Relation Between UV Suppression of Polarity in \( \phi \)X174 and UV Sensitivity of \( \rho \)o Mutants

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The suppression of polarity by UV irradiation was similar to the suppression by \( \rho \)o mutants. This was demonstrated for a polar nonsense mutant of phage \( \phi \)X174. Treatment of the host for 30 min with 100 \( \mu \)g of the radiomimetic drug mitomycin C per ml was about as effective as 550 J of UV irradiation per m² in relieving polarity. The shape of the UV survival curves for \( \rho \)o 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 \( \rho \)o mutations. UV suppression of polarity is independent of the Hcr\(^{-} \) and RecA\(^{-} \) phenotypes. An explanation for the UV sensitivity of \( \rho \)o mutants is provided, and several ways are considered in which UV irradiation may deplete cellular \( \rho \)o 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 \( \rho \)o 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 \( \rho \)o protein is needed to terminate transcription for either natural or mutational polarity (6, 25). A model for \( \rho \)o 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 \( \phi \)X174 (21). Since \( \rho \)o mutations also suppress polarity (18, 22, 24), Pollock et al. (21) suggested that UV irradiation of the cells may lead to reduced \( \rho \)o activity, an idea that further seemed plausible because of the known effect of \( \rho \)o mutations in creating UV-sensitive cells (4). Such a relationship between UV irradiation and \( \rho \)o activity could have far-reaching consequences. However, Pollock et al. provided no direct evidence that the effect of UV irradiation paralleled the effect of \( \rho \)o 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 \( \rho \)o 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 \( \rho \)o is involved in the repair of UV damage prompted us to examine the effect of \( \rho \)o 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 \( \rho \)o activity depletion by UV irradiation provides an explanation for both the suppression of polarity by UV irradiation and the UV sensitivity of \( \rho \)o 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 (\( \psi \)su17) and C3108 (\( \psi \)su4) were supplied by Richard Goldstein and are derivatives of *E. coli* \( \psi \)RF4704 (17) carrying the \( \rho \)o102 and \( \rho \)o103 alleles, respectively, which were isolated by Korn and Yanofsky (15, 16). *E. coli* C3103 is the corresponding \( \rho \)o\(^{+} \) strain. AD1600 (\( \rho \)o \( \tau \)15) and its parent *E. coli* SA1030 (4) were provided by Asia Das. AP1 is a UV-sensitive (Hcr\(^{-} \)) derivative of *E. coli* C. Strains AB1157 (rec\(^{-} \)), AB2463 (recA), and AB2500 (\( \psi \)uvR) were obtained from Barbara J. Bachmann.

**Bacteriophage.** \( \phi \)X174 and S13 mutants were from our own collection.

**Media.** Labeled protein extracts were made in HFS-T medium which consisted of \( 5 \times 10^{-2} \) M Tris-hydro-
chloride (pH 7.4), 2 \times 10^{-2} \text{ M NaCl}, 1 \times 10^{-2} \text{ M NaCl},
1 \times 10^{-3} \text{ M MgSO}_4, 1 \times 10^{-4} \text{ M CaCl}_2, 5 \times 10^{-4} \text{ M K-
HPO}_4, (\text{pH} 7.4), and 1 \times 10^{-4} \text{ 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-\text{H}]leucine (120
Ci/ml, 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 \mu l) 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,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 \phi X174. The previous studies on suppression of
polarity in \phi X174 and S13 involved several polar mutants; one of these mutants, \phi XamF1005, was
used here. We compared the polar effects of amF1005 in a rho* (psu*) strain and rho (psu2
and psu3) strains; the behavior of \phi X* 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 \phi X* infections of psu* and psu2, 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/m²). 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 psu2, the G and H bands were restored. The reap-
pearance 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

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**Fig. 1.** Fluorogram showing UV suppression of polarity in psu* compared with suppression by the rho
mutants psu2 (lanes 1 through 8) and psu3 (lanes 9 through 18). Cells were infected with a polar mutant of \phi X,
\phi XamF1005. 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/

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that the \textit{rho} mutation restored 20 to 25% of the activities of genes \textit{G} and \textit{H}, with the intensity of the \textit{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 \textit{psu*} (Fig. 1, lane 7). Densitometry indicated that the restoration resulted in 35 to 45% of the amounts of proteins \textit{G} and \textit{H} found in \textit{\phi X*}. The effect of the combination of UV irradiation and the \textit{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 \textit{rho} mutation added to the effect of UV irradiation (data not shown).

Results with \textit{psu} \textit{\delta} 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 \textit{H}. In general, a large UV dose was somewhat more effective than a viable \textit{rho} mutation in suppressing polarity.

MC suppression of polarity. MC cross-links DNA (12), and because of its general radiometric 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 \textit{\phi X} polar mutant \textit{amF1005} was again used, and the following four doses of MC were tested: 0, 1, 10, and 100 \text{\mu g/ml} for 30 min. A comparison of lanes 2 and 3 shows that the polarity of \textit{amF1005} was again demonstrated by the elimination of bands \textit{G} and \textit{H} in addition to band \textit{F}. There was no restoration of bands \textit{G} and \textit{H} with MC doses of 1 and 10 \text{\mu g/ml}. However, at 100 \text{\mu g/ml} of MC per ml (lanes 10 through 12) polarity was suppressed, as shown by the reappearance of bands \textit{G} and \textit{H}. The MC dose of 100 \text{\mu g/ml} for 30 min was massive compared with the dose of 1 \text{\mu g/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 \text{\mu g/ml} for 30 min) was comparable to the effect of UV irradiation (550 J/m²). In \textit{amF1005} MC restored 26% of the \textit{H} protein (lanes 16 through 18), whereas UV irradiation restored 38% (lanes 19 through 21).

**UV sensitivity of \textit{rho} strains.** An intriguing aspect of the \textit{Rho}− phenotype is the sensitivity of \textit{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 \textit{rho} and also suppressed polar mutations in the \textit{gal} operon, all were qualitatively UV sensitive. We determined the inactivation curves for AD1600 (\textit{rho ts15}) and its parent strain SA1030 (\textit{rho*}) (Fig. 3). It was striking that although the \textit{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 \text{\mu g/ml} 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 \textit{\phi X*} or the polar mutant \textit{amF1005}, and labeled as described in the legend to Fig. 1. (B) Comparison of MC treatment (100 \text{\mu g/ml} for 30 min) with UV irradiation (550 J/m²). UN, Untreated cells.
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 psu3 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 psu3 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 psu3 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 psu3 and psu+, respectively.

A physical interpretation of the extrapolated intercepts is beyond the scope of this paper. Several considerations, such as whether the activation 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 psu3 and psu+ behaved exactly like ts15 and ts15+, respectively (data not shown).

Comparison of W- reactivation of phage G4 in psu3 and psu+ also did not show any clear difference. For G4 initially inactivated to a survival of $10^{-3}$, these strains produced reactivation by factors of 4.5 for psu+ and 3.5 for psu3, 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...
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 psu2 and psu3 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...
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 psu2. 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/m² (21; this paper) is about 10 times greater than the value of about 20 J/m² 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 the 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/m² 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/m² 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.

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