Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 6805–6809, October 1987
Genetics

Recovery from ultraviolet light-induced inhibition of DNA synthesis requires *umuDC* gene products in *recA718* mutant strains but not in *recA*$^+$ strains of *Escherichia coli*

(SOS response/*recA430*/ultraviolet mutagenesis/*lexA*)

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Contributed by Evelyn M. Witkin, June 1, 1987

ABSTRACT **Ultraviolet light (UV) inhibits DNA replication in *Escherichia coli* and induces the SOS response, a set of survival-enhancing phenotypes due to derepression of DNA damage-inducible genes, including *recA* and *umuDC*. Recovery of DNA synthesis after UV irradiation ("induced replisome reactivation," or IRR) is an SOS function requiring RecA protein and postirradiation synthesis of additional protein(s), but this recovery does not require UmuDC protein [Khidhir, M. A., Casaregola, S. & Holland, I. B. (1985) *Mol. Gen. Genet.* 199, 133–140]. IRR occurs in strains carrying either *recA718* (which does not reduce recombination, SOS inducibility, or UV mutagenesis) or *umuC36* (which eliminates UV mutability), but not in *recA718 umuC36* double mutants. In *recA430* mutant strains, IRR does not occur whether or not functional UmuDC protein is present. IRR occurs in *lexA*-(Ind$^-$) (SOS noninducible) strains if they carry an operator-constitutive *recA* allele and are allowed to synthesize proteins after irradiation. We conclude the following: (*i*) that UmuDC protein corrects or complements a defect in the ability of RecA718 protein (but not of RecA430 protein) to promote IRR and (*ii*) that in *lexA*(Ind$^-$) mutant strains, IRR requires amplification of RecA$^+$ protein (but not of any other LexA-repressed protein) plus post-UV synthesis of at least one other protein not controlled by LexA protein. We discuss the results in relation to the essential, but unidentified, roles of RecA and UmuDC proteins in UV mutagenesis.**

In wild-type *Escherichia coli*, DNA damage activates RecA protein for its roles in induction of the SOS response and in expression of some individual SOS functions (1, 2). RecA in its activated state (RecA*) is bound to single-stranded DNA in a ternary complex that includes a nucleotide cofactor, possibly dATP (3). RecA* induces the SOS response by facilitating proteolytic cleavage of LexA protein, the repressor of numerous DNA damage-inducible genes (2, 4). This regulatory activity of RecA* is rendered unnecessary by a *lexA*-defective [*lexA*(Def)] mutation that inactivates LexA repressor and causes constitutive synthesis of gene products normally controlled by LexA (2).

Khidhir *et al.* (5) have investigated the requirements in *E. coli* strain K-12 for recovery from the inhibition of DNA replication that occurs after UV irradiation. They concluded that the ability to resume DNA synthesis in cells containing UV-blocked replication forks is an inducible SOS phenotype requiring both the regulatory function of RecA protein and its direct participation in the recovery process, a process they called "induced replisome reactivation," or IRR. Khidhir *et al.* showed, too, that IRR requires the postirradiation synthesis of another protein (IRR factor) but does not require the products of the *uvrA*, *recBC*, or *umuC* genes.

In contrast to the apparent indifference of RecA protein to the presence or absence of UmuDC protein in recovery of DNA synthesis, UV mutagenesis in *E. coli* requires both RecA* and UmuDC proteins. Mutations inactivating UmuC or UmuD protein eliminate all bacterial SOS mutagenesis and cause a moderate degree of UV sensitivity (6, 7). RecA* has an essential nonregulatory role in SOS mutagenesis, in addition to its antirepressor activity (8, 9). Although the specific roles of these gene products in UV mutagenesis are not known, UmuDC and RecA* are thought to mediate error-prone translesion replication across noncoding UV photoproducts (1, 10–12).

Because UV-induced mutations are largely targeted opposite UV photoproducts (13), RecA* and UmuDC proteins probably act in physical proximity to the target lesion and to each other. If so, some mutations affecting the structure of one of these proteins might be expected to influence the activity of the other, whether they interact directly with each other or indirectly—either as members of a multiprotein complex or by their mutual affinity for a common DNA target site. We have sought evidence for functional or physical interaction between RecA and UmuDC proteins by combining mutant *recA* alleles with mutant alleles of *umuC* and *umuD* and analyzing any synergistic or epistatic effects observed in the double mutants. Our most telling results were obtained with *recA718*, an allele that causes a complex phenotype described fully elsewhere (14, 15). Briefly, *recA718* strains are recombination-proficient, moderately UV-sensitive, and UV-hypermutable at low radiation doses. RecA718 protein requires DNA damage to become activated for SOS induction and expression when present at low baseline levels, but amplified levels of RecA718 protein are constitutively activated without DNA damage. Thus, *recA718 lexA*(Def) strains (but not *recA718 lexA*$^+$ strains) express SOS mutator activity and are constitutive for other SOS phenotypes requiring RecA* for their expression (15).

In this report, we describe and interpret the synergistic effects on UV sensitivity and on recovery from UV-induced inhibition of DNA replication that occurred when the mutant allele *recA718* was combined with *umuC36*, *umuC122*::Tn*5*, or *umuD44*. We also compare *recA718* with another mutant allele, *recA430*, which has been reported to cause delayed recovery of replication after UV irradiation (5). We suggest that active UmuDC protein can complement or correct a defect in the ability of RecA718 to contribute to IRR, whereas RecA430 protein is unconditionally defective in this activity.

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Abbreviations: IRR, "induced replisome reactivation"; UV, ultraviolet light; RecA*, RecA protein in activated state; *lexA*(Def), *lexA*-defective mutation that inactivates LexA repressor.

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MATERIALS AND METHODS

Bacterial Strains. Table 1 lists and describes the strains used in this study.

Culture Media and Growth Conditions. Liquid medium was MCHT, minimal medium E (18) supplemented with 0.4% Casamino acids and 20 μg of tryptophan per ml. Cultures were grown overnight in MCHT on a shaker at 37°C, diluted 200-fold in fresh MCHT, and incubated to an OD (450 nm) of 0.02–0.04. Plating medium was MCHT solidified with 1.5% Bacto Agar (Difco). Glucose (0.4%) was the carbon source. Chloramphenicol (25 μg/ml) (Sigma) was added, where indicated, immediately after UV irradiation. Kanamycin or ampicillin or both (50 μg/ml each) (Sigma) were added to the overnight culture media of strains resistant to one or both antibiotics. All incubations were at 37°C.

UV Irradiation. UV was administered under conditions previously described (19), except that the bacteria were irradiated in 15-ml aliquots of growth medium, instead of in saline solution, to minimize interruption of exponential growth. The only important difference between cultures irradiated in growth medium and control cultures irradiated either in saline solution or in unsupplemented minimal medium was an effective dose reduction of 25–30% caused by the presence of amino acids. Neither UV survival curves nor the rates of post-UV DNA synthesis were otherwise significantly altered. UV doses shown are not corrected for the dose-reduction effect.

Measurement of DNA Synthesis. The rate of DNA synthesis was measured essentially as described by Khidhir *et al.* (5). Cultures growing exponentially at 37°C were pulse labeled for 2 min at various times before and after UV irradiation with [^{3}H]thymidine (20 μCi/ml; specific activity 20 Ci/mM; 1 Ci = 37 GBq). Growth was monitored by determining OD of the cultures at 450 nm at the time of each pulse labeling.

RESULTS

Synergistic UV Sensitivity in Double Mutants *recA718 umuC36*. Fig. 1 shows the synergistic effect on UV sensitivity caused by combining *recA718* and *umuC36* in the double mutant strain SC18-UM36, compared with strains carrying only one of these mutant alleles and with a strain carrying both wild-type alleles at these loci. Strains carrying *recA718* in combination with either *umuC122*::Tn*5* or *umuD44* exhibit the same degree of UV sensitivity as the double mutant carrying *umuC36*. The double mutant does not degrade its DNA after UV irradiation any more extensively than either of the single mutants, neither of which exhibits "reckless" DNA degradation (data not shown).

Recovery from Postirradiation Inhibition of DNA Replication. RecA protein (but not UmuC protein) is necessary for the recovery from UV-induced inhibition of DNA replication in *E. coli* K-12 (5). We considered that *recA718* strains, unlike *recA*$^+$ strains, might depend upon the presence of functional UmuDC protein for IRR. Fig. 2 compares the kinetics of inhibition and recovery of DNA synthesis after exposure to a UV dose of 3 J/m^2 in the set of strains compared in Fig. 1. The rate of DNA synthesis falls after UV irradiation in all four strains, reaching a significantly lower level in both the *recA718* single mutant and the *recA718 umuC36* double mutant than in either the *recA*$^+$ *umuC*$^+$ strain or the *recA*$^+$ *umuC36* strain. The presence of *recA718*, regardless of the *umuDC* genotype, results initially in a more severely de-

Table 1. Bacterial strains

Strain	Relevant genotype*			Description and/or reference†
	recA	*lexA*	*umu*	
SC18	*718*	+	*DC*$^+$	Ref. 14
SC18-UM36	*718*	+	*C36*	This study; Kan^r transductant of SC18
SC18-UM122	*718*	+	*C122*::Tn*5*	This study; Kan^r transductant of SC18
SC18-UM44	*718*	+	*D44*	This study; Tet^r recombinant in conjugation of SC18 × (F′ *umuD44 fadR*::Tn*10* donor)
SC18-RP1	+	+	*DC*$^+$	Ref. 15
SC18-RP-UM36	+	+	*C36*	This study; Kan^r transductant of SC18-RP1
SC18-SP	*718*	*71*::Tn*10*(Def)	*DC*$^+$	Ref. 15
SC18-UM36-SP	*718*	*51*(Def)	*C36*	This study; Mal$^+$ transductant of SC18-UM36
SC30-OC	*o281*	+	*DC*$^+$	This study; Srl$^+$ transductant of SC30 *srlC300*::Tn*10*; ref. 14
SC30-LM-OC	*o281*	*102*(Ind$^-$)	*DC*$^+$	This study; Tet^r transductant of SC30-LM; ref. 16
SC30-RP1	+	+	*DC*$^+$	Ref. 16
SC30-RP-LM	+	*102*(Ind$^-$)	*DC*$^+$	This study; Mal$^+$ transductant of SC30-RP1
SC430	*430*	+	*DC*$^+$	This study; Srl$^+$ transductant of SC30 *srlC300*::Tn*10*; ref. 14
SC430-SP	*430*	*71*::Tn*5*(Def)	*DC*$^+$	This study; Kan^r transductant of SC430
SC430-UM36	*430*	+	*C36*	This study; Kan^r transductant of SC430
SC18(pSE117)	*718*	+	*DC*$^+$	This study; Amp^r transformant of SC18 with pSE117 [pBR322 derivative carrying cloned *umuDC*$^+$]; ref. 17

All hyphenated strains were constructed in one or two steps of P1 transduction.

*All strains listed are also *uvrA155 trpE65 sulA1*; the SC strains are derived from a single recombinant in a cross of an F′ K-12 donor × an F$^-$ B/r recipient (14). They have the restriction/modification pattern of their B/r-derived parent, strain WP2$_s$, and retain most of its known markers.

†Donor strains used as sources of the *umu*$^-$ alleles were: MV50 (*umuC36* linked to Tn*5*), from M. Volkert (University of Massachusetts Medical School, Worcester, MA); DE367 (*umuD44* linked to Tn*10*) and DE372 (*umuC122*::Tn*5*), from D. Ennis (University of Arizona College of Medicine, Tucson, AZ). MV50 is a B/r derivative; DE367 and DE372 are K-12 strains derived from DE192 (9). The donor strain used as a source of *recAo281* (formerly *rnmB281*) was MV2, from M. Volkert.

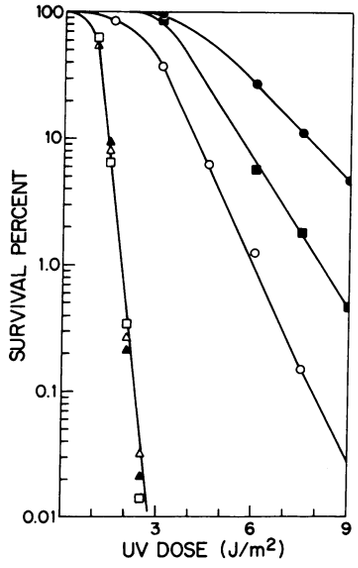

FIG. 1. Ultraviolet survival curves. SC18-RP1 [*recA*$^+$ *umuDC*$^+$] (●); SC18-RP-UM36 [*recA*$^+$ *umuC36*] (■); SC18 [*recA718 umuDC*$^+$] (○); SC18-UM36 [*recA718 umuC36*] (△); SC18-UM122 [*recA718 umuC122*::Tn*5*] (▲); and SC18-UM44 [*recA718 umuD44*] (□).

pressed rate of DNA synthesis after UV irradiation than is observed in *recA*$^+$ strains. This effect of *recA718* is seen after irradiation with 2 and 2.5 J/m^2 as well (data not shown) and is highly reproducible. In the *recA*$^+$ *umuC36* strain, the loss of UmuC protein activity makes little or no difference in the degree of the initial inhibition of DNA synthesis, but this loss does slightly retard recovery after exposure to 3 J/m^2. In the *recA718 umuC*$^+$ strain, the rate of DNA synthesis begins to increase slightly later than in the two *recA*$^+$ strains, increases very rapidly for a short time, then continues to increase more slowly. Both single mutants achieve the control rate slightly later than the *recA*$^+$ *umuDC*$^+$ population. However, in the double mutant *recA718 umuC36*, no detectable recovery occurs after the initial inhibition. Similar results were obtained in *recA718 umuC122*::Tn*5* and *recA718 umuD44* double mutants after exposure to 3 J/m^2 (data not shown). Thus, the loss of UmuC or UmuD protein activity prevents recovery of DNA synthesis after UV irradiation in the presence of *recA718*, but not in the presence of *recA*$^+$.

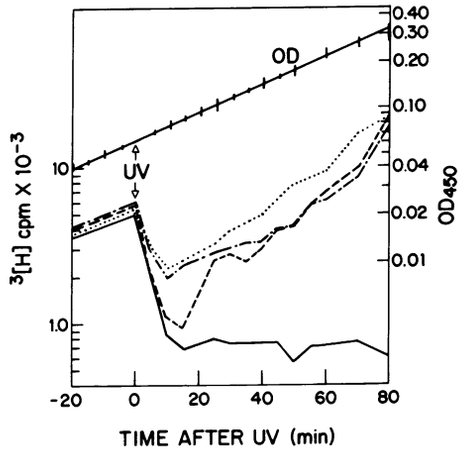

FIG. 2. Recovery from inhibition of DNA synthesis after UV irradiation: synergism in double mutants combining *recA718* and *umuC36*. SC18-RP1 [*recA*$^+$ *umuDC*$^+$] (····); SC18-RP-UM36 [*recA*$^+$ *umuC36*] (—·—); SC18 [*recA718 umuDC*$^+$] (– – –); and SC18-UM36 [*rec718 umuC36*] (——). UV dose was 3 J/m^2. Vertical lines on the OD curve define the range of values obtained for all strains.

Effect of a *lexA*(Def) Allele and of pSE117 on Recovery from Postirradiation Inhibition of DNA Replication. We introduced a *lexA*(Def) allele into both Umu$^+$ and Umu$^-$ strains carrying *recA718* and examined the effects of elevated pre-UV levels of LexA-repressed gene products (including RecA718) on post-UV DNA replication. Fig. 3*a* shows that the *lexA*(Def) derivative of the double mutant *recA718 umuC36* exhibits the same severe inhibition of DNA synthesis after UV irradiation and the same failure to recover as the *lexA*$^+$ double mutant strain SC18-UM36. In the Umu$^+$ *lexA*(Def) strain SC18-SP (Fig. 3*b*), inhibition of DNA synthesis shortly after UV irradiation is considerably less extreme than in the *lexA*$^+$ strain SC18, although the two strains recover the normal rate at about the same time. Constitutive expression of the LexA regulon in *recA718* strains, including synthesis of high levels of RecA718 protein, alleviates post-UV inhibition of DNA replication only if functional UmuDC protein is among the constitutively derepressed gene products. Fig. 3*b* shows also that transformation of strain SC18 with pSE117, a multicopy plasmid carrying the *umuDC*$^+$ operon, has the same effect as the *lexA*(Def) allele. Selective pre-UV amplification of UmuDC protein, with no concomitant pre-UV amplification of RecA718 or any other LexA-controlled protein, is sufficient to lessen the initial radiation-induced inhibition of DNA synthesis otherwise seen in *recA718* strains. We tested the ability of pSE117 to cleave LexA protein, which could result in amplification of RecA718 as well as of UmuDC proteins. No significant increase in β-galactosidase synthesis was detected in *recA718*-containing strain K-250 (15) transformed with pSE117 (data not shown). [K-250 carries *sfiA*::Mu *cts* d(Ap *lac*) (Mu *c*$^+$).] It is reasonable to conclude that in strain SC18 (pSE117) UmuDC protein is selectively amplified.

IRR in *recA430* Strains. We observed (data not shown) that the Umu$^+$ *recA430* strain SC430 is as UV-sensitive as the double mutant *recA718 umuC36* (Fig. 1) and that this extreme UV sensitivity is neither increased by *umuC36* in SC430-UM36 nor decreased by *lexA*(Def) in SC430-SP. Fig. 4 shows that SC430 is just as unable to recover from UV-induced inhibition of DNA synthesis as its *umuC36* transductant and that the IRR deficiency is not alleviated by the *lexA*(Def) allele in SC430-SP, which constitutively amplifies both RecA430 and UmuDC proteins.

Does Recovery Require a LexA-Controlled Gene Product Other Than RecA Protein? Fig. 5 shows that, whereas no recovery occurs in the *lexA*(Ind$^-$) *recA*$^+$ strain SC30-RP-

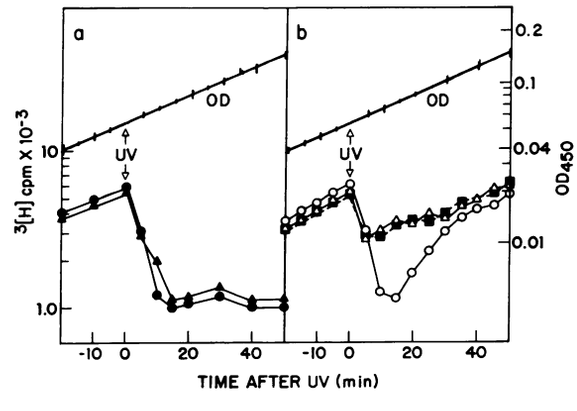

FIG. 3. Effect of a *lexA*(Def) allele and of pSE117 on post-UV inhibition of DNA synthesis in *recA718* strains. (*a*) SC18-UM36 [*recA718 umuC36 lexA*$^+$] (●); SC18-UM36-SP [like SC18-UM36 except *lexA*(Def)] (▲); (*b*) SC18 [*recA718 umuDC*$^+$ *lexA*$^+$] (○); SC18-SP [like SC18 except *lexA*(Def)] (△); SC18(pSE117) (■). pSE117 is a multicopy plasmid carrying *umuDC*$^+$. UV dose was 3 J/m^2. Vertical lines on the OD curve define the range of values obtained for all strains.

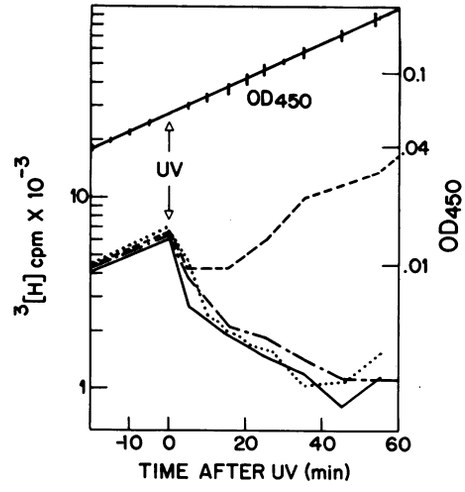

FIG. 4. Recovery from inhibition of DNA synthesis after UV irradiation in *recA430* strains. SC30-RP1 [*recA*$^+$ *umuDC*$^+$ *lexA*$^+$] (---); SC430 [*recA430 umuDC*$^+$ *lexA*$^+$] (——); SC430-UM36 [*recA430 umuC36 lexA*$^+$] (···); and SC430-SP [*recA430 umuDC*$^+$ *lexA*(Def)] (——). Vertical lines on the OD curve define the range of values obtained for all strains.

LM, recovery does occur in a *lexA*(Ind$^-$) strain carrying the operator-constitutive *recAo281* allele if protein synthesis is permitted to occur after UV irradiation, but not if post-UV protein synthesis is inhibited by chloramphenicol. These results show that, at least in these strains, amplification of RecA$^+$ is a necessary but insufficient condition for recovery, and that the postirradiation synthesis of at least one additional protein is also required. Because the repressor encoded by *lexA102*(Ind$^-$) is not cleavable in the presence of RecA*, the results in Fig. 5 establish that RecA is the only protein regulated under the LexA regulon that must be amplified as a requirement for the post-UV recovery of DNA replication in strain SC30-LM-OC and that LexA does not control the additional protein(s) required for this recovery.

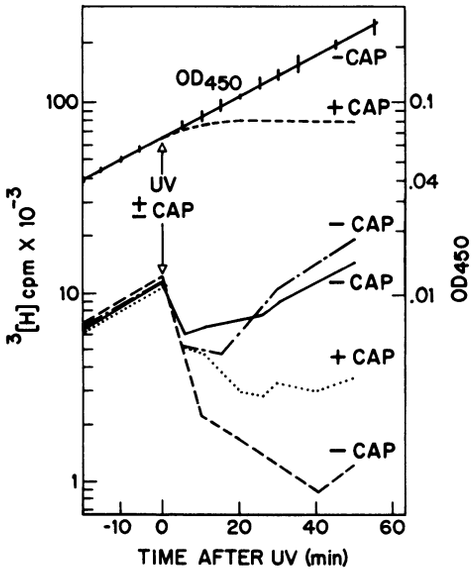

FIG. 5. Recovery from inhibition of DNA synthesis after UV irradiation in *lexA*(Ind$^-$) strains. SC30-OC [*recAo281 lexA*$^+$] (—·—); SC30-LM-OC [*recAo281 lexA*(Ind$^-$)] −CAP (——), +CAP (···); SC30-RP-LM [*recA*$^+$ *lexA*(Ind$^-$)] (——). UV dose was 3 J/m^2. All strains shown are *umuDC*$^+$. Vertical lines on the OD curve defines the range of values obtained for all strains; CAP, chloramphenicol.

DISCUSSION

IRR does not occur in *umuC* or *umuD* mutants carrying *recA718* (Fig. 2), although loss of UmuDC function does not prevent recovery of DNA synthesis after UV irradiation in *recA*$^+$ strains. In *recA430* mutants, no such recovery occurs whether or not UmuDC products are functional (Fig. 4). It is likely that the extreme UV sensitivity of *recA718* Umu$^-$ double mutants (Fig. 1), as well as that of *recA430* strains, is due primarily to their inability to recover from UV-induced inhibition of DNA synthesis.

The Regulatory Role of RecA in IRR. Amplification of RecA$^+$ is necessary for IRR in a *lexA*(Ind$^-$) strain (Fig. 5), and if the same requirement applies to *lexA*$^+$ strains, cleavage of LexA protein by RecA* protein is a prerequisite for recovery. We confirm the conclusion of Khidhir *et al.* (5) that amplified levels of RecA are not sufficient for IRR, which requires, additionally, the synthesis of at least one other protein (IRR factor) after UV irradiation. We conclude, further, that LexA protein does not control the additional protein(s) required for recovery in our *lexA*(Ind$^-$) strain (Fig. 5) and that RecA is the only protein controlled by LexA protein that must be amplified for IRR. Thus, at least two regulatory activities of RecA protein could be essential for IRR: cleavage of LexA protein to induce RecA protein synthesis and cleavage of another repressor if IRR factor is DNA damage-inducible, rather than constitutive but short-lived.

The Direct Role of RecA Protein in IRR. Khidhir *et al.* (5) have summarized the evidence that replisome reactivation requires two distinct steps: release of the blocked replisome from the damaged site and reinitiation of DNA synthesis downstream. RecA protein could participate in one or both of these steps. The observations of Lu *et al.* (12) suggest that RecA binds the single-stranded regions that flank helix-distorting UV lesions in double-stranded DNA, and the requirement for both RecA and UmuDC proteins for targeted UV mutagenesis makes it seem likely that both of these proteins operate near the target photoproducts. The two-step model of UV mutagenesis (11) also implies proximity of both RecA and UmuDC proteins to premutational lesions. Because of the synergistic interaction of *recA718* and *umu*$^-$ alleles in inhibiting IRR, we propose that RecA and UmuDC proteins are present, in close proximity, at all UV-blocked replication sites, not only at those lesions (presumably a small subset) that are ultimately tolerated by mutagenic translesion replication rather than by an error-free process. Thus, both RecA and UmuDC proteins may be positioned to influence the release of a blocked replisome at every site of blocked replication.

Implications for UV Mutagenesis. If both RecA and UmuDC proteins can influence events at every UV lesion that causes a transient replication block, error-prone translesion replication may be an available option at every such site, albeit one that is rarely utilized. Translesion replication may occur only at "trouble spots" that are refractory to error-free repair or lesion tolerance mechanisms, such as sites having two or more lesions close together on opposite strands (10). Mutations or treatments that reduce the efficiency of error-free mechanisms could enhance UV mutability by allowing translesion replication to operate at some uncomplicated sites that would normally be repaired more rapidly by excision or tolerated by recombination. Support for this competition hypothesis is provided by the UV hypermutability of *mfd* mutants (20) and of a *dnaB* mutant (21), both of which excise pyrimidine dimers much more slowly than normal without any increase in UV sensitivity.

Does UmuDC Correct or Complement RecA718? There are two ways to interpret the synergistic loss of IRR activity in *recA718 umuC36* double mutants. UmuDC$^+$ protein could

correct a conditional defect in the ability of RecA718 to affect IRR by exerting a direct or indirect allosteric effect upon the structure of RecA718. Direct interaction between RecA and UmuDC proteins is indicated by recent evidence for RecA*-dependent proteolytic processing of UmuD protein *in vivo* (H. Shinagawa, personal communication) or *in vitro* (H. Echols, personal communication). UmuDC protein could influence the structure and thereby the activity of RecA718 protein indirectly, if both proteins were members of a multiprotein complex such as the SOS-modified replisome. Several observations hint that RecA protein interacts with replisome components in SOS-induced cells: for example, a specific temperature-sensitive $dnaB_{ts}$ allele suppresses expression of *recA441* (22), and in $recA_{ts}$ bacteria undergoing SOS-induced "stable DNA replication" (replication not requiring new protein synthesis for reinitiation) (23) DNA synthesis is also temperature-sensitive (24).

Alternatively, RecA718 protein may be unconditionally defective in the direct role of RecA protein in IRR. If so, $RecA^+$ and UmuDC proteins must each be capable of facilitating IRR independently of each other. In a *recA718* $UmuDC^+$ strain, recovery would then occur with almost normal efficiency because only the regulatory activities of RecA718 protein (which are normal as far as is known), but not its direct role, would be required for operation of the UmuDC-dependent recovery pathway. The data in Figs. 2 and 3 are consistent with this possibility. DNA synthesis shortly after UV irradiation, before any substantial synthesis of inducible proteins, is much more severely inhibited in *recA718* than in $recA^+$ strains, whether Umu^+ or Umu^-, $lexA^+$ or *lexA*(Def), possibly reflecting the relative ability of baseline levels of RecA718 and $RecA^+$ proteins to limit the inhibition. Furthermore, whereas constitutive synthesis of $RecA^+$ enhances IRR (5), constitutive synthesis of RecA718, in the absence of active UmuDC protein, does not (Fig. 3*a*).

Ability of UmuDC protein to relieve post-UV inhibition of DNA synthesis, independently of the direct participation of RecA protein, could account for the similar effects of a *lexA*(Def) allele and pSE117 in a *recA718* strain (Fig. 3*b*). Selective amplification of UmuDC protein is as effective as constitutive derepression of the LexA regulon in reducing the initial inhibition, a result suggesting nonstoichiometric complementation rather than stoichiometric correction of RecA718 protein by UmuDC protein.

IRR in *recA430* Strains. The inability of *recA430* strains, whether Umu^+ or Umu^-, $lexA^+$ or *lexA*(Def), to recover from UV-induced inhibition of DNA synthesis (Fig. 4) is probably indicative of an unconditional defect in the ability of RecA430 to perform an essential RecA function in IRR. *recA430* strains are unable to effect cleavage of λ repressor (25) and are entirely nonmutable by UV, even in *lexA*(Def) strains (26). An unconditional defect in IRR could result from inability to promote cleavage of the unknown repressor of IRR factor (if it is, in fact, inducible) or from inability to bind the single-stranded DNA flanking UV photoproducts, a defect that could also account for the inactivity of RecA430 in UV mutagenesis. Inability of RecA430 to process UmuD (if such processing is necessary for UmuDC activity) could explain why UmuDC does not complement the IRR defect of RecA430 and could also explain the UV nonmutability of RecA430 protein. RecA430 is recombination-proficient (27) and probably, therefore, this protein does not owe its IRR defect to loss of any activity required for standard modes of genetic recombination.

The Role of RNase in IRR. Khidhir *et al.* (5) have proposed that IRR factor is an inhibitor of RNase H and that such an inhibitor may be required for reinitiation of DNA replication downstream from a blocked replisome, especially on the leading strand, which normally initiates replication only at *oriC*. This possibility was suggested by the properties of mutants mapping in the *rnh* gene, which encodes RNase H (28). These mutants, selected for constitutive expression of "stable DNA replication," reinitiate DNA synthesis without new protein synthesis and do not require *oriC* or *dnaA* activity (29). High levels of RNase H cause UV sensitivity and inhibit DNA synthesis (30), possibly by preventing the stable formation of RNA primers at secondary origins (31) and thereby preventing IRR. In SOS-induced cells, transcription of the *rnh* gene is reduced (32), as would be predicted if IRR factor is an inhibitor of RNase transcription. However, because the regulatory regions of *rnh* and *dnaQ* overlap and because SOS induction increases *dnaQ* transcription, the reduced transcription of *rnh* seen in some SOS-induced cells may be a trivial byproduct of the induction of *dnaQ* (32). Although elimination of RNase H clearly permits reinitiation of DNA synthesis at secondary origins, it is not necessarily the way UV-irradiated wild-type *E. coli* achieves the same capability. In SOS-induced cells, RNase H activity does not appear to be reduced (33). The possibility that various *E. coli* mutants may recover from replication blocks in quite different ways suggests caution in extrapolating the requirements for IRR observed in wild-type strains to mutant strains and vice versa.

This work was supported by Grant AI10778 (to E.M.W.) from the National Institutes of Health and by a Merck Predoctoral Fellowship (to J.B.S.).

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