JOURNAL OF BACTERIOLOGY, Oct. 1976, p. 317-324

Vol. 128, No. 1
Printed in U.S.A.

Postirradiation Recovery Dependent on the *uvr-1* Locus in *Bacillus subtilis*

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Received for publication 12 July 1976

A mutant (*uvr-1*) of *Bacillus subtilis* that is deficient in excision of ultraviolet (UV)-induced pyrimidine dimers from deoxyribonucleic acid (DNA) shows a marked increase in ability to survive UV irradiation when plated on amino acid-supplemented agar medium compared with its survival ability when plated on nutrient agar. Since the extent of killing depends on the richness of the plating medium, the effect is considered to be one of growth-dependent lethality. Irradiated stationary phase *uvr-1* cells, incubated in liquid medium lacking amino acids required for growth, recover from this sensitivity to rich medium within 3 to 4 h after irradiation. Recovery is greatly reduced in the absence of glucose or in the presence of NaCN, although it is not completely eliminated. Exponentially growing cells have a limited ability to recover from sensitivity to rich medium. Growth-dependent lethality can also occur in liquid medium. In nutrient broth the ability of irradiated stationary-phase *uvr-1* cells to form colonies on defined agar medium decreases during postirradiation incubation, but treatment with chloramphenicol inhibits the loss of colony-forming ability. Recovery from sensitivity to rich media is inhibited by caffeine but not by 6-(*p*-hydroxyphenylazo)-uracil, an inhibitor of DNA replication. Alkaline sucrose gradient profiles show that conditions allowing recovery also favor maintaining intact DNA strands, whereas DNA strand breakage or degradation is associated with loss of viability. Recovery from sensitivity to rich medium has not been observed in the Uvr$^+$ parent or in strains carrying the mutations *uvs-42* (another deficiency in dimer excision), *recA1*, or *polA59*. A *uvr-1 recA1* mutant shows a higher level of recovery than does the *recA1* single mutant, but a much lower level than the *uvr-1* single mutant. Apparently, both the *uvr-1* defect and Rec$^+$ and PolI$^+$ functions are essential for recovery from sensitivity to rich medium. For optimal recovery, growth immediately after irradiation must be delayed. The process requires energy, apparently involves recombination, and probably results in rejoining of DNA strands in which incision but not excision has occurred.

A number of conditions have been observed in which the sensitivity of an organism to ultraviolet (UV) irradiation is modified by suboptimal growth conditions. In liquid-holding recovery, irradiated bacteria or yeast recover from UV damage while being held in buffer before plating (16, 24–26). UV-irradiated *Escherichia coli* K-12 recover when incubated in or plated on minimal medium (10, 11), *E. coli* 15 show at least a transient restoration in any liquid medium (3), and irradiated *E. coli* B plated on acid agar medium survive better than if plated on alkaline medium (30).

UV-irradiated cells of the *B. subtilis* strain GSY1027 (*uvr-1*), which is deficient in excision of pyrimidine dimers from deoxyribonucleic acid (DNA), appear much more resistant to UV irradiation when plated on amino acid-supplemented minimal agar (SAA) medium than when plated on nutrient agar (NA). This survival enhancement also occurs if irradiated cells are incubated in liquid minimal medium lacking required amino acids before being plated on NA. Since the observed sensitivity to UV irradiation depends on the richness of the medium to which the cells are exposed immediately after irradiation, and since only stationary-phase cells are stable in liquid medium after irradiation, there seems to be some process that occurs slowly and which, given sufficient time before growth resumes, allows the cell to survive. This process will be referred to as recovery from sensitivity to rich medium, or simply as recovery.

Recovery from sensitivity to rich medium occurs only in *uvr-1* mutants. Another Uvr⁻ mutation, *uvs-42*, does not allow recovery. Recovery depends at least partially on RecA⁺ activity and is inhibited by caffeine, so recombination may be involved. Energy metabolism is essential for recovery, but protein synthesis is not. In fact, inhibiting protein synthesis during incubation in nutrient broth allows recovery to progress despite the richness of the medium. Sensitivity to rich medium appears to result from a failure of recombinational repair, because more single-strand nicks are found in the DNA of cells incubated in nutrient broth or in the presence of caffeine than under conditions favoring viability.

MATERIALS AND METHODS

Bacterial strains. The strains used are listed in Table 1. GSY1027, FB56, GSY1025, and GSY1063 were prepared by transformation or transduction of GSY1026 and are essentially isogenic.

Growth of bacteria. Cells were grown to stationary phase at 37°C in modified (4) Spizizen salts (SM) containing 0.5% glucose, 0.05% acid-hydrolyzed casein (CAA) (Difco) or, for SAA medium, the synthetic amino acid mixture described below for SAA agar, and 50 μg of required amino acids per ml. For exponentially growing cells, a stationary-phase culture was diluted in the same medium and grown to a titer of 1.5×10^8 to 2.5×10^8/ml.

Irradiation and incubation of bacteria. Cells were centrifuged, washed with ice-cold SM + 0.5% glucose (SMG), and for most experiments resuspended in SMG at a Klett reading (no. 42 filter) of 100 (1.5×10^8 to 2.5×10^8 colony-forming units [CFU]/ml). They were irradiated with two 15-W germicidal lamps (General Electric) as previously described (14).

In dose-response experiments, samples were diluted with SSC (0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0) and plated either on Difco NA or on the SAA agar described previously (5), modified by substituting 1.0% Calbiochem granulated agar for 1.5% agar (Difco). The amino acid supplement consisted of L-asparagine (25 mg/liter), L-glutamic acid (25 mg/liter), L-proline (6 mg/liter), L-alanine (4 mg/liter), L-valine (2.8 mg/liter), L-phenylalanine (2.4 mg/liter) and L-tyrosine (2.4 mg/liter), and L-cysteine HCl (0.8 mg/liter).

TABLE 1. *Bacterial strains studied*

Strain designation	Genotype	Reference/ source
GSY1026	*trpC2 metB4*	(21)
GSY1027	*trpC2 metB4 uvr-1*	(21)
FB56	*trpC2 metB4 sfr uvs-42*	FB50-X GSY1026
GSY1025	*trpC2 metB4 recA1*	(21)
GSY1063	*trpC2 uvr-1 recA1*	(20)
FB50	*trpC2 met sfr thyA thyB uvs-42*	(23)
FB52	*trpC2 met sfr uvs-42 recA1*	GSY1025-X FB50
HA160	*his leu metB polA59*	(19)

In most experiments on the time course of recovery in SMG, 1- to 2-ml samples of irradiated and unirradiated cells were transferred to warm culture tubes and shaken at 37°C. Samples were taken at intervals and plated on NA plates.

In the experiments involving nutrient broth and chloramphenicol, cells were irradiated in SMG at a Klett reading of 200. Samples (1 ml) were diluted with 1 ml of either SMG, double-strength nutrient broth (Difco), or double-strength nutrient broth containing 100 μg chloramphenicol per ml (Sigma). These cultures were then incubated at 37°C, sampled, and plated on NA or SAA agar plates. In other experiments, concentrated irradiated cells were similarly diluted twofold in SMG containing caffeine (Sigma) or 6-(*p*-hydroxyphenylazo)-uracil (HPUra) (kindly furnished by B. L. Langley, Imperial Chemical Co.) at twice the desired concentration.

Alkaline sucrose gradient centrifugation. Cells were prepared as described above, except that the growth medium contained 30 μg of 2'-deoxyadenosine per ml and 20 μCi of [*methyl*-³H]thymidine (Amersham, 50 Ci/mmol) per ml. After 10 min or 3 h of postirradiation incubation under the indicated conditions, samples were diluted with an equal volume of buffer containing 0.04 M tris(hydroxymethyl)aminomethane and 0.06 M ethylenediaminetetraacetate (EDTA) at pH 8.1. The cells were centrifuged and resuspended in the same buffer at half the concentration containing 0.3 mg of egg white lysozyme per ml (Sigma). After 3 to 4 min of incubation at 37°C, 50 μl of cell suspensions was layered onto 5-ml gradients of 5 to 20% sucrose containing 0.5 M NaCl, 0.2 N NaOH, and 1.0 mM EDTA (28), as well as a 0.1-ml lysing layer of 0.5 M NaCl, 0.2 N NaOH, and 1.0 mM EDTA. Gradients were centrifuged for 90 min at 28,000 rpm at 20°C in the SW50.1 rotor, using a Beckman L5-50 ultracentrifuge. Fractions were collected on strips of Whatman no. 17 chromatography paper, which were then soaked successively in 5% trichloroacetic acid and 95% ethanol, dried, and counted by liquid scintillation spectrometry.

RESULTS

Although the survival of many strains of *Bacillus subtilis* was somewhat higher after UV irradiation when the cells were plated on defined agar medium than on NA, the survival of stationary-phase cells of *uvr-1* mutants was relatively much greater on defined medium (Fig. 1). At UV doses resulting in 1% survival on NA, the survivals on defined agar medium of GSY1026 (Uvr⁺) and FB56 (*uvs-42*) are 7.6 and 5.8%, respectively, whereas that of GSY1027 (*uvr-1*) is 22%. As shown below, this effect appears to be a result of recovery during the lag phase.

The liquid culture medium used to grow cells before irradiation contained CAA, whereas the

amino acid supplement in the plates (SAA) lacked a number of amino acids present in CAA. This means that plating cells on the defined medium results in temporary starvation for several amino acids, until the previously repressed biosynthetic pathways become fully active again. To test the hypothesis that nutritional step-down allows recovery from sensitivity to rich medium, irradiated cells of GSY1027 (*uvr-1*) previously grown in media containing either CAA or SAA (i) were plated on NA and incubated at 37°C or at room temperature, or (ii) were plated on minimal agar supplemented with SAA, 0.05% CAA, or a combination of CAA, SAA, and 50 μg each of adenine, thymidine, and uracil per ml. The results presented in Table 2 imply that nutritional step-down plays a minor role in reducing the observed sensitivity to UV irradiation (line 1, cells grown in CAA compared with cells grown in SAA). Evaluation of Student's *t* test for the difference between values of D_0 gave $P > 0.35$. The richness of the agar medium is more important, since CAA-grown cells plated on CAA agar (line 2) are more sensitive than SAA-grown cells plated on SAA agar (line 1, $P < 0.025$) or CAA-grown cells plated on SAA agar (line 1, $P < 0.05$). In a nutritional step-up, the richness of the agar medium also appears to be more significant than the quantitative difference between the media. As shown in line 2, cells grown in CAA were not significantly more resistant to UV ($P > 0.2$) when plated on CAA (with no nutritional shift) than SAA-grown cells plated on CAA, and the liquid medium had no effect on sensitivity of cells plated on NA (line 4). Maximum sensitivity can also be achieved using a defined medium (compare lines 3 and 4). Since survival is independent of temperature on NA plates (lines 4 and 5), the recovery process must be coordinated with growth in a different way than the temperature-dependent phenomena observed in X-irradiated *E. coli* by Stapleton et al. (29) or heat-induced reversal of filamentation in *E. coli* B after UV irradiation (1).

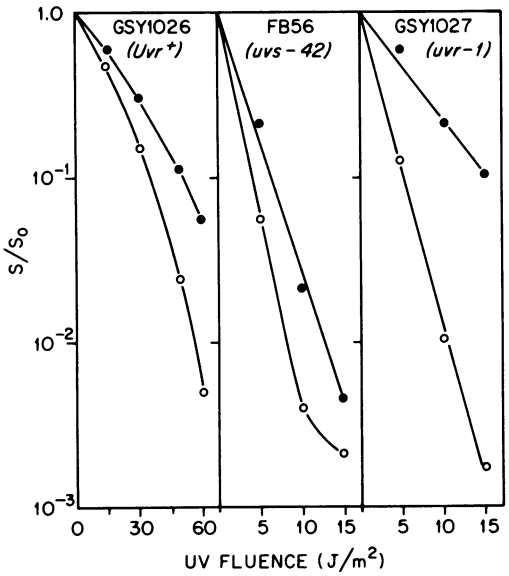

FIG. 1. *Effect of plating medium on survival of UV-irradiated cells. Stationary-phase cells were UV irradiated and plated on NA* (○) *or on defined agar medium* (●).

Amino acid starvation of irradiated *uvr-1* cells in liquid medium should also allow the recovery processes to occur. Figure 2 shows that when UV-irradiated cells of GSY1027 (*uvr-1*) are incubated in SMG, their ability to survive plating on NA increases approximately 50-fold in 3 to 4 h. It is also evident from Fig. 2 that maximal recovery in liquid requires glucose. In the absence of glucose, recovery occurs at a lower rate and after a lag of about 2 h. Incubation for longer than 4 h without glucose did not appreciably increase the number of survivors, so recovery is limited in extent as well as in rate by the absence of an energy source. Incubation in SMG plus 0.1 M NaCN resulted in

TABLE 2. *Effect of plating conditions on UV sensitivity*[a]

Line no.	Plating condition	$D_0 \pm \sigma$ (J/m²) for cells grown in:	
		CAA	SAA
1	SAA	5.22 ± 0.74	5.43 ± 1.34
2	CAA	3.49 ± 0.56	4.96 ± 0.58
3	CAA + SAA + ATU[b]		2.2
4	NA (37°C)	2.0 ± 0.12	2.2
5	NA (20°C)		2.1

[a] Cells of GSY1027 were grown to stationary phase in medium supplemented with either SAA or 0.05% CAA. Washed cells were irradiated and plated on NA or defined agar with the indicated supplements. D_0 is the fluence required for a 63% reduction in survival on the exponential portion of the fluence-response curve. Determinations for which standard deviations are given were made in triplicate.

[b] ATU, Adenine, thymidine, uracil.

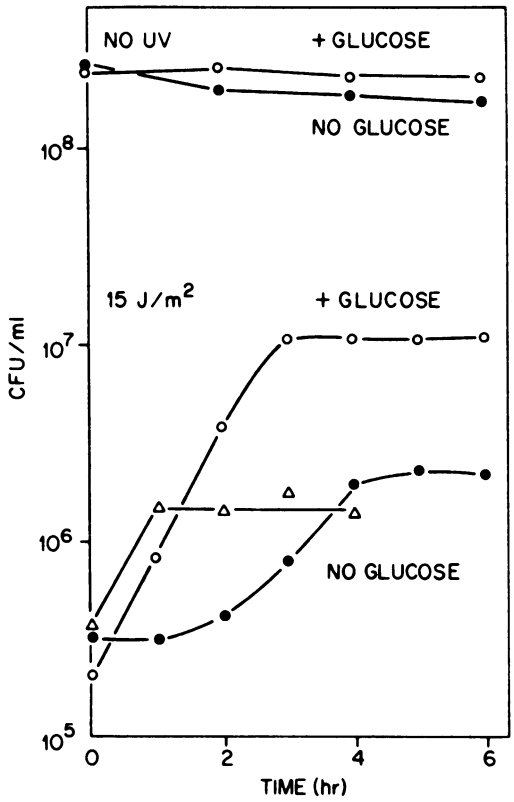

FIG. 2. *Requirement for glucose for recovery of GSY1027 (uvr-1). Irradiated stationary-phase or exponentially growing cells were incubated in SM with or without 0.5% glucose and plated on NA at the indicated times. The UV fluence was 15 J/m². Open symbols, SMG; closed circles, Sm only; ○ and ●, stationary-phase cells; △, exponentially growing cells.*

recovery kinetics essentially identical to those with SM alone (not shown).

Exponentially growing cells have greatly reduced ability to recover in SMG (Fig. 2). During the first hour of incubation the level of recovery is similar to that of stationary-phase cells, but thereafter the log-phase cells show no further recovery. Thus, it appears that recovery requires a prior nongrowing condition.

If recovery from sensitivity to rich media depends on failure or inhibition of growth, then incubation of cells in nutrient broth immediately after irradiation should mimic the effect of plating on NA and decrease the potential to survive on defined agar medium. Figure 3 shows the results of such an experiment. During a 4-h incubation in SMG, survival on NA increased 20-fold with about a doubling in survival on SAA agar (Fig. 3a). However, in nutrient broth there was a eightfold drop in survival of the same cells on SAA agar and an increase of about 2.5-fold in titer on NA (Fig. 3b), an increase which probably includes growth of the survivors. Protein synthesis is involved in the loss of viability on defined agar medium, because addition of 50 μg of chloramphenicol per ml to the nutrient broth prevented the death of the cells initially surviving on defined agar (Fig. 3c).

Recovery from sensitivity to NA by cells in nutrient broth plus chloramphenicol was slightly greater than in nutrient broth alone, but considerably reduced compared with the recovery by cells incubated in SMG. Table 3 shows that adding chloramphenicol to SMG also reduces recovery. Since amino acid starvation does not prevent protein synthesis but

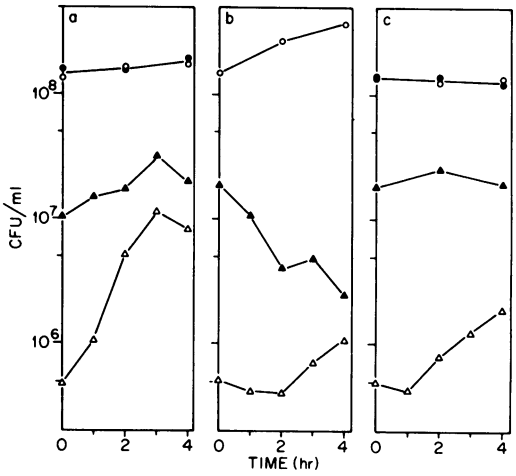

FIG. 3. *(a–c) Effects of nutrient broth and chloramphenicol on recovery of irradiated stationary-phase cells of GSY1027 (uvr-1) in liquid and on defined agar medium. (a) Cells incubated in SMG; (b) cells incubated in nutrient broth; (c) cells incubated in nutrient broth + 50 μg of chloramphenicol per ml. Open symbols, plated on NA. Closed symbols, plated on defined agar medium.* (○, ●) *unirradiated cells;* (△, ▲) *15 J/m².*

TABLE 3. *Effect of chloramphenicol on recovery in SMG*[a]

Time (h)	CFU/ml (×10⁸)		
	SMG	SMG + chloramphenicol	SMG + HPUra
0	0.163	0.165	0.164
2	2.39	1.24	3.92
4	12.75	3.80	13.05

[a] Stationary-phase cells of GSY1027 were irradiated with 15 J of UV per m², incubated in SMG or SMG plus 50 μg of chloramphenicol per ml, and plated on NA plates at the times indicated. The cell titer was about 2.5 × 10⁸ colony-forming units per ml before irradiation.

merely limits the total amount of protein that can be synthesized, it may be that to remain viable the irradiated cells must be allowed to carry out turnover of proteins, whereas recovery requires only that they be prevented from carrying out net protein synthesis.

Figure 4 shows the effect of incubating UV-irradiated cells in SMG containing various concentrations of caffeine. Caffeine at a concentration of 1 mg/ml markedly inhibits recovery without affecting survival of unirradiated cells. Recovery can apparently be nearly completely blocked by 1.5 or 2 mg of caffeine per ml; but since unirradiated cells are killed at these concentrations, it is impossible to determine whether all of the inhibition of recovery is due to intervention by caffeine in the recovery processes or whether cells that are especially sensitive to rich medium are also more sensitive to the lethal effects of caffeine. However, it seems likely that a caffeine-sensitive step is essential for recovery from sensitivity to rich medium.

Ganesan and Smith (12) have implicated postreplication repair in minimal medium recovery of *E. coli*. The possibility that replication in GSY1027 (*uvr-1*) cells during incubation in SMG was responsible for recovery from sensitivity to rich medium was ruled out by including in the SMG 0.3 mM HPUra, an inhibitor of DNA replication (6, 7). There was no inhibition of recovery by HPUra, so recovery could not require prior replication of DNA (Table 3).

After UV irradiation of exponentially growing *uvr-1* cells, single-strand breaks appear in the DNA, but unlike the Uvr⁺ parent, this strain is very slow to restore the nicked molecules to high molecular weight (15). This raises the possibility that in rich medium, DNA strands are nicked more rapidly or repaired more slowly than in SMG. Figure 5 shows that in stationary-phase cells both the rate of nicking and the extent of degradation of restoration of DNA molecules are dependent on the medium. Cells labeled with [³H]thymidine were irradiated, lysed, and centrifuged on alkaline sucrose gradients after 10 min or 3 h of incubation in SMG, SMG plus 1 mg of caffeine per ml, nutrient broth, or nutrient broth plus 50 μg of chloramphenicol per ml. The results shown in Fig. 5 indicate that nicking was much more rapid in nutrient broth than in SMG, and that chloramphenicol initially inhibited nicking in nutrient broth. After 3 h there was considerably more high-molecular-weight DNA in cells incubated in SMG than in cells incubated in

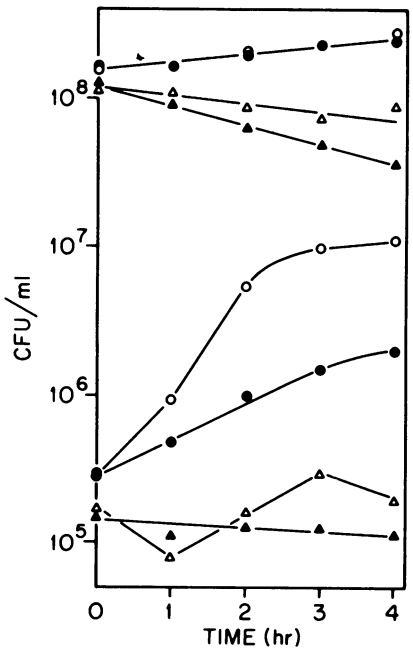

FIG. 4. *Inhibition of recovery of GSY1027 (uvr-1) by caffeine in liquid. Stationary-phase cells were irradiated in SMG and incubated in SMG containing various concentrations of caffeine and plated on NA. Symbols: ○, no caffeine; ●, 1.0 mg/ml; from a different experiment △, 1.5 mg/ml; ▲, 2.0 mg/ml. Upper set of curves, unirradiated cells; lower set, irradiated with 15 J of UV per m².*

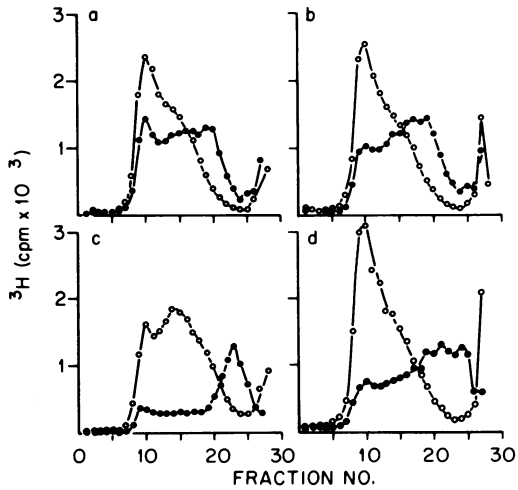

FIG. 5. *(a–d) Alkaline sucrose gradient analysis of DNA from cells of GSY1027 during recovery. Stationary-phase cells labeled with [³H]thymidine were irradiated with 5 J of UV per m² and incubated as described in the text. They were lysed on the gradients and centrifuged as described in Materials and Methods. The direction of sedimentation is from right to left. Symbols: ○, 10 min of incubation; ●, 3 h of incubation. (a) SMG; (b) SMG + 1 mg of caffeine per ml; (c) nutrient broth; (d) nutrient broth + 50 μg of chloramphenicol per ml.*

SMG plus caffeine or in nutrient broth. In nutrient broth alone most of the DNA was degraded, but that degradation was inhibited by chloramphenicol. It is not possible to conclude from this experiment whether caffeine inhibits rejoining of nicked strands or hastens the nicking observed in SMG alone.

Genetic analysis has shown that for recovery to be possible the irradiated cell must be *uvr-1*, RecA$^+$, PolI$^+$. Figure 6a shows the responses of GSY1026 (wild type for repair), FB56 (*uvs-42*), and HA160 (*polA59*) compared with GSY1027 (*uvr-1*). Neither wild-type nor *uvs-42* cells, which are dimer-excision deficient, respond at all to incubation in SMG, while PolI$^-$ cells show a further loss of viability. In Fig. 6b, the responses of several other mutants are compared. The *recA1* mutant GSY1025 does not exhibit a significant amount of recovery. However, GSY1063, a double mutant containing both *recA* and *uvr-1*, does recover in SMG. In this strain recovery is slower than in GSY1027 (*uvr-1*), in keeping with the reduced, but not completely absent, recombination ability of *recA* cells, but recovery is considerably faster than in the *recA* strain. It is not addition of a Uvr$^-$ mutation to a *recA* strain that allows recovery to occur, since the double mutant FB52 (*uvs-42 recA1*) does not recover during incubation in SMG. It is clear that the *uvr-1* mutation is essential to allow recovery, and RecA$^+$ activity is necessary for maximal recovery. DNA polymerase I deficiency results in further death of UV-irradiated cells during incubation in SMG, probably by failure of another pathway since the *uvr-1* mutation is not involved.

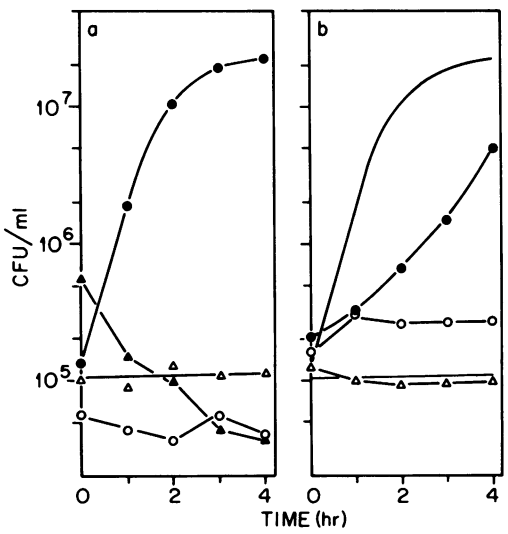

FIG. 6. *(a-b) Genetic requirements for recovery. Stationary-phase cells of various mutants were irradiated at a concentration of 1 × 10^8 to 2 × 10^8 colony-forming units per ml, incubated in SMG, and plated on nutrient agar. The UV fluences were: (a) GSY1026 (wild type)* (○), *150 J/m^2; GSY1027 (uvr-1)* (●) *and FB56 (uvs-42)* (△), *15 J/m^2; HA160 (polA59)* (▲), *40 J/m^2. (b) GSY1025 (recA1)* (○), *60 J/m^2; GSY1063 (uvr-1 recA1)* (●), *2.4 J/m^2; FB52 (uvs-42 recA1)* (△), *2.0 J/m^2. The solid lines without data points are for GSY1027 and FB56 (from part a).*

DISCUSSION

The markedly reduced UV sensitivity of *uvr-1* cells plated on SAA agar compared with cells plated on NA (Fig. 1) can be accounted for by a recovery process that occurs before cell growth resumes. Kinetic studies of recovery in liquid medium show that recovery is maximal at 3 h after irradiation (Fig. 2-4). At this time, the number of cells forming colonies on NA is the same as the number initially surviving on SAA plates (Fig. 3a). This implies that a large fraction of the UV damage is repaired in all of the cells during the recovery period, even though at the fluence chosen for kinetic studies only about 5% of the cells survived after maximum recovery occurred.

Recovery of irradiated *uvr-1* cells of *B. subtilis* from sensitivity to rich medium seems to be different from recovery processes in *E. coli*. It is not typical liquid-holding recovery, since liquid-holding recovery in *E. coli* requires Uvr$^+$ activity and does not require an energy source. *E. coli* K-12 derivatives must in addition be RecA$^-$ (10). The disappearance of photoreactivable lesions during liquid-holding recovery (17) further implies that dimer excision is necessary for liquid-holding recovery. In contrast, recovery in *B. subtilis* requires an energy source, and in the strains studied recovery can be observed only in strains carrying the *uvr-1* mutation.

E. coli B exhibits markedly less sensitivity to UV irradiation when plated on minimal media than when plated on rich media (2, 18, 26), as do either Rec$^-$ or Uvr$^-$ K-12 strains (10). In the case of *E. coli* B, this appears to be because filament formation is reduced on minimal agar (1). K-12 *recA* strains, which carry out liquid-holding recovery, probably carry out the same process (excision repair before growth) on minimal agar medium but not on complex agar (10). K-12 Uvr$^-$ strains incubated in liquid minimal medium or on minimal agar medium lose sensitivity to complex media, but net protein synthesis is required for this effect (10, 13).

The similarities and differences between

these phenomena and the recovery from sensitivity to rich medium exhibited by *B. subtilis uvr-1* are summarized in Table 4. The marked medium-dependent radiation response shown by GSY1027 (*uvr-1*) has little in common with the *E. coli* processes described above except that for minimal medium recovery the cells must be Rec⁺. However, minimal medium recovery appears to be a postreplicational repair process (12), whereas there is little or no net DNA synthesis in GSY1027 incubated in SMG after irradiation (C. Hadden, unpublished observations), and inhibiting replication with HPUra does not inhibit recovery (Table 3).

Unlike the *uvs-42* mutant FB56, which does hardly any incision at pyrimidine dimer sites, GSY1027 (*uvr-1*) can carry out a limited amount of incision (15). However, there is no more release of dimers into acid-soluble material by GSY1027 than by FB56 (C. Hadden, manuscript in preparation), implying that dimer excision can be initiated by GSY1027 but not completed in the normal manner.

Perhaps the recovery of viability of irradiated *uvr-1* cells reflects a different type of excision repair, dependent on the RecA gene product, which is slow to be completed but which must be completed before growth resumes; or perhaps recovery reflects a prereplication recombinational repair taking place in cells with duplicated chromosomes. (In *B. subtilis* 168 derivatives, most of the stationary-phase cells contain partially replicated chromosomes [31].) Recovery in SMG containing HPUra implies that replication is not required for recovery. Growth before completion of the recovery process probably leads to degradation of DNA, especially in nutrient broth, in which incision is more rapid. In nutrient broth the defective prophage PBSX might be induced (27). However, PBSX packages DNA molecules of about 1.2×10^7 daltons (27), and the size of the DNA in the low-molecular-weight peak in Fig. 5c is about 10^7 daltons, twice the size of single-stranded DNA from PBSX. There is even more low-molecular-weight DNA in the 3-h sample of Fig. 5d (nutrient broth plus chloramphenicol), and chloramphenicol is reported to prevent induction of PBSX (27). Therefore, it does not seem likely that sensitivity to NA is a result of prophage induction.

The amount of DNA recovered in the experiment shown in Fig. 5 was lowest after 3 h of incubation in nutrient broth (Fig. 5c). Thus, the cause of death may be degradation at sites of single-strand breaks, occurring because incision is faster than closure of the breaks by the recovery process. This recovery then would be masked by normal excision repair in the Uvr⁺ parent strain. Recovery would not be possible without incision, which depends on the *uvs-42* gene product. Because of the dependence of recovery on *recA*⁺ activity and the inhibition of recovery by caffeine which also inhibits recombination (22), it seems likely that recovery involves recombination, specifically the class of recombination defective in the *recA* mutant (8). This suggests that dimers may persist during recovery as they do during recombinational repair in *uvr* cells of *E. coli* K-12 (9).

In summary, it appears that lack of the *uvr-1* gene product initiates the sequence of events leading to recovery of viability under nongrowing conditions, probably after incision at pyrimidine dimer sites. Inability to complete dimer excision blocks completion of normal excision repair and shunts the damage into another repair pathway, which is recombination dependent. This pathway is relatively slow, and unrepaired nicks are lethal if growth resumes before they are repaired. Maximum recovery requires at least 3 h of delayed growth after a UV dose of 15 J/m².

ACKNOWLEDGMENTS

I thank A. S. Angel for technical assistance and D. Billen, G. R. Hellermann, and J. F. Lemontt for their critical reading of the manuscript. This work stemmed from observations made by Alonzo T. White, undergraduate research trainee, Carnegie Corporation Program. The work was supported in part by Atomic Energy Commission contract AT (40-1)-3596 (1974).

TABLE 4. *Requirements for recovery processes in E. coli and B. subtilis uvr-1*

Process	Property	*E. coli* type		*B. subtilis*
		B	K-12	
Liquid-holding recovery	Uvr⁺ character	+	+	−
	Rec⁻ character	−	+	−
	Energy requirement	−	−	+
Minimal medium recovery	Uvr⁻ character (retention of pyrimidine dimers)		+	−[a]
	Rec⁺ character		+	+
	Protein synthesis		+	−

[a] Scored as negative because the Uvr⁻ strain FB56 (*uvs-42*) does not exhibit recovery.

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