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THE EXPRESSION OF LIQUID HOLDING RECOVERY IN ULTRAVIOLET-IRRADIATED *ESCHERICHIA COLI* REQUIRES A DEFICIENCY IN GROWTH MEDIUM-DEPENDENT DNA REPAIR

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Abstract—Ultraviolet (UV) irradiated *Escherichia coli* K-12 *recA* cells (but not *rec*$^+$ cells) show enhanced survival if they are held in buffer prior to plating for viability. To understand the role of the *recA* mutation in this liquid holding recovery (LHR) phenomenon, we have studied LHR in a temperature sensitive *recA*200 mutant. The detection of LHR requires that the irradiated cells be *recA*$^-$ when they are plated on growth medium, but the *recA* deficiency plays no role during liquid holding (LH). We conclude that it is the extreme sensitivity of *recA* cells in growth medium to unrepaired DNA daughter-strand gaps that magnifies the beneficial effects of the excision repair of DNA lesions during LH. Furthermore, we demonstrate a correlation between a strain's inability to perform growth medium dependent repair and its ability to express LHR. The relative amount of LHR was: *recA* > *recF* > *lexA* > *recB* > wild type (with the *recB* and wild-type strains showing negative LHR). Two strains did not show this correlation; the *uvrD* strain showed less LHR than expected from its UV radiation sensitivity, while the *polA* strain showed more. The molecular bases for these exceptions are explored.

INTRODUCTION

The first indication that bacterial cells might have the capacity to repair ultraviolet (UV) radiation-induced damage was the demonstration of an increase in cell viability by minor modifications in the postirradiation handling of the cells. Thus, Roberts and Aldous (1949) showed that holding UV-irradiated bacteria in non-nutrient liquid medium before plating on solid nutrient medium resulted in an enhancement of survival, compared to cells plated immediately after irradiation. This phenomenon has been termed liquid holding recovery (LHR).

It has been concluded both from genetic (Harm, 1966, 1968a; Ganesan and Smith, 1968, 1969) and biochemical evidence (Castellani *et al.*, 1964; Tang and Patrick, 1977a) that the major repair process that occurs during liquid holding (LH) is excision repair. However, Ganesan and Smith (1968) reported that the detection of LHR in *E. coli* K-12 cells required the presence of a *recA* mutation. The reason that a *recA* mutation permits the detection of LHR remains obscure. We have reinvestigated this problem with the view of better understanding the genetic control and molecular basis of LHR.

We observed that the detection of LHR in a temperature sensitive *recA* strain requires that the strain be *recA*$^-$ in the growth medium after LH, but it can be either *recA*$^+$ or *recA*$^-$ during LH. Therefore, the beneficial effect of the excision repair that occurs during LH is only expressed if cells are deficient in growth medium-dependent DNA repair. In confirmation of this, we have observed a correlation between the amounts of LHR and the UV radiation sensitivities of the *recA*, *recF*, *lexA*, *recB*, and wild-type strains. Two strains (*uvrD* and *polA*) did not fit this correlation, and the reasons are explored.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* strains used are listed in Table 1.

Growth conditions. The growth medium used was a minimal salts medium (DTM) (Kaplan *et al.*, 1962) (4×10^{-2} *M* K_2HPO_4, 1.5×10^{-2} *M* KH_2PO_4, 4.1×10^{-4} *M* $MgSO_4$, 7.6×10^{-3} *M* $(NH_4)_2SO_4$, 1.4×10^{-3} *M* sodium citrate, 3.4×10^{-5} *M* $CaCl_2$, 9.0×10^{-7} *M* $FeSO_4$) supplemented with glucose [at 0.4% (w/v)], and as required, with amino acids (at 10^{-3} *M*), thymine (at 4 μg/mℓ), biotin (at 0.5 μg/mℓ). Bacteria were grown exponentially in minimal medium at 37°C (or 30°C) for at least five generations to a titer of $1–3 \times 10^8$ cells/mℓ. The cultures were collected on Millipore filters (HA, 0.45 μm pore size), and washed three times with and resuspended in the same volume of DTM. The cell suspensions were kept at 37°C (or 30°C) in a shaking water bath for 2 h prior to experimental treatment.

UV irradiation and liquid holding. A cell suspension was placed in an open glass petri dish on a rotary shaker and irradiated at room temperature with an 8-W General Electric germicidal lamp (254 nm). Depending upon the strain, the fluence rate was reduced to between 0.15 and 0.76 J m^{-2} s^{-1} by placing wire grids between the source and the cell sample. The fluence rates were determined with a Germicidal Photometer (International Light Co., Model IL-254). The UV radiation fluence was corrected for sample absorption according to the method of Youngs and Smith (1976).

After exposure to UV radiation, the cell suspension was held at the desired temperature (30, 37, or 42°C) in a shaking water bath for 24 h (LH procedure). Cells with or without LH were assayed for viability after diluting with DTM and plating 0.1 mℓ on yeast extract nutrient agar (YENB) (23 g Difo Nutrient Agar, and 7.5 g Difco Yeast Extract per

Table 1. *E. coli* strains used

Stanford Radiology Number	Repair Associated Markers	Other genotype*	Source or derivation**
SR144	*polA1*	*thyA36 deo(C2?) lacZ53 rha-5 rpsL151*	JG138, J. D. Gross
SR192	*lexA101*	*metE thyA36 deo(C2?) lacZ53 rpsL151*	DY99 (Youngs *et al.*, 1974)
SR248	+	*leuB19 metE70 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151* Su^-	KH21, R. B. Helling
SR255	*recB21*	*argE3 his-4 leuB6 proA2 thr-1 deoB16 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rpsL31 supE44*	(Youngs and Smith, 1976)
SR318	*recB21*	*metE deo(C2?) lacZ53 rpsL151*	SR752 × P1·SR255 (select Thy^+)
SR352	*sulA1*	*trpE65*	WP2, E. M. Witkin
SR392	+	*leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	DY174 (Smith, 1976)
SR393	*uvrD3*	*leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	DY175 (Smith, 1976)
SR395	+	*ilv leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	SR248 × P1·SR462 (select Met^+)
SR444	*polA1*	HfrR1 *metE rpoB*	JG78, J. D. Gross
SR455	+	*metE70 thyA36 deo(C2?) lacZ53 malB45 rha-5 rpsL151* Su^-	JG123, J. D. Gross
SR462		HfrKL16 *ilv thr rpsE*	JG89, J. D. Gross
SR556	+	*metE thyA36 deo(C2?) lacZ53 malB45 rpsL151*	SR444 × SR455 (select Rha^+
SR669	*recA56*	HfrP045 *ilv318 thr300 srlA300*::Tn*10 rpsE300*	JC10240, A. J. Clark
SR688	*lexA101*	*leuB10 metE70 thyA36 deo(C2?) lacZ53 rha-5 bioA2 rpsL151*	SR248 × P1kc·SR192 (select Mal^+)
SR752	+	*metE thyA36 deo(C2?) lacZ53 rpsL151*	SR556 × P1·SR192 (select Mal^+)
SR755	*recA56*	*leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 srlA300*::Tn*10 bioA2 rpsL151*	SR392 × P1::Tn*9c*·SR669 (select Tet^R)
SR758	*recA200*	*cys-43 hisF860 leu-6 metE proC36 thyA54 ara-14 lac836 malA38 mtl-1 xyl-5 thi-1 rpsE15 rpsL109*	KL399, A. K. Ganesan
SR759	*recA200 sulA1*	*trpE65 srlA300*::Tn*10*	SR352 × P1::Tn*9c*·SR758 (select Tet^R)
SR760	*polA1*	*deo(C2?) lacZ53 rha-5 rpsL151*	SR144 (select spontaneous Thy^+)
SR781	*recF143*	*argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rpsL31 supE44*	JC3875, A. J. Clark
SR812	*recF143*	*leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	SR395 × P1kc·SR781 (select Ilv^+)

*Genotype nomenclature is that of Bachmann *et al.* (1976). All strains are F^- and λ^- unless otherwise noted. The insertion element Tn*10* confers resistance to tetracycline.

**Strains SR318, SR395, SR556, and SR752 were constructed by D. A. Youngs. Strain SR688 was constructed by N. J. Sargentini. Thy^+, Met^+ and Ilv^+ = nonrequirement for thymine, methionine, and isoleucine and valine, respectively; Rha^+ and Mal^+ = ability to utilize rhamnose and maltose, respectively, as sole carbon and energy sources; Tet^R = resistant to tetracycline.

ml). Supplemented minimal medium plates solidified with 1.6% Difco Noble agar (Var der Schueren *et al.*, 1974) were used for *polA* cells, since we found that it gives a higher plating efficiency than YENB plates for unirradiated *polA* cells, although essentially the same LH effect was observed on both kinds of plates. In experiments on *recA*200 cells at 42°C, the diluent and YENB plates were preheated to 42°C. Immediately after spreading the plates with 0.1 ml of cell suspension, they were returned to the 42°C incubator. All plates were counted after a 24–48 h incubation at the desired temperature (30, 37 or 42°C).

All experiments were performed under General Electric 'gold' fluorescent lights to prevent photoreactivation. The surviving fraction (SF_t) was determined by the formula $N_r(t)/N_0(t)$, where N_r is the number of colony-forming units (CFU) per ml of cell suspension in the irradiated population, N_0 is the number of CFU/ml in the unirradiated population, and (t) is the time of LH after exposure of the irradiated population to UV radiation. LHR is defined as the ratio $SF_t/SF_{t=0}$. An LH time of 24 h was chosen for all the experiments, since during this time the unirradiated cells maintained a high viability, and LHR was near maximal.

Measurement of DNA degradation. Cells prelabeled with [^{3}H-methyl]-thymine (5 μCi/ml, 30 Ci/mmol, Amersham) during five generations of growth were used to measure the degradation of DNA in cells held in buffer. After labeling, unincorporated radioactive thymine was removed by filtration through a Millipore filter (HA, 0.45 μm pore size), and washing three times with ~10 ml of prewarmed medium. Cells were resuspended in prewarmed medium and incubated for 1–2 h before harvesting. At various times during LH, 0.5 ml of the cell suspension was mixed with 2 ml of cold 10% trichloroacetic acid (TCA). The TCA precipitate was collected on a Millipore filter (EH, 0.5 μm pore size) that had been pretreated with thymine at 1 mg/ml, and the

precipitate was washed three times with ~5 m/ of cold 0.1% TCA. The filters were dried under a heat lamp, placed in 5 m/ of scintillation solution [4 g Omnifluor (New England Nuclear) in 1 / of toluene], and assayed for [^{3}H] in a liquid scintillation spectrometer.

RESULTS AND DISCUSSION

LHR in the recA200 *mutant at permissive and non-permissive temperatures*

Three hypotheses could explain why a *recA* mutation is needed in order to demonstrate LHR in *E. coli* K-12: (1) the *recA* mutation permits the expression of a new excision repair system during LH, (2) the beneficial effect of the excision repair that occurs during LH can only be observed in strains like *recA* whose growth medium-dependent DNA repair efficiency is greatly reduced, and (3) the *recA* mutation permits the expression of a new excision repair system during LH, and the beneficial effect of this repair can only be observed in strains like *recA* whose growth medium-dependent DNA repair efficiency is greatly reduced. These three possibilities have mutually exclusive features. In hypothesis 1, the *recA* mutation is only needed during LH, in contrast to hypothesis 2 where the *recA* mutation is only needed when the cells are plated on growth medium. In hypothesis 3, the *recA* mutation is needed both during LH and in growth medium. These three possibilities should yield different results, as listed in Table 2, when UV-irradiated *recA200* cells are held in buffer at permissive (30°C) and nonpermissive (42°C) temperatures, and are plated at these two temperatures. Figure 1a shows

Table 2. Predicted LHR in the *recA200* strain as a function of holding and plating temperature

LH temp (°C)	Plating temp (°C)	LHR hypotheses		
		1	2	3
30	30	–	–	–
30	42	–	+	–
42	30	+	–	–
42	42	+	+	+

that when UV irradiated *E. coli* K-12 *recA200* cells were plated at 42°C, significant LHR was observed regardless of the LH temperature. The cells held in buffer at 42°C consistently showed a higher recovery than did the cells held at 30°C; probably due to an enhanced rate of excision repair at 42°C as compared to 30°C. Similar results were also obtained for the *E. coli* B/r *recA200* strain (Fig. 1b). Regardless of the LH temperature, no LHR was observed when UV irradiated [up to a UV radiation fluence of 50 J/m^2 (data not shown)] *E. coli* K-12 *recA200* cells were plated at 30°C. [Note that after 50 J/m^2 the UV radiation survival of *E. coli* K-12 *recA200* cells at 30°C was about the same as after 2 J/m^2 and incubation at 42°C (data not shown).]

Therefore, these results exclude hypotheses 1 and 3, but completely fulfil the expectations of the second hypothesis, i.e. the repair that occurs during LH is independent of *recA* gene functions, but enhanced survival after LH is detected only when the capacity

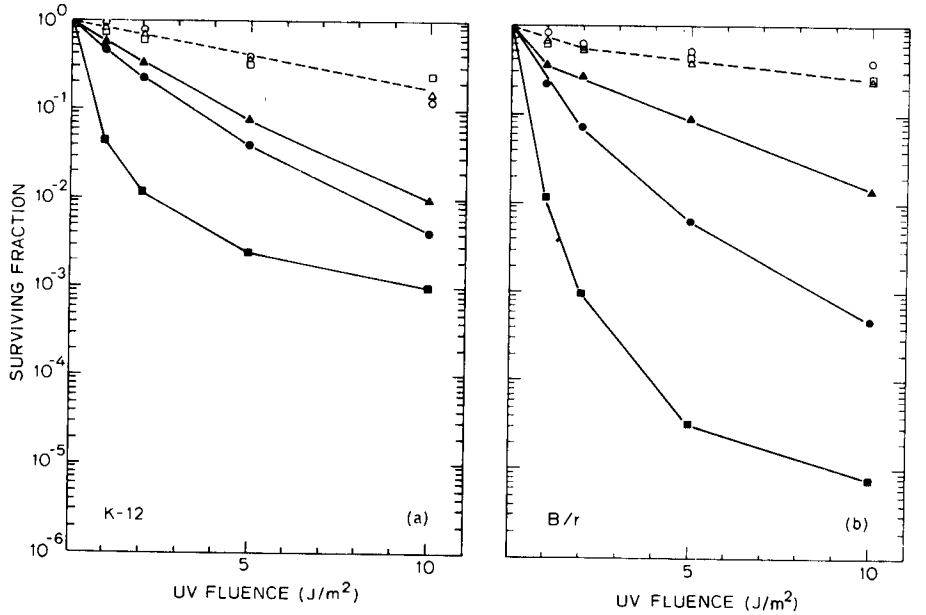

Figure 1. UV radiation survival curve of *recA200* cells under different conditions. (a) *E. coli* K-12 *recA200* (SR758), (b) *E. coli* B/r *recA200* (SR759). Solid lines (closed symbols) represent cells that were plated and incubated at 42°C, and the dashed line (open symbols) represents cells that were plated and incubated at 30°C. The different symbols represent cells under different liquid holding (LH) treatment after UV irradiation: (□,■) no LH; (○,●) LH for 24 h at 30°C; (△,▲) LH for 24 h at 42°C. Each point is the average of two experiments.

for growth medium-dependent DNA repair is greatly reduced, as is the case for *recA* strains (Youngs and Smith, 1976).

LHR in UV-irradiated wild-type recA56, recB21, recF143 *and* lexA101 *strains*

The UV radiation survival, with and without a 24 h LH treatment, of the wild-type, *recA*, *recB*, *recF*, and *lexA* strains is shown in Fig. 2. UV-irradiated wild-type cells showed a large decrease in cell viability after 24 h of LH treatment, as compared to cells plated immediately after irradiation (Fig. 2a). A similar negative LH effect has been observed for *E. coli* K-12 (AB1157) (Moss and Davies, 1974), and *E. coli* C (Harm and Haefner, 1968). The LH killing effect was greatly reduced in UV-irradiated *recB* cells (Fig. 2c). LH treatment had no significant effect on viability in UV-irradiated *lexA* cells (Fig. 2d). A slight enhancement of cell survival due to LH was observed in UV-irradiated *recF* cells (Fig. 2e), but a large amount of LHR was observed for *recA* cells (Fig. 2f). Overall, the extent of LHR after UV irradiation correlates with the UV radiation sensitivities of the individual strains (Fig. 3). It has been shown that *recB*, *recF*, and *lexA* strains are partially defective in postreplication repair (Smith, 1978, and references therein). These observations, together with the results in Fig. 3, support the hypothesis that the detection of LHR afrer UV irradiation requires that the cells be deficient in growth medium-dependent DNA repair.

LHR in uvrD3 *and* polA1 *strains*

Harm (1966, 1968a) reported that in *E. coli* B strains, LHR only occurred in cells that were able to reactivate UV-irradiated phage (i.e. were able to perform host cell reactivation, and were termed Hcr^+). Subsequently Ganesan and Smith (1968, 1969) found that *uvrA*, *uvrB*, or *uvrC* mutations block LHR in *E. coli* K-12 *recA* strains, and concluded that LHR reflects the excision repair that occurs during LH. This conclusion was further supported by Tang and Patrick's (1977a) observation that UV radiation-induced incision, excision, repair replication, and ligation occur during LH in Hcr^+, but not in Hcr^- derivatives of *E. coli* B. In confirmation of the requirement that cells must be Hcr^+ in order for them to exhibit LHR, we found that the *uvrD* strain [which is Hcr^- (Ogawa *et al.*, 1968)] shows no LHR (Fig. 2b).

Tang and Patrick (1977b) reported that *polA* cells were able to excise pyrimidine dimers as well as wild-

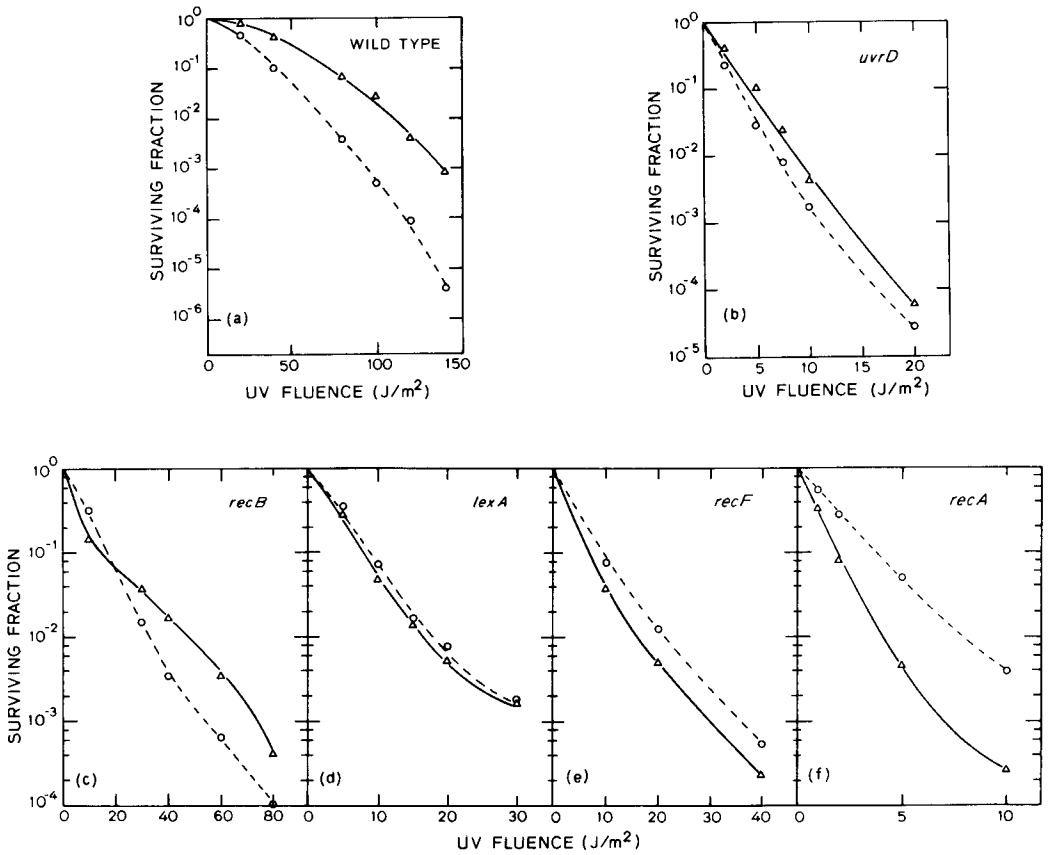

Figure 2. UV radiation survical curves for different *E. coli* K-12 strains with or without LH (24 h) treatment. (a) wild type (SR248), (b) *uvrD3* (SR393), (c) *recB21* (SR318), (d) *lexA101* (SR688), (e) *recF143* (SR812), and (f) *recA56* (SR755) cells. Solid lines represent the viability of cells without LH, and dashed lines represent cells with 24 h of LH. Each point is the average of two experiments.

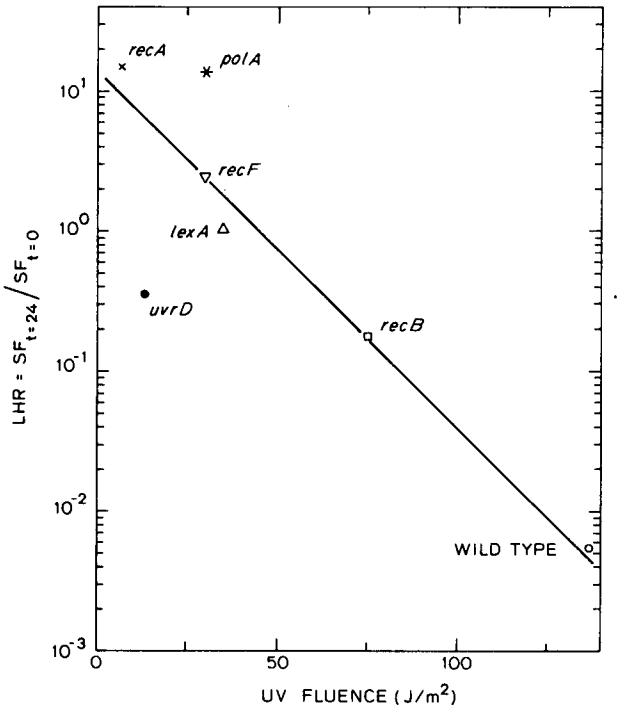

Figure 3. The relationship between the amount of LHR (after 24 h of LH) and the UV radiation sensitivities of the various strains (as the UV radiation fluence to give 10^{-3} survival without LH treatment). The data are derived from survival curves in Fig. 2. The same relationship was observed for these strains when compared at a surviving fraction of 10^{-1} or 10^{-2} (data not shown).

type cells during LH and moreover, they observed a significant extent of LHR in a *polA* strain. Our results (Fig. 4a, b) confirm that LHR occurs in *polA* cells. However, we wish to call attention to the fact that in a *polA* strain auxotrophic for thymine, different extents of LHR resulted depending on whether or not thymine was present in the buffer during LH treatment (Fig. 4a). In a Thy$^+$ revertant of this same *polA* strain, LHR was observed regardless of the presence or absence of thymine in the LH buffer (Fig. 4b). This requirement for thymine in the LH buffer for the expression of LHR was not observed for the *recA*, *recB*, *recF*, *uvrD* or wild-type strains, even when they were Thy$^-$ (data not shown).

Thymine auxotrophs of *polA* cells have been shown to be very sensitive to thymineless death (Sedgwick and Bridges, 1971, and references therein). We observed higher amounts of DNA degradation in UV-irradiated *polA* Thy$^-$ cells held in buffer without thymine compared to cells held in the presence of thymine (Table 3). It has been reported (Tang and Patrick, 1977b) that DNA polymerase I is the major enzyme that performs dimer excision and 'DNA turnover' synthesis when cells are held in buffer. Therefore, we assume that holding UV-irradiated *polA* Thy$^-$ cells in buffer without thymine could result in more DNA single-strand breaks or wider excision gaps that could lead to a higher probability of producing lethal DNA double-strand breaks.

Models for LHR

Since both genetic and biochemical evidence (Castellani *et al.*, 1964; Harm, 1966, 1968a; Ganesan and Smith, 1968, 1969; Tang and Patrick, 1977a) have shown that excision repair is the major repair process occurring during LH, it is reasonable to assume that

Table 3. The effect of thymine on DNA degradation during LH of UV-irradiated *polA1* Thy$^-$ cells

LH buffer	UV fluence (J/m^2)	[^{3}H]-Thy remaining in DNA after 24 h of LH (UV irradiated cells/unirradiated cells)
−Thy	35	0.45, 0.46**
+Thy*	35	0.61, 0.70**

*At 4 μg/mℓ.
**Separate experiments.

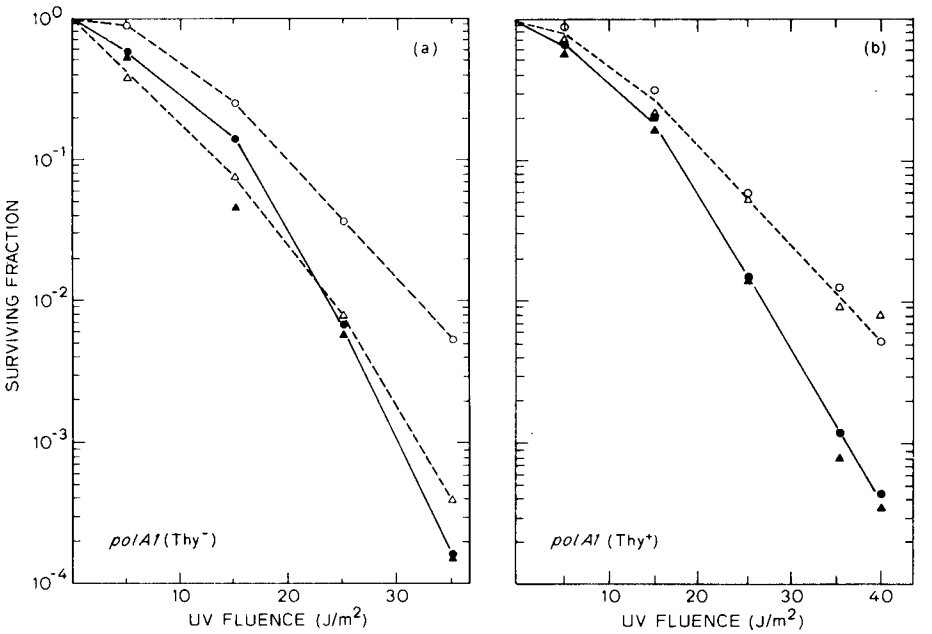

Figure 4. (a) UV radiation survival curves of *E. coli* K-12 *polA1* Thy$^-$ (SR144) under different experimental conditions. Solid line and closed symbols represent cells without LH treatment; dashed lines and open symbols represent cells with LH for 24 h. Symbols: (○,●) cells held in DTM containing 4 μg/mℓ thymine; (△,▲) cells held in DTM only. The cells were plated on minimal medium. Each point is the average of two experiments. (b) UV radiation survival curves of *E. coli polA1* Thy$^+$ (SR760) under different experimental conditions. Lines and symbols represent the same experimental conditions as described in Fig. 4a.

the increased viability of UV-irradiated cells after LH treatment is due to an increase in the time allowed for excision repair before the cells start to replicate and repair their DNA in growth medium. The results in Fig. 1 suggest that excision repair taking place during LH is independent of the *recA* gene product, and demonstrate that the expression of LHR in *recA* cells depends upon their deficiency in growth medium-dependent DNA repair.

Since *recA* cells are deficient in postreplication repair, the major repair pathway left in these cells is excision repair, which can occur in buffer (Tang and Patrick, 1977a, b; Youngs *et al.*, 1974). Thus, the lethality that would certainly occur in *recA* cells from unrepaired DNA daughter-strand gaps formed when DNA replication resumes in growth medium is prevented by the removal of DNA lesions during the LH treatment. In more general terms, LHR should be observed in any excision repair proficient cell whose growth medium-dependent DNA repair systems are impaired, as for example in *recA* cells.

The reason LHR was observed in *polA* cells could be due to its small deficiency in growth medium-dependent repair (Barfknecht and Smith, 1978), and/or that attempted repair produces DNA double-strand breaks (Bonura and Smith, 1975). However, due to the extensive DNA degradation of UV irradiated *polA* cells during LH, molecular level experiments on DNA repair would be difficult to interpret.

The reason that *uvrD* cells do not perform LHR could be due to a decreased ability to perform excision repair in buffer [*uvrD* cells are Hcr$^-$ (Ogawa *et al.*, 1968)]. We will discuss more fully the effect of a *uvrD* mutation on DNA repair in buffer in a separate paper (Tang and Smith, 1980).

The molecular defect in *E. coli* B and B/r that permits the detection of LHR (Castellani *et al.*, 1964; Harm, 1966, 1968a) still remains to be determined. That a repair defect exists is confirmed by the fact that wild-type *E. coli* K-12 cells are more resistant to UV radiation than are *E. coli* B/r cells (data not shown), and that *E. coli* B/r shows LHR (Harm, 1966), while wild-type *E. coli* K-12 cells do not (Fig. 2a). We predict that *E. coli* B/r is defective in some pathway of growth medium-dependent repair.

While LH resulted in enhanced survival in UV-irradiated *recA* and *polA* strains, decreased survival was observed in UV-irradiated wild-type cells, although excision occurred in these cells during LH (Tang and Patrick, 1977b). Harm (1968b) and Moss and Davies (1974) proposed that the LH killing effect that occurs in UV-irradiated wild-type cells may be due to the formation of lethal DNA double-strand breaks by the overlapping of excision repair-gaps. However, the studies of Tang and Patrick (1977a, b) on the excision of pyrimidine dimers in cells held in buffer argue against this hypothesis. A more likely hypothesis involves the production of a 'non-repair site' when a gap is formed, due to DNA turnover, in the strand opposite a pyrimidine dimer (Tang *et al.*,

1979). Therefore, the killing effect observed in UV-irradiated wild-type cells may be due to a high yield of 'non-repair sites', due to the relatively larger UV radiation fluence employed, compared to that used for *recA* strains. For example, the surviving fraction used for detecting the LH effect in many of our experiments was 10^{-2}. The UV radiation fluence needed for *recA* cells to reach this survival was about 4 J/m^2 (Fig. 2f), while in wild-type cells it was 110 J/m^2 (Fig. 2a). Therefore, there should be a greater probability of DNA turnover occurring opposite a pyrimidine dimer in the wild-type vs the *recA* cells. While *recA* cells benefit from the excision repair that occurs during LH, this beneficial effect is erroded in the wild-type cells by the formation of 'non-repair sites'.

In conclusion, whether or not postirradiation LH will benefit a cell requires that there be a minimal interference with the excision repair of UV radiation-induced damage by DNA turnover (or other causes) during LH. If the formation of 'non-repair sites' (which is a function of the original amount of UV damage; Tang *et al.*, 1979) is extensive enough (e.g. as in wild-type cells) then no enhancement of cell survival will be observed, despite the fact that excision repair occurs during LH.

A requirement for the detection of LHR is that cells be deficient in growth medium-dependent DNA repair; i.e. lesions that are not repaired by excision repair will become lethal when their repair is attempted by a deficient growth medium-dependent process. Thus, except for the *uvrD* and *polA* strains, the differences in LHR observed in the excision repair *proficient* strains (Fig. 3) is a reflection of their different efficiencies for performing growth medium-dependent DNA repair.

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REFERENCES

Bachmann, B. J., K. B. Low and A. L. Taylor (1976) *J. Bacteriol.* **40**, 116–167.
Barfknecht, T. R. and K. C. Smith (1978) *Mol. Gen. Genet.* **167**, 37–41.
Bonura, T. and K. C. Smith (1975) *Photochem. Photobiol.* **22**, 243–248.
Castellani, A., J. Jagger and R. B. Setlow (1964) *Science* **143**, 1170–1171.
Ganesan, A. K. and K. C. Smith (1968) *J. Bacteriol.* **96**, 365–373.
Ganesan, A. K. and K. C. Smith (1969) *J. Bacteriol.* **97**, 1129–1133.
Harm, W. (1966) *Photochem. Photobiol.* **5**, 747–760.
Harm, W. (1968a) *Mutat. Res.* **6**, 25–35.
Harm, W. (1968b) *Photochem. Photobiol.* **7**, 73–86.
Harm, W. and K. Haefner (1968) *Photochem. Photobiol.* **8**, 179–192.
Kaplan, H. S., K. C. Smith and P. A. Tomlin (1962) *Radiat. Res.* **16**, 98–113.
Moss, S. H. and D. J. G. Davies (1974) *J. Bacteriol.* **120**, 15–23.
Ogawa, H., K. Shimada and J-i. Tomizawa (1968) *Mol. Gen. Genet.* **101**, 227–244.
Roberts, R. B. and E. Aldous (1949) *J. Bacteriol.* **57**, 363–375.
Sedgwick. S. G. and B. A. Bridges (1971) *J. Bacteriol.* **108**, 1422–1423.
Smith, K. C. (1978) *Photochem. Photobiol.* **28**, 121–129.
Tang, M. and M. H. Patrick (1977a) *Photochem. Photobiol.* **26**, 247–255.
Tang, M. and M. H. Patrick (1977b) *Photochem. Photobiol.* **26**, 257–262.
Tang, M. and K. C. Smith (1980) *Mutat. Res.*, submitted.
Tang, M., T. V. Wang and M. H. Patrick (1979) *Photochem. Photobiol.* **29**, 511–520.
Van der Schueren, E., D. A. Youngs and K. C. Smith (1974) *Photochem. Photobiol.* **20**, 9–13.
Youngs, D. A. and K. C. Smith (1976) *J. Bacteriol.* **125**, 102–110.
Youngs, D. A., E. Van der Schueren and K. C. Smith (1974) *J. Bacteriol.* **117**, 717–715.