and phages. The bacterial strains employed in this study are listed in Table 1, and the extent of each deletion is shown in Fig. 1. *polA*$^+$ revertants were selected on 0.05% MMS plates. The *polA* revertant strains were examined for resistance to UV and ability to support growth of λ *red* and γ mutants (λ *red* and γ mutants fail to grow in *polA* strains [16]). HS323 is a single lysogen of P3478 for λ *cI857xis1*. λ *cI857xis1* is deficient in site-specific excision of lambda genome from the host chromosome but can integrate into the host chromosome normally at 30 C (7, 9). Phage P1*kc* was used for generalized transductions experiments.

Media. Tryptone broth (2) and tryptone agar (2) were routinely used for bacterial growth. Eosin methylene blue agar containing galactose (EMBG) was prepared as described by Campbell (2). The *pgl* test (*blu*) was done as described by Sato and Campbell (15). Selection for chlorate-resistant deletions and the chlorate test were done by using the media and procedures described by Adhya, Cleary, and Campbell (1). Synthetic agar and synthetic medium (2) were supplemented with 10^{-3} μg of biotin per ml to provide for the biotin requirement, thymine (10 μg/ml), and casein amino acids (150 μg/ml).

P1 transduction procedure. Phage P1 was added to 5×10^8 cells per ml grown in L broth (10) containing 0.25 mM $CaCl_2$ (multiplicity of infection of 0.1). Twenty minutes were allowed for adsorption. The mixture was spun down, resuspended into 0.85% sodium chloride, and plated on selective plates. After the transductants were purified once, they were tested.

Test for UV sensitivity. UV sensitivity was tested by two methods. (i) Colonies were "stabbed" onto tryptone agar plates (master plates), incubated overnight at 37 C, and then replicated onto previously dried tryptone agar plates (daughter plates). The daughter plates were exposed to UV. (ii) A colony was suspended in a drop of 0.01 M $MgSO_4$. A loopful of each suspension was streaked on previously dried tryptone agar plates. After drying, half of each streak was irradiated by UV. Since *polA* is only moderately sensitive to UV and the *polAuvrB* double mutant has essentially the same sensitivity as *uvrB* alone, an appropriate dose (200 ergs/mm^2) of UV exposure distinguishes a *polA*$^-$ *uvrB*$^+$ strain from a *polA*$^-$ *uvrB*$^-$ strain. Method i was used to determine whether or not the *uvrB* gene had been deleted in the survivors of the lysogens. Method ii

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was employed for P1 transduction experiments. Any questionable colonies when method i was used were tested again with method ii.

Isolation of deletion mutants. When cells carrying λ *cI857* prophage are heated to 42 C, repression is released and phage development and cell death proceed in almost every cell. Most of the heat-resistant survivors derived from a λ *cI857* lysogen are spontaneously cured cells (13). To prevent extensive curing, a λ*cI857xis1* lysogen was used. The rare heat-resistant survivors derived from this lysogen often have deleted the prophage genes responsible for cellular death following induction. In some cases the deletion extends into adjacent bacterial genes.

To obtain deletions, overnight shaking cultures were appropriately diluted and spread on tryptone plates with 1 ml of anti-λ serum (K = 10 min^{-1}) per plate. The plates were incubated overnight at 42 C.

RESULTS

Array of deletions isolated from strains HS323 and HS340. Upon heating the λ *cI857xis1* lysogens, the average frequency of survival was 1.8×10^{-7} for HS323 and 2.1×10^{-7} for HS340. About 500 survivors, obtained from five independent tubes (about 100 per tube), were examined for utilization of galactose and UV sensitivity. The results are shown in Table 2. The majority of the survivors for both HS340 and HS323 were *gal*$^+$ and *uvrB*$^+$;

TABLE 1. *Bacterial strains and bacteriophages*[a]

Strain	Characteristics	History or source
P3478	*polA1*, *thy*$^-$	DeLucia & Cairns (4)
C173	Δ(*bioD-chlA*), *his*$^-$	P. Cleary, Ph.D. thesis, Univ. of Rochester, 1971
SA420	Δ(*uvrB-chlA*), *his*$^-$	S. Adhya (1)
HS351	Δ(att λ-*uvrB*), *thy*$^-$	Heat-resistant survivor of HS340
HS352	Δ(*gal-bioA*), *polA1*, *thy*$^-$	Heat-resistant survivor of HS323
HS353	Δ(*gal-bioA*), *thy*$^-$	*polA* revertant of HS323
MM383	*polA12*, *thy*$^-$	M. Monk & J. Kinross (11)
HS405	Δ(*gal-chlA*), *polA12*, *thy*$^-$	Chlorate-resistant isolate of MM383
HS406	Δ(*gal-chlA*), *thy*$^-$	*polA* revertant of HS405
HS323	*polA1*, *thy*$^-$, λ*cI857xis1*	λ lysogen of P3478
HS340	*thy*$^-$, λ*cI857xis1*	*polA* revertant of HS323
N3-1	*trp*$^-$, *gal*$^-$, *uvrB51*	H. Ogawa (14)
N7-1	*trp*$^-$, *gal*$^-$, *uvrB53*	H. Ogawa (14)
N314	*trp*$^-$, *uvrB52*, *uvrD3*	H. Ogawa (14)
λ *cI857xis1*	Excision-negative mutant	A. Campbell (7)
P1*kc*		A. Campbell

[a] All bacterial strains are derivatives of *E. coli* K-12; λ *cI857xis1* and P1*kc* are bacteriophages.

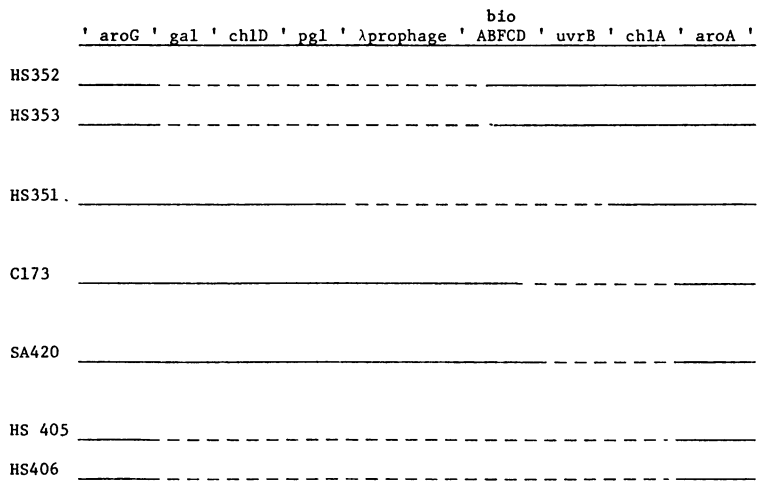

FIG. 1. *Deletion map of the bacterial strains employed. Dotted lines represent deleted segments. HS405 and HS406 were obtained from nonlysogens.*

12/513 were *gal*$^-$ *uvrB*$^+$ in HS340 and 7/525 were this genotype in HS323. Thus, deletions removing *gal* but not *uvrB* can be found with approximately the same frequency in the two strains. There were 134/513 *gal*$^+$ *uvrB*$^-$ survivors for the HS340 strains, and no *gal*$^+$ *uvrB*$^-$ survivors for the HS323 strains. Likewise, there were 21/513 *gal*$^-$ *uvrB*$^-$ survivors for HS323. From another tube of HS323, a deletion was isolated which extended into *bio* but not into *uvrB*. This is strain HS352. Whereas deletions of various extent including deletions of *gal* and *uvrB* were produced in HS340, no deletions extending into the *uvrB* gene could be found in HS323.

Transduction of a uvrB deletion into HS352 and HS353. One explanation for this failure to obtain deletions through *uvrB* in a *polA* strain might be that the deletion mechanism is affected by the *polA* mutation. To check the possibility that deletions of *uvrB* are viable in *polA*, but just arise very infrequently, a deletion was transferred to *polA* cells with P1 transduction. The recipients used for transduction were HS352, which is a *polA* strain with deletion from *gal* into *bioA*, and its *polA* revertant, HS353. The deletion in strain C173, which extends from *bioD* through *chlA*, was transduced into HS352 and HS353. The *gal*$^+$ transductants were selected and tested for UV sensitivity. Thirty-two out of 39 of the *gal*$^+$ transductants tested from HS353 carried the deletion of *uvrB*, whereas none out of 95 of the *gal*$^+$ transductants tested from HS352 carried the deletion of *uvrB*. As shown in Table 3, the number of the *gal*$^+$ transductants per input phage in HS352 was 12% of the number in HS353. Seven out of 39 or 18% of the *gal*$^+$ transductants of HS353 were *uvrB*$^+$. Thus, the frequency of *gal*$^+$ transductants seen in *polA* cells nearly equals the frequency of *gal*$^+$ *uvrB*$^+$ transductants in *polA*$^+$ cells. This suggests that the deletion is transduced at the same frequency to both HS353 and HS352, but that the deletion carried by the donor is lethal in HS352.

Mapping of the lethal factor. Overlapping deletions were tested for viability with *polA* to determine the location of the gene. As shown in Fig. 1, the deletion in HS351 overlaps the deletion in the recipient strains, HS352 and HS353. No *gal*$^+$ transductants of HS352 were obtained from HS351 (Table 3). And, as expected, all the *gal*$^+$ transductants of HS353 include the deletion of *uvrB*. The short deletion from *uvrB* to *chlA*, carried by SA420, also could not be transferred into HS352. All the *gal*$^+$ transductants in HS352 were *uvrB*$^+$. These results indicate that the lethal factor is between the left end of the deletion in SA420 and the right end of the deletion in HS351. It may be either the *uvrB* gene itself, or another gene to the left or the right of *uvrB* gene.

Absence of the *uvrB* gene might be lethal in *polA* cells. To test this, three point mutants of *uvrB* from Ogawa et al. (14), which were isolated independently, were transduced into HS352 and HS353 using phage P1*kc*. The *bio*$^+$ transductants were selected on synthetic agar plates without the biotin or casein amino acids added and then tested for sensitivity to UV. The results of these studies are shown in Table 4. The frequency of *bio*$^+$ transductants did not differ in HS353 and HS352. The percentage of cotransduction of *bio* and *uvrB* is similar in both HS353 and HS352. Three-day-old *uvrB52 polA1* double mutants kept on tryptone agar

TABLE 2. *Analysis of the bacterial survivors of heating a λ cI857xis1 lysogen*[a]

Galactose utilization (*gal*)	UV resistance (*uvrB*)	No. of survivors tested	
		HS340	HS323
+	+	346	518
+	−	134	0
−	+	12	7
−	−	21	0
Total		513	525

[a] The test for galactose utilization and UV sensitivity was described in Materials and Methods; +, presence of the gene; −, absence of the gene.

TABLE 3. *Transduction of the deletion of uvrB gene into polA1*[a]

Donor	Recipient	*gal*$^+$ transductants/ input phage	*gal*$^+$*uvrB*$^-$/ *gal*$^+$[b]	*gal*$^+$*bio*$^+$ *uvrB*$^+$/ *gal*$^+$[c]
C173	HS352	4.0×10^{-7}	0/95	95/95
	HS353	3.3×10^{-6}	32/39	7/39
HS351	HS352	$<1.5 \times 10^{-8}$	0/0[d]	
	HS353	3.4×10^{-6}	19/19	
SA420	HS352	1.4×10^{-6}	0/33	
	HS353	3.4×10^{-6}	23/31	

[a] The recipients were treated with lysates of P1*kc* grown on the donors at a multiplicity of 0.1 and plated on EMBG plates.

[b] The number of *gal*$^+$ transductants which are UV sensitive, divided by the total number of *gal*$^+$ transductants which were purified and tested.

[c] This applies only to the first experiment. It is the number of transductants tested which carried neither deletion, divided by the total number tested.

[d] No transductants were recovered.

TABLE 4. *Transduction of the uvrB point mutation into polA1*[a]

Donor	Recipient	*bio*$^+$ transductants/ input phage	*bio*$^+$*uvrB*$^-$/ *bio*$^+$[b]
N3-1	HS352	5.7×10^{-6}	22/24
	HS353	5.7×10^{-6}	15/22
N7-1	HS352	7.3×10^{-6}	14/23
	HS353	5.7×10^{-6}	19/23
N314	HS352	1.7×10^{-5}	7/36
	HS353	1.9×10^{-5}	12/37

[a] The recipients were treated with lysates of P1*kc* grown on the donors at a multiplicity of infection of 0.1 and plated on minimal media containing thymine.

[b] This is the fraction of the *bio*$^+$ transductants tested which were sensitive to UV.

plates at room temperature did not survive. The same results were obtained in *uvrB polA1* and *uvrB53 polA1* transductants.

Robert Helling (*personal communication*) constructed a *polA1 uvrB* strain by using the presumptive *uvrB* mutation in *E. coli* B_{s-1} (8). He found that this strain grows on synthetic medium but not on complex media. We find that *polA1 uvrB51* is less viable on tryptone than on synthetic medium.

Construction of the double mutant containing a deletion of uvrB with a temperature-sensitive polA mutation. Recently, Monk and Kinross (11) have isolated a strain (MM383) with a temperature-sensitive mutation *polA12* which is as sensitive to MMS and UV as *polA1* at 42 C but less sensitive at 30 C. We made deletion mutants of *uvrB* gene in this strain by selecting chlorate-resistant strains (the method is described in reference 1). The *chlA* locus has been mapped between *uvrB* and *aroA* (1), and some deletion mutations may extend into the *uvrB* gene. Thirty-eight chlorate-resistant, UV-sensitive strains were isolated at 30 C. All of these and only these were the ones which failed to make colonies at 42 C. One of those clones (HS405) has been studied further.

Growth of the double mutant. A *polA*$^+$ revertant (HS406) of HS405 (which contains a deletion from *gal* through *chlA* as well as the *polA12* mutation) was isolated as an MMS-resistant colony. Figure 2 shows the growth curves of MM383 (panel a), HS405 (panel b), and HS406 (panel c) at 30 C and after transfer to 42 C. After the shift to 42 C the number of cells of HS405 increased continuously up to 90 min at a reduced rate of growth as compared with HS406 and MM383. After that, the number of viable cells in HS405 decreased gradually. Only 40% of the viable cells of HS405 at 90 min die within 6 hr. The optical density of HS405 continued to increase up to the plateau density at 42 C. At this time most of the cells are filaments as judged by microscopic examination. At 2 hr after the shift the cultures were diluted 10-fold into fresh T broth at 42 C. About 99% of the cells of HS405 died within 2 hr. Such dilution was not lethal to HS405 at 30 C. This suggests that the cells need to be growing for the lethal effect of the *polA*$^-$ *uvB*Δ to be expressed. To test this, another series of cultures of HS405, HS406,

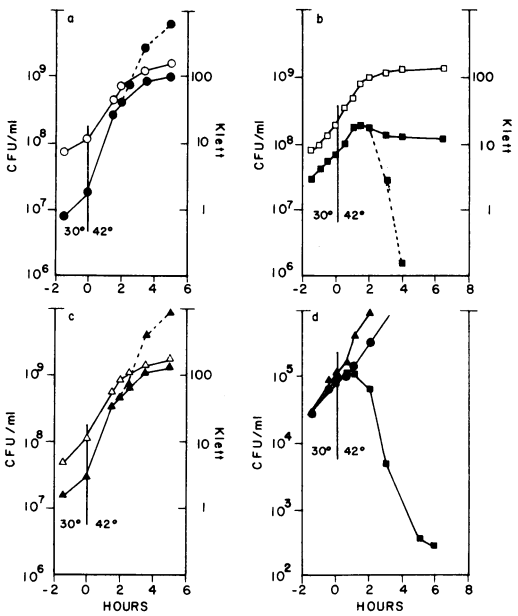

FIG. 2. *a, b, and c, Growth properties of MM383 (polA12), HS405 (polA12, uvrB deletion), and HS406 (polA*$^+$ *revertant of HS405). Strains grown in tryptone broth supplemented with thymine (10 μg/ml) were brought to exponential growth phase at 30 C. The cultures were then transferred to a 42 C water bath. After 2 hr at 42 C a portion of the cultures was diluted 10-fold into prewarmed, fresh tryptone broth and incubated at 42 C. Viable cells were measured by spreading 0.1 ml of dilute samples on tryptone plates. Colony-forming units (CFU) were counted after overnight incubation at 30 C. The number of viable cells in the diluted cultures was multiplied by 10 when the data were plotted. Optical densities were followed in a Klett-Summerson colorimeter. Closed symbols signify CFU; open symbols, optical density. For each graph, the solid line represents the original culture and the broken line represents the diluted culture. d, The viable count of the three cultures followed at low cell density using the same procedure as above. MM383 and HS406 continued to grow exponentially at the same rate until the experiment was terminated at the end of 6 hr. ●, MM383; ■, HS405; ▲, HS406.*

and MM383 were set up at a low density so that the culture would not reach stationary phase until more than 6 hr after the shift to 42 C. Figure 2, panel d, shows the growth curves for viable cells for these three cultures. The growth rate of the three cultures was the same at 30 C. After the shift to 42 C the growth rates of MM383 and HS406 were the same after the initial adjustment. However, the number of colony-forming units of HS405 increased for only 30 min. By 90 min, the rate of death of the HS405 culture was similar to that of the diluted culture. Ninety-nine percent of the cells died within 3 hr.

Another culture of HS405 was grown the same way as the one shown in Fig. 2b. After 2 hr at 42 C the culture was divided into three parts. One part continued growing at 42 C; the other two were centrifuged. One of these was resuspended in its own medium, another in the same volume of fresh tryptone. All three cultures had the same number of cells per milliliter. The control culture and the culture which was resuspended in its own medium showed little death, but the culture suspended in fresh tryptone showed rapid death as the diluted culture had.

This procedure was then repeated with the cells of strain HS405 being resuspended in a salt solution (the same salts as were used in synthetic medium). Even though the medium was fresh, the cells did not die as they did in the analogous experiment using fresh tryptone.

Cells of HS405 were grown in synthetic medium at 30 C, washed, and suspended in salts at a density of about 10^5 cells/ml. These cells were kept at 30 C for 0.5 hr to permit any residual growth and then shifted to 42 C. After 3.5 hr at the nonpermissive temperature there was no decrease in the number of colony-forming units. When a culture of HS405 was diluted to about 10^5 cells/ml in synthetic medium, the cells died at 42 C, even though MM383 and HS406 grew. The salts in the synthetic medium did not prevent death when growth was possible.

DISCUSSION

Because the strains compared here carry various mutations of *polA* and revertants, the *polA* mutation is necessary for the lethal effect. We were unable to get deletions of *uvrB* in HS352, a strain possessing the *polA1* mutation, either by selecting for spontaneous deletions or recombination between HS352 and strains carrying such deletions. It is unlikely that the failure to obtain deletions of *uvrB* in HS352 is due to an abnormal deletion mechanism, since the *polA* mutation does not alter the distribution of end points of *tonB-trp* deletions (3) and does not affect genetic recombination (5).

As shown in Fig. 2, we found no increase in the frequency of deletions from *gal* through the λ prophage in strains carrying a *polA* mutation. This result is different from the result found by Coukell and Yanofsky (3). They found an increased deletion frequency of the *tonB-trp* region in *polA*$^-$ strains over *polA*$^+$ strains. They suggested that the increased frequency was due to an increased frequency of spontaneous deletions caused by an increased number of unrepaired single-strand breaks in the DNA. This explanation suggests that an increased frequency of deletions in *polA*$^-$ strains would be found for all regions of the *E. coli* chromosome which can be deleted. It is difficult to reconcile this explanation with our result. Instead, we suggest that the effect of the *polA* mutation on the frequency of deletions is different in different parts of the chromosome, depending upon the local structure of the chromosome in the deleted region.

There is a difference between the viability of *uvrB* point mutations and deletions covering *uvrB* in *polA*$^-$ strains. The point mutation survives on synthetic medium whereas the deletion does not. The poor viability or inviability of the *uvrB* point mutation with *polA1* on tryptone suggests that the loss of the *uvrB* gene in the deletion is at least part of the cause of the lethal effect. The difference between the point mutations and the deletion may be explained either by postulating that the viability of the point mutations results because they are a little leaky or by postulating that the inviability of the deletion is caused by the loss of an unrecognized gene which is closely linked to *uvrB*.

The effects of mutations in genes involved in DNA repair and genetic recombination have been tested with *polA* mutations. A *uvrA6 polA1* strain is viable on tryptone (12). The difference in the viability of *uvrB* and *uvrA* mutations with *polA1* is a phenotypic difference between two genes which previously have been very similar phenotypically. However, if the main effect on viability in the deletions is due to an unknown linked gene, then the suspected difference between *uvrB* and *uvrA* with *polA1* may disappear with further examination.

A *polA1 recA* double mutant is inviable (6). Both *polA12 recA56* and *polA12 recB21* are

inviable at the nonpermissive temperature (11). Thus, both the UV repair system and the *rec* system seem to be linked to DNA polymerase I in some important way. However, we have reconfirmed other observations that *recB uvrB* double mutants are quite viable.

Strain HS405 (*polA12 uvrB*Δ) dies rapidly at the nonpermissive temperature when it is growing, but not when suspended in salts or at stationary phase. That there is no rapid death of HS405 in salts suggests that the death is not caused by leakage of metabolites. That there is death in synthetic medium suggests that the death is not caused by some undefined factor in tryptone. Rather, the linkage of the lethality with growth suggests that DNA polymerase I and some gene product, either that from the *uvrB* gene or from some unknown gene linked to *uvrB*, functions in growth, presumably in some manner related to DNA synthesis.

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