JOURNAL OF VIROLOGY, Mar. 1981, p. 955-962
0022-538X/81/030955-08$02.00/0

Vol. 37, No. 3

Relation Between UV Suppression of Polarity in φX174 and UV Sensitivity of *rho* Mutants

JAN S. FASSLER AND IRWIN TESSMAN*

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

The suppression of polarity by UV irradiation was similar to the suppression by *rho* mutants. This was demonstrated for a polar nonsense mutant of phage φX174. Treatment of the host for 30 min with 100 μg of the radiomimetic drug mitomycin C per ml was about as effective as 550 J of UV irradiation per m^2 in relieving polarity. The shape of the UV survival curves for *rho* mutants could be linked to a proposed mechanism of UV relief of polarity. Host cell reactivation of phage lambda and W-reactivation of phage G4 were unaffected by *rho* mutations. UV suppression of polarity is independent of the Hcr^- and $RecA^-$ phenotypes. An explanation for the UV sensitivity of *rho* mutants is provided, and several ways are considered in which UV irradiation may deplete cellular rho activity and thereby cause UV relief of polarity. We propose a novel theory that relates the UV inactivation of normal repair-proficient cells to a decrease in rho activity.

Polarity, a reduction in the activity of genes downstream from a promoter (13), can be produced by point nonsense mutations and insertion sequences (14, 27, 28); it can also exist in the natural state of an operon (6, 15). It is caused by termination of transcription (6, 11), and in the case of mutational polarity this termination is premature.

In vitro experiments demonstrate that the rho protein is needed to terminate transcription for either natural or mutational polarity (6, 25). A model for rho involvement in this termination (1) provides a needed connection between translational termination at a nonsense codon and subsequent transcriptional termination at a signal further downstream.

An effect of UV irradiation is to suppress polarity. This was shown in a study of the contiguous capsid genes *F*, *G*, and *H* of bacteriophages S13 and φX174 (21). Since *rho* mutations also suppress polarity (18, 22, 24), Pollock et al. (21) suggested that UV irradiation of the cells may lead to reduced rho activity, an idea that further seemed plausible because of the known effect of *rho* mutations in creating UV-sensitive cells (4). Such a relationship between UV irradiation and rho activity could have far-reaching consequences. However, Pollock et al. provided no direct evidence that the effect of UV irradiation paralleled the effect of *rho* mutations in the phage system which they studied.

In the work reported here, we continued the investigation of UV suppression of polarity. The initial question was whether the effect of UV irradiation indeed parallels the effect of *rho* mutations. This question was answered by comparing the two effects in the same system. We also examined whether mitomycin C (MC) mimics UV irradiation in suppressing polarity.

The hypothesis that rho is involved in the repair of UV damage prompted us to examine the effect of *rho* mutations on repair processes. We also studied the roles of two repair mechanisms, excision repair and recA-inducible repair, in the UV suppression of polarity.

A model of rho activity depletion by UV irradiation provides an explanation for both the suppression of polarity by UV irradiation and the UV sensitivity of *rho* mutants. The depletion theory is also proposed as an answer to the old question of why normal cells die after irradiation.

(A preliminary presentation of this work has been made [J.S. Fassler and I. Tessman, Abstr. Phage Meet., Cold Spring Harbor Laboratory, p. 131, 1979].)

MATERIALS AND METHODS

Bacterial strains. *Escherichia coli* C3104 (*psu*$_2$) and C3108 (*psu*$_3$) were supplied by Richard Goldstein and are derivatives of *E. coli* HF4704 (17) carrying the *rho102* and *rho103* alleles, respectively, which were isolated by Korn and Yanofsky (15, 16). *E. coli* C3103 is the corresponding rho^+ strain. AD1600 (*rho ts15*) and its parent *E. coli* SA1030 (4) were provided by Asis Das. AP1 is a UV-sensitive (Hcr^-) derivative of *E. coli* C. Strains AB1157 (rec^+), AB2463 (*recA*), and AB2500 (*uvrA*) were obtained from Barbara J. Bachmann.

Bacteriophage. φX174 and S13 mutants were from our own collection.

Media. Labeled protein extracts were made in HFS-T medium which consisted of 5×10^{-2} M Tris-hydro-

chloride (pH 7.4), 2×10^{-2} M NH_4Cl, 1×10^{-2} M NaCl, 1×10^{-3} M $MgSO_4$, 1×10^{-4} M $CaCl_2$, 5×10^{-4} M K-H-PO_4 (pH 7.4), and 1×10^{-5} M $FeCl_3$; this medium was supplemented with 0.2% glucose.

Chemicals. MC (lot 49C-0411) was obtained from Sigma Chemical Co. We found that some lots prepared earlier were ineffective in reducing host protein synthesis and suppressing polarity. [4,5-^{3}H]leucine (120 Ci/mmol, 1 mCi/ml) was obtained from Amersham Corp.

UV irradiation. UV irradiation was performed with a 15-W General Electric germicidal lamp; the intensity was determined by the current generated in a calibrated UV-sensitive photocell.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The preparation of labeled extracts of phage-infected cells was essentially as described previously (20). Samples (10 μl) were electrophoresed for 2.5 h at 140 V through a 13.4% acrylamide gel. The gel composition was 13.4% (wt/vol) acrylamide (Miles Laboratories, Inc.), 0.067% (wt/vol) *N,N'*-methylbisacrylamide (Aldrich Chemical Co.), 0.089% (vol/vol) *N,N,N',N'*-tetramethylenediamine (Miles), 0.089% (vol/vol) 2-mercaptoethanol (Eastman Organic Chemicals), 25% (vol/vol) denaturing buffer (0.4 M Tris-acetate, pH 9.1, 4.0 M urea, 0.4% sodium dodecyl sulfate), and 0.22% (wt/vol) ammonium persulfate (Mallinckrodt).

RESULTS

Effect of *rho* mutations on polarity in φX174. The previous studies on suppression of polarity in φX174 and S13 involved several polar mutants; one of these mutants, φX*amF1005*, was used here. We compared the polar effects of *amF1005* in a *rho*$^+$ (*psu*$^+$) strain and *rho* (*psu*$_2$ and *psu*$_3$) strains; the behavior of φX$^+$ provided a standard reference (Fig. 1). The effect of UV irradiation (21) was retested in order to provide a parallel study.

Figure 1, lanes 1 and 2, show extracts from φX$^+$ infections of *psu*$^+$ and *psu*$_2$, respectively. Bands corresponding to proteins F, G, and H are prominent. Lanes 3 and 4 correspond to the same infections but with cells that were pre-irradiated (550 J/m^2). The main effect of the UV irradiation was to increase the proportion of the D protein, as previously observed (21).

Infections with the polar mutant *amF1005* are shown in lanes 5 through 8. Lane 5 shows the absence of the F, G, and H bands. Faint host bands, which were observed with uninfected cells, remained in the positions of F and G, so that complete absence of these phage proteins was not proven. Fortunately, however, host bands were completely absent at the position of H. The key results are shown in lane 6; in *psu*$_2$ the G and H bands were restored. The reappearance of the H band is striking, but to measure restoration of G we had to rely on the considerable increase in the intensity of the G band. Densitometry of the fluorogram showed

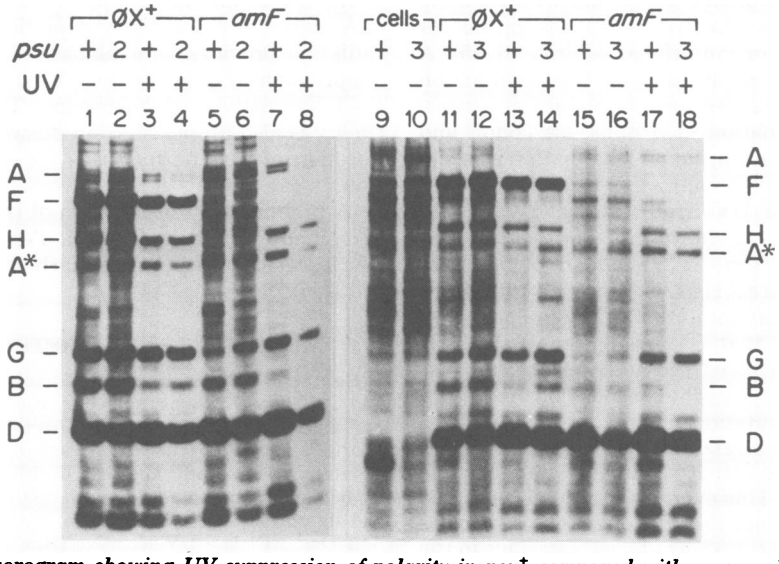

FIG. 1. *Fluorogram showing UV suppression of polarity in psu$^+$ compared with suppression by the rho mutants psu$_2$ (lanes 1 through 8) and psu$_3$ (lanes 9 through 18). Cells were infected with a polar mutant of φX, φXamF1005. Polarity and its suppression were measured by comparing the densities of affected bands G and H with the density of the unaffected band A*. Fluorography and densitometry were performed as previously described (20). Unirradiated cells were labeled from 19 to 24 min after infection; pre-irradiated cells (550 J/m^2) were labeled from 15 to 45 min after infection. Extracts were electrophoresed in a sodium dodecyl sulfate-polyacrylamide gel.*

that the *rho* mutation restored 20 to 25% of the activities of genes *G* and *H*, with the intensity of the A* bands serving as a reference. These quantitative measurements were made with presensitized films exposed within the linear response range.

As expected, UV irradiation suppressed the polarity in *psu*$^+$ (Fig. 1, lane 7). Densitometry indicated that the restoration resulted in 35 to 45% of the amounts of proteins G and H found in ϕX^+. The effect of the combination of UV irradiation and the *rho* mutation (lane 8) was comparable to the effect of UV alone in relieving polarity, suggesting that these effects are not additive at the saturating UV dose. At a UV dose below saturation, a *rho* mutation added to the effect of UV irradiation (data not shown).

Results with *psu*$_3$ were qualitatively the same (Fig. 1, lanes 11 through 18). Figure 1, lanes 9 and 10, show the host proteins and particularly the absence of any major band at the position of H. In general, a large UV dose was somewhat more effective than a viable *rho* mutation in suppressing polarity.

MC suppression of polarity. MC cross-links DNA (12), and because of its general radiomimetic properties (3, 9, 19, 26) it might be expected to suppress polarity. Preliminary results (22; Pollock, personal communication) suggested that MC does indeed suppress polarity. We confirmed that here (Fig. 2). The ϕX polar mutant *amF1005* was again used, and the following four doses of MC were tested: 0, 1, 10, and 100 μg/ml for 30 min. A comparison of lanes 2 and 3 shows that the polarity of *amF1005* was again demonstrated by the elimination of bands G and H in addition to band F. There was no restoration of bands G and H with MC doses of 1 and 10 μg/ml. However, at 100 μg of MC per ml (lanes 10 through 12) polarity was suppressed, as shown by the reappearance of bands G and H. The MC dose of 100 μg/ml for 30 min was massive compared with the dose of 1 μg/ml for 10 min that is needed to cause lysis of a lambda lysogen (19), a dose which we confirmed for our lot of MC.

The effect of MC (100 μg/ml for 30 min) was comparable to the effect of UV irradiation (550 J/m^2). In *amF1005* MC restored 26% of the H protein (lanes 16 through 18), whereas UV irradiation restored 38% (lanes 19 through 21).

UV sensitivity of *rho* strains. An intriguing aspect of the Rho$^-$ phenotype is the sensitivity of *rho* mutants to UV irradiation. This was noted by Das et al. (4), who found that in a group of 16 mutants with mutations that mapped in the region of *rho* and also suppressed polar mutations in the *gal* operon, all were qualitatively UV sensitive. We determined the inactivation curves for AD1600 (*rho ts15*) and its parent strain SA1030 (*rho*$^+$) (Fig. 3). It was striking that although the rho defect produced a more sensitive strain, the ultimate slope was not affected.

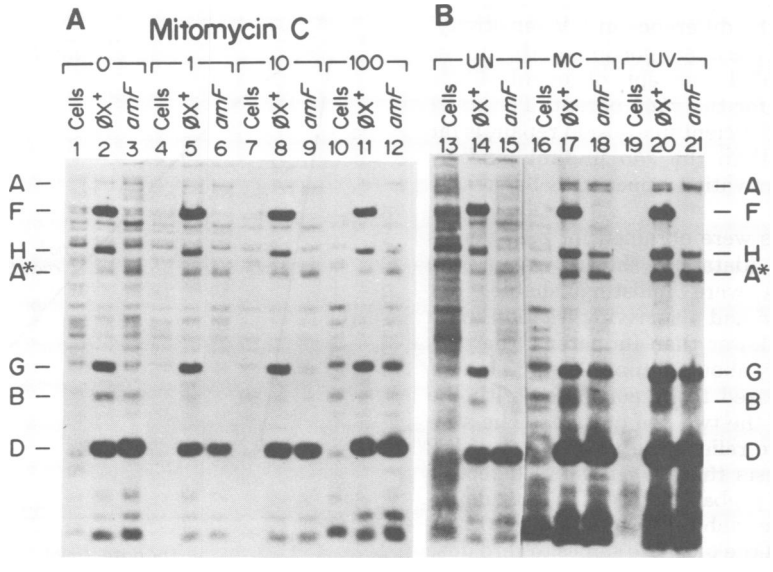

FIG. 2. *Fluorogram showing MC suppression of polarity. (A) Effect of pretreating strain AP1 for 30 min with doses of 0, 1, 10, and 100 μg of MC per ml. After treatment the cells were washed two times in HFS-T medium containing glucose, resuspended in HFS-T medium containing glucose, infected with either ϕX^+ or the polar mutant amF1005, and labeled as described in the legend to Fig. 1. (B) Comparison of MC treatment (100 μg/ml for 30 min) with UV irradiation (550 J/m^2). UN, Untreated cells.*

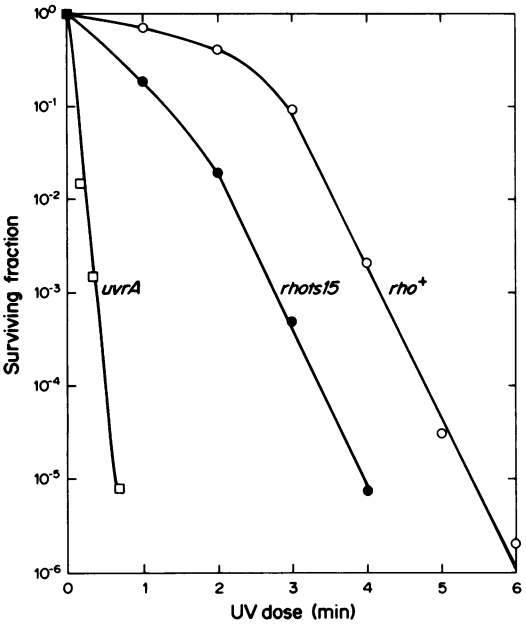

FIG. 3. *UV inactivation of E. coli strains SA1030 (rho+), AD1600 (rho ts15), and AB2500 (uvrA) at a dose rate of 45 J/m² per min. The cells were grown at 30°C overnight to stationary phase in tryptone broth (1.3% tryptone [Difco], 0.7% NaCl) and diluted 20-fold in 0.05 M ammonium acetate (pH 7.0) just before irradiation.*

Thus, the difference in the initial shoulder was responsible for the difference in UV sensitivity. Extrapolation of the linear portions of the curves to zero dose yielded intercepts of roughly 40 for *ts15* and 10,000 for the *rho*+ parent. The *uvrA* strain, which is deficient in excision repair, is far more sensitive than the *rho* mutant, and by contrast its inactivation appears to be strictly exponential.

Similar curves were obtained for psu_3 versus its parent *psu*+ (data not shown). Again, the ultimate slopes were indistinguishable, but whereas *rho ts15* had a surviving fraction more than 100 times lower than its parent, the psu_3 survival was only about 10 times lower. The UV sensitivity appeared to be correlated with the defectiveness of the two *rho* mutants. The ability of *rho ts15* to relieve the polar effect of IS2 insertions suggests that *ts15* is more defective than psu_3 and is probably one of the most defective of all of the viable *rho* mutants (4, 6, 23). Extrapolation of the ultimate slopes to zero dose yielded intercepts of roughly 6,000 and 50,000 for psu_3 and *psu*+, respectively.

A physical interpretation of the extrapolated intercepts is beyond the scope of this paper. Several considerations, such as whether the inactivation is multiunit (multitarget in current terminology) or multihit (2), critically determine the significance of the intercepts.

Ability of *rho* mutants to repair DNA damage. We examined the ability of *rho* mutants to perform host cell reactivation by measuring the survival of UV-irradiated lambda (Fig. 4). There was no difference in the abilities of the *rho*+ and *ts15* strains to repair the irradiated phage. In contrast, survival of lambda was much reduced on strain AP1. Strains psu_3 and psu_3^+ behaved exactly like *ts15* and *ts15*+, respectively (data not shown).

Comparison of W-reactivation of phage G4 in psu_3 and psu_3^+ also did not show any clear difference. For G4 initially inactivated to a survival of 10^{-5}, these strains produced reactivation by factors of 4.5 for *psu*+ and 3.5 for psu_3, a difference that is within the range of fluctuations found for W-reactivation of G4 (Tessman, unpublished data).

Role of excision repair and recombination repair in UV suppression of polarity. UV suppression of polarity had been demonstrated only in strain AP1, an Hcr⁻ derivative of *E. coli* C. To test whether an Hcr⁻ phenotype is essential for the UV effect, we used *E. coli C* (Hcr+) (Fig. 5). In quantitative experiments on the polar mutant *G4amF223*, the G band was restored to 25 and 70% of the wild-type amount at doses to the Hcr+ host of 70 and 550 J/m², respectively. These results are comparable to

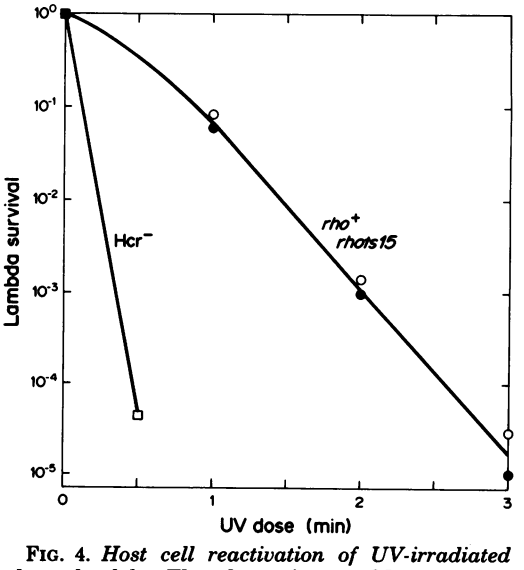

FIG. 4. *Host cell reactivation of UV-irradiated phage lambda. The phage, in 0.05 M ammonium acetate (pH 7.0), was irradiated at a dose rate of 165 J/m² per min and plated at 35°C for assay on rho+ (○), rho ts15 (●), and the Hcr⁻ strain AP1 (□).*

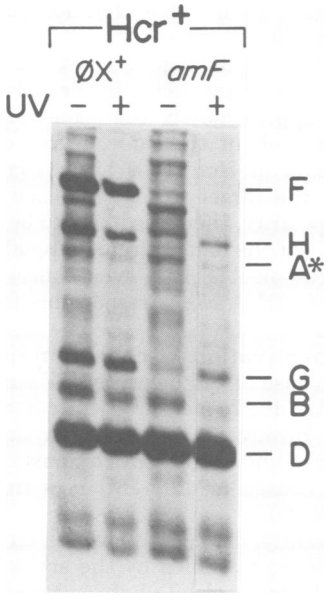

FIG. 5. *Fluorogram showing UV suppression of polarity of φXamF1005 in an Hcr⁺ host (E. coli C) preexposed to 550 J of UV irradiation per m². The results of a comparable infection but with an Hcr⁻ host (AP1) are shown in Fig. 2, lanes 19 through 21.*

what was found previously for the Hcr⁻ strain AP1 (21). Thus, it appears that excision repair has little effect even on the UV dose needed to suppress polarity.

Previous studies had been done only in *recA*⁺ cells. To test the need for recA protein, we studied polarity in the *recA* strain AB2463 and the parental *recA*⁺ strain AB1157. These K12 strains had been made sensitive to S13 and φX (31). Because S13 adsorbed to the cells better than φX, we used the polar S13 gene *G* mutant *amG83*, which has a reduced level of H protein. UV irradiation restored the H protein to roughly the same extent in both *rec*⁺ and *recA*, with S13⁺ serving as a standard (Fig. 6). Because of the large background of host proteins in the extracts, accurate comparisons were not possible. However, qualitatively it was evident that the RecA function is not required for UV suppression of polarity.

DISCUSSION

Parallel effects of UV irradiation and *rho* mutations. In a comparative study, we found that UV irradiation and Rho⁻ defects were similar in the ability to suppress the polar effects of nonsense mutations within gene *F* of φX174. The *rho* strains containing the psu_2 and psu_3 mutations were used because they adsorb φX, which the *rho* strain AD1600 (*ts15*) does not. Our results support the view (21) that UV irradiation reduces rho activity in *E. coli*.

A complementary observation is that rho-defective cells are UV sensitive (4), which immediately suggests that rho might function in the repair of UV damage. We confirmed that *rho* mutants are UV sensitive. Significantly, however, our detailed inactivation curves showed that *rho* mutants are actually inactivated at the same rate as *rho*⁺ strains once the initial shoulder is passed. The key point is that *rho* mutations are equivalent to an increase in dose by an absolute amount rather than by a constant factor, for if a *rho* mutation were equivalent to a dose increase by a constant factor (as is the case for an Hcr⁻ strain), the ultimate slope of the inactivation curve for the mutant would be steeper.

In irradiated cells involvement of rho in repair would reduce its availability for the termination function and thereby provide a reasonable explanation for the effect of UV irradiation on polarity. However, it is also quite conceivable that rho is not involved in repair. Figure 7 shows a general approach to the two UV effects (on transcription termination and on survival of *rho* mutants). This figure provides a unified way to view the UV effects and avoids the assumption

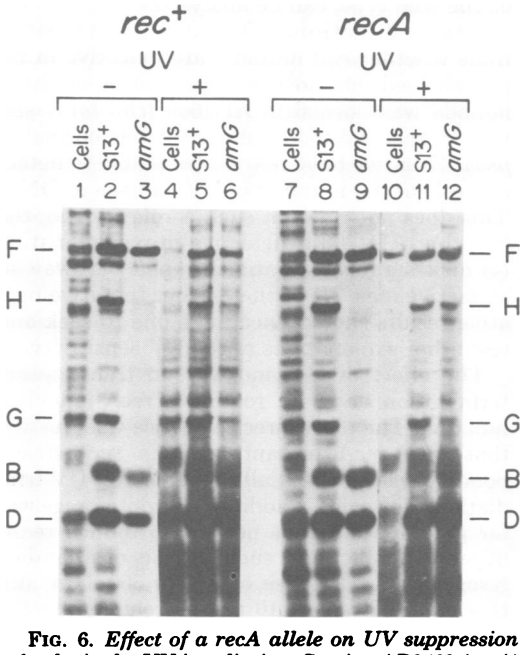

FIG. 6. *Effect of a recA allele on UV suppression of polarity by UV irradiation. Strains AB2463 (recA) and AB1157 (rec⁺) were irradiated with 550 J/m² and infected with S13⁺ or the polar mutant amG83. Restoration of H protein was examined.*

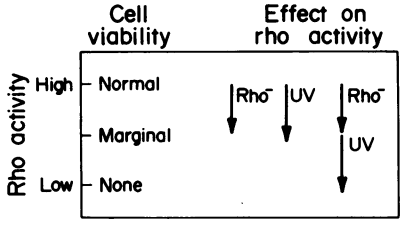

FIG. 7. *General model to explain the UV sensitivity of rho mutants in terms of the lethal consequence of severely reduced rho activity and a postulated reduction of rho activity by UV irradiation.*

that rho has a repair function.

In Fig. 7, normal growth is assumed to require full rho activity. If viable, a *rho* mutant would still have some rho activity, but the reduced level would lower the ability to function, as in transcription termination. From the results on polarity suppression, UV irradiation is pictured as having an effect that parallels the effect of a *rho* mutation in reducing rho activity. Thus, since rho activity in a *rho* mutant is already at a marginal level for survival (4, 11), the mutant would be extrasensitive to UV irradiation; the combination of the mutation and UV irradiation would reduce rho activity to a level too low for growth. This model provides a framework in which the UV sensitivity of *rho* mutants, as well as the wild type, can be analyzed.

Repair functions. Our attempts to determine whether *rho* mutants are defective in repair showed that host cell reactivation of phage lambda was normal in AD1600 (*rho ts15*) and that W-reactivation of phage G4 was normal in psu_2. Thus, we have not yet been able to detect any role of rho in repair of UV damage to DNA. This does not rule out such a role for rho; the fact that AD1600 is defective in recombination (4) makes a recombinational repair pathway an attractive idea. Nevertheless, our tentative negative results should encourage one to seek and test other explanations of the UV sensitivity.

The effect of UV and MC on transcription termination does not require a recA-inducible function. The most direct evidence was the fact that in a *recA* mutant, as in a rec^+ strain, polarity was substantially reversed by UV irradiation. However, in addition the dose range was far greater than that needed to induce recA-dependent functions, such as lysis of a lambda lysogen. The UV dose of 150 to 550 J/m^2 (21; this paper) is about 10 times greater than the value of about 20 J/m^2 needed to induce lambda (32). However, even more striking was the relatively large MC dose which was needed for polarity reversal (see above). Therefore, the damage needed for maximum polarity suppression is considerably greater than that needed to induce recA functions.

Repair of UV damage to DNA does not seem to be an important factor in the polarity effect because approximately the same range of UV doses gave about the same degree of polarity suppression in Hcr^+ cells as in Hcr^- cells. If DNA is the target for UV damage that suppresses polarity, then it is likely to be the initial damage, not the damage remaining after repair, that is responsible. It is even conceivable that damage to DNA is not responsible for polarity reversal.

UV inactivation of cells. Surprisingly, the specific cause of cell death by UV irradiation is unknown (death measured by colony-forming ability). However, there are many hypotheses, and indeed under different circumstances death may be caused in different ways (reviewed in reference 30).

Apparently, damage to DNA is an important initial event, as shown by the action spectrum for cell killing and by photoreactivation. However, under conditions where the major DNA repair mechanisms are functioning, the actual cause of cell death may not be the continued presence of damaged DNA; rather, the initial DNA damage may upset a chain of essential physiological processes that is needed for survival and cell duplication. This general approach is like one proposed by Swenson (30).

The theory outlined in Fig. 7 provides a specific explanation of how UV irradiation causes cell death: lethality is caused by the reduction of rho activity. The argument is simple. UV irradiation lowers rho activity, lowered rho activity is lethal, and therefore, a cause of UV lethality is the reduction of rho activity. That this is not merely a potential cause but rather an actual cause of lethality is shown by the increased UV sensitivity of *rho* mutants, which indicates that rho activity is a limiting factor in survival.

It follows from this theory that the inactivation curves should have a shoulder because death does not occur unless rho activity is reduced below the so-called marginal level indicated in Fig. 7. Because rho activity is already reduced in a *rho* mutant, one expects the shoulder in the inactivation curve to be smaller and tbe final slope to be unchanged, as observed. In the case of *rho ts15*, the survival curve can be explained by assuming that the mutant receives a dose 60 J/m^2 higher than the parent strain. This can be thought of as the dose equivalent to the Rho^- defect (i.e., the dose that would be needed to reduce the rho activity in wild-type cells to that found in *rho ts15*). A UV dose of about 60 J/m^2 is roughly in the dose range

needed to suppress polarity to the extent that the *rho* mutant does (21).

An implication of our work is that in normal repair-proficient cells repair is so efficient that ultimately cell death occurs for reasons of rho deficiency rather than lack of repair. However, it is possible that lack of repair could contribute to the slow rate of inactivation in the shoulder region of the inactivation curve.

Since *rho* mutations have a pleiotropic effect (5), we cannot immediately say what might be the most critical aspect of a rho deficiency. Two likely possibilities are clearly the transcription termination function of rho and the polycytidylic acid-dependent ATPase activity. Although we have focused on rho activity, it should be recognized that anything that varies in amount in the same way as rho activity could be the actual weak link in the chain of processes essential for survival.

Mechanisms by which UV could reduce rho activity. The idea that UV irradiation and radiomimetic agents reduce the in vivo activity of rho is based on indirect evidence. To seek direct proof, we must bear in mind that the amount of rho protein itself need not necessarily be altered, for there are other ways to reduce rho activity. For example, (i) the coupled ATPase activity is essential for transcription termination (7, 8), and UV irradiation may deplete the pool of cellular ATP; and (ii) rho may be occupied in the degradation of abnormal proteins (29) and thus be less available for transcription termination. UV irradiation produces abnormally short transcripts, which could increase the amount of short polypeptides needing degradation.

Despite the complications, the proposed explanation for the suppression of polarity by UV irradiation provides a novel approach to the understanding of the effects of UV irradiation and other cell-damaging treatments.

ACKNOWLEDGMENTS

Ethel S. Tessman generously provided assistance, criticism, and encouragement at every stage of this work. The technical assistance of Joyce Dodd Forestal and Ruth Ann Gyure is also appreciated.

This research was supported by Public Health Service grant CA-22239 from the National Cancer Institute. J.S.F. was the recipient of a traineeship supported by Public Health Service Training Grant GM-7211 from the National Institutes of Health.

LITERATURE CITED

1. **Adhya, S., and M. Gottesman.** 1978. Control of transcription termination. Annu. Rev. Biochem. **47**:967–996.
2. **Atwood, K. C., and A. Norman.** 1949. On the interpretation of multi-hit survival curves. Proc. Natl. Acad. Sci. U.S.A. **35**:696–709.
3. **Boyce, R., and P. Howard-Flanders.** 1964. Genetic control of DNA breakdown and repair in *E. coli* K12 treated with mitomycin C or ultraviolet light. Mol. Gen. Genet. **95**:345–350.
4. **Das, A., D. Court, and S. Adhya.** 1976. Isolation and characterization of conditional lethal mutants of *Escherichia coli* defective in transcription termination factor rho. Proc. Natl. Acad. Sci. U.S.A. **73**:1959–1963.
5. **Das, A., D. Court, and S. Adhya.** 1978. Pleiotropic effect of *rho* mutation in *Escherichia coli*, p. 459–468. *In* M. Chakravarty (ed.), Molecular basis of host-virus interactions. Science Press, Princeton, N.J.
6. **de Crombrugghe, B., S. Adhya, M. Gottesman, and I. Pastan.** 1973. Effect of rho on transcription of bacterial operons. Nature (London) New Biol. **241**:260–264.
7. **Galluppi, G., C. Lowery, and J. P. Richardson.** 1976. Nucleoside triphosphate requirement for termination of RNA synthesis for rho factor, p. 657–665. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Howard, B. H., and B. de Crombrugghe.** 1976. ATPase activity required for termination of transcription by the *Escherichia coli* protein factor ρ. J. Biol. Chem. **251**: 2520–2524.
9. **Iijima, T., and A. Hagiwara.** 1960. Mutagenic action of Mitomycin C on *Escherichia coli*. Nature (London) **185**:395–396.
10. **Imamoto, F.** 1970. Evidence for premature termination of transcription of the tryptophan operon in polarity mutants of *Escherichia coli*. Nature (London) **228**:232–235.
11. **Inoko, H., K. Shigesada, and M. Imai.** 1977. Isolation and characterization of conditional-lethal rho mutants of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**: 1162–1166.
12. **Iyer, V. N., and W. Szybalski.** 1963. A molecular mechanism of Mitomycin action: linking of complementary DNA strands. Proc. Natl. Acad. Sci. U.S.A. **50**:355–362.
13. **Jacob, F., and J. Monod.** 1961. On the regulation of gene activity. Cold Spring Harbor Symp. Quant. Biol. **26**: 193–209.
14. **Jordan, E., H. Saedler, J. Lengeler, and P. Starlinger.** 1967. Changes in the specific activities of the galactose enzymes in *E. coli* under different growth conditions. Mol. Gen. Genet. **100**:203–209.
15. **Korn, L. J., and C. Yanofsky.** 1976. Polarity suppressors increase expression of the wild-type tryptophan operon of *Escherichia coli*. J. Mol. Biol. **103**:395–409.
16. **Korn, L. J., and C. Yanofsky.** 1976. Polarity suppressors defective in transcription termination at the attenuator of the tryptophan operon of *Escherichia coli* have altered rho factor. J. Mol. Biol. **106**:231–241.
17. **Lindqvist, B. H., and R. L. Sinsheimer.** 1967. Process of infection with bacteriophage φX174. XIV. Studies on macromolecular synthesis during infection with a lysis-defective mutant. J. Mol. Biol. **28**:87–94.
18. **Morse, D. E., and P. Primakoff.** 1970. Relief on polarity in *E. coli* by "suA." Nature (London) **226**:28–31.
19. **Otsuji, N., M. Sekiguchi, T. Iijima, and Y. Takagi.** 1959. Induction of phage formation in the lysogenic *Escherichia coli* K-12 by Mitomycin C. Nature (London) **184**:1079–1080.
20. **Pollock, T. J.** 1977. Gene *D* of bacteriophage φX174: absence of transcriptional and translational regulatory properties. J. Virol. **21**:468–474.
21. **Pollock, T. J., E. S. Tessman, and I. Tessman.** 1979. Suppression of polar effects of nonsense mutations by ultraviolet irradiation. J. Bacteriol. **138**:122–125.
22. **Ratner, D.** 1976. Evidence that mutations in the *suA* polarity suppressing gene directly affect termination factor rho. Nature (London) **259**:151–153.
23. **Reyes, O., M. Gottesman, and S. Adhya.** 1976. Sup-

pression of polarity of insertion mutations in the *gal* operon and *N* mutations in bacteriophage lambda. J. Bacteriol. **126**:1108–1112.

24. **Richardson, J. P., C. Grimley, and C. Lowery.** 1975. Transcription termination factor rho activity is altered in *Escherichia coli* with *suA* gene mutations. Proc. Natl. Acad. Sci. U.S.A. **72**:1725–1728.
25. **Roberts, J. W.** 1969. Termination factor for RNA synthesis. Nature (London) **224**:1169–1174.
26. **Roberts, J. W., and C. W. Roberts.** 1975. Proteolytic cleavage of bacteriophage lambda repressor in induction. Proc. Natl. Acad. Sci. U.S.A. **72**:147–151.
27. **Shapiro, J. A.** 1969. Mutations caused by the insertion of genetic material into the galactose operon of *E. coli*. J. Mol. Biol. **40**:93–105.
28. **Shapiro, J. A., and S. L. Adhya.** 1969. The galactose operon of *E. coli* K12. II. A deletion analysis of operon structure and polarity. Genetics **62**:249–264.
29. **Simon, L. D., M. Gottesman, K. Tomczak, and S. Gottesman.** 1979. Hyperdegradation of proteins in *Escherichia coli rho* mutants. Proc. Natl. Acad. Sci. U.S.A. **76**:1623–1627.
30. **Swenson, P. A.** 1976. Physiological responses of *E. coli* to far UV radiation, p. 269–387. *In* K. C. Smith (ed.), Photochemical and photobiological review, vol. 1. Plenum Press, New York.
31. **Tessman, I.** 1966. Genetic recombination of phage S13 in a recombination-deficient mutant of *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **22**:169–174.
32. **Tomizawa, J.-I., and T. Ogawa.** 1967. Effect of ultraviolet irradiation on bacteriophage lambda immunity. J. Mol. Biol. **23**:247–263.