Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 619–623, February 1986
Biochemistry

Capacity of RecA protein to bind preferentially to UV lesions and inhibit the editing subunit (ε) of DNA polymerase III: A possible mechanism for SOS-induced targeted mutagenesis

(fidelity of DNA replication/LexA cleavage)

CHI LU, RICHARD H. SCHEUERMANN, AND HARRISON ECHOLS

Department of Molecular Biology, University of California, Berkeley, CA 94720

Communicated by Allan Campbell, September 23, 1985

ABSTRACT **The RecA protein of *Escherichia coli* is required for SOS-induced mutagenesis in addition to its recombinational and regulatory roles. Most SOS-induced mutations probably occur during replication across a DNA lesion (targeted mutagenesis). We have suggested previously that RecA might participate in targeted mutagenesis by binding preferentially to the site of the DNA damage (e.g., pyrimidine dimer) because of its partially unwound character; DNA polymerase III (polIII) will then encounter RecA-coated DNA at the lesion and might replicate across the damaged site with reduced fidelity. In this report, we analyze at a biochemical level two major predictions of this model. With respect to lesion recognition, we show that purified RecA protein binds more efficiently to UV-irradiated double-stranded DNA than to nonirradiated DNA, as judged by filter-binding and gel electrophoresis assays. With respect to replication fidelity, Fersht and Knill-Jones [Fersht, A. R. & Knill-Jones, J. W. (1983) *J. Mol. Biol.* 165, 669–682] have found that RecA inhibits the 3′ → 5′ exonuclease (editing function) of polIII holoenzyme. We extend this observation by demonstrating that RecA inhibits the exonuclease of the purified editing subunit of polIII, ε protein. Thus, we suggest that the activities of RecA required for targeted mutagenesis are lesion-recognition, followed by localized inhibition of the editing capacity of the ε subunit of polIII holoenzme. In this proposed mechanism, one activation signal for RecA for mutagenesis is the lesion itself. Because UV-irradiated, double-stranded DNA efficiently activates RecA for cleavage of the LexA repressor, the lesion itself may also often serve as an activation signal for induction of SOS-controlled genes.**

The introduction of a replication-inhibiting lesion into the DNA of *Escherichia coli* results in a marked increase in mutation rate (1–3). This mutagenesis is one consequence of the induced, multigene response to DNA damage termed the SOS pathway (2–5). There are two components to SOS-induced mutagenesis: (*i*) an intrinsic reduction in replication fidelity, termed untargeted mutagenesis because known lesions are not required in the mutated DNA; (*ii*) a much larger increase in mutation rate dependent on the presence of DNA lesions, termed targeted mutagenesis (1, 5, 6). Targeted mutagenesis probably derives from replication across the site of the DNA lesion (e.g., the pyrimidine dimer for UV damage) (7–9). At least two proteins are required for targeted mutagenesis, RecA and UmuC/D (3, 5, 10–12). RecA has a regulatory function in the SOS response through its capacity to catalyze cleavage of the multioperon LexA repressor (4). In addition, RecA is probably involved directly in SOS-induced mutagenesis (13–15).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

From the preceding summary, targeted mutagenesis requires two SOS-induced events: localization of the site (e.g., pyrimidine dimer for UV) and a mechanism for replication errors at that site. One suggested error mechanism is an inhibition of the editing capacity (3′ → 5′ exonuclease) of the replicating DNA polymerase, allowing replication past the dimer with random base insertion (16). A modification of this proposal, specialized to RecA, is that RecA recognizes the DNA lesion and locally modifies the DNA polymerase III (polIII) holoenzyme at the dimer site to a reduced fidelity form; the enzyme then replicates through the dimer site, using the residual base-pairing capacity of the dimerized bases in spite of their distorted configuration (a localized relaxed-specificity mechanism) (17). Some recent studies have made this proposed mechanism more attractive. Fersht and Knill-Jones (18) have shown that RecA inhibits the editing exonuclease of polIII holoenzyme. The editing function of polIII holoenzyme has been found to reside on a subunit, ε, that is distinct from the polymerization subunit, α (19). Thus, fidelity can be controlled through editing largely independently of capacity to extend DNA chains. The relaxed-specificity model is shown in updated form in Fig. 1. This model makes two specific predictions: (*i*) RecA should bind more effectively to UV-irradiated, double-stranded DNA than to nonirradiated DNA; (*ii*) RecA should inhibit the 3′ → 5′ exonuclease activity of the isolated ε protein. In this report, we present evidence in support of each of these expectations.

MATERIALS AND METHODS

Proteins. RecA protein, purified as described by Cox *et al.* (24), was a gift of I. R. Lehman. polIII holoenzyme, prepared as reported by McHenry and Kornberg (25), was a gift of H. Maki and A. Kornberg. The ε subunit of polIII holoenzyme was purified as reported (19). The *E. coli* single-stranded-DNA binding protein (Ssb) was prepared as described (26). LexA protein, purified by the procedure of Little (27), was the gift of J. W. Little.

DNA. The substrate for RecA-binding experiments was the double-stranded, supercoiled form of φX174 (RFI). ^{3}H-labeled RFI was prepared by addition of [^{3}H]thymidine to phage-infected cells (28). Cells were lysed, and supercoiled RFI was prepared by two cycles of CsCl sedimentation in the presence of ethidium bromide (29). The specific activity of the DNA preparation was 100 cpm/ng. The substrate for exonuclease assays was the mispaired-copolymer substrate poly(dA)·[(dT)$_{18}$([^{3}H]dC)$_{2.8}$]$_n$, prepared as described (19, 23).

DNA-Binding Assays. To measure binding of RecA to φX174 supercoiled DNA, two assays were used. The first measured the retention of ^{3}H-labeled DNA on a nitrocellu-

Abbreviations: polIII, DNA polymerase III; ATP[γ-S], adenosine 5′-[γ-thio]triphosphate.

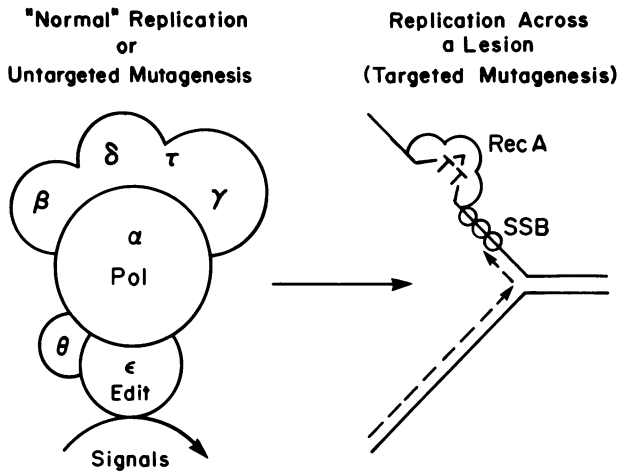

FIG. 1. Possible mechanism for SOS-induced mutagenesis. The α subunit of polIII holoenzyme carries the polymerization activity (refs. 20 and 42; our unpublished work with K. Young); the ε subunit provides the editing exonuclease; the β, δ, γ, and τ subunits are responsible for processivity and primer recognition (21, 22); and θ serves an unknown role in the "polIII core" of α, ε, and θ (21, 22). Based on the properties of mutator mutations, we presume that variations in replication fidelity occur mainly through changes in the activity of ε (19, 23). To accomplish targeted mutagenesis, we suggest that RecA protein binds to the double-stranded DNA at the site of the pyrimidine dimer. When the strands separate for replication, the RecA remains bound, and polIII will copy RecA-coated DNA at this site rather than its normal substrate, DNA coated with single-strand binding protein (Ssb). In the absence of RecA, replication will normally stop at the dimer site because the distorted base configuration renders the base-pairing required for polymerization difficult and makes even a correct base pair look like a mispair to the editing system. If RecA inhibits the editing capacity of ε, polymerization will proceed past the dimer more often but with reduced fidelity.

lose filter, dependent on added RecA (30). The standard assay (10 μl) contained 20 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM EDTA, 30 mM NaCl, 1 mM dithiothreitol, 40 ng of DNA, and RecA as indicated. The mixture was incubated at 37°C for 5 min, and then adenosine 5'-[γ-thio]triphosphate (ATP[γ-S]) was added to 200 μM and incubation was continued for another 10 min. Samples were diluted to 100 μl with cold reaction buffer and applied directly to a nitrocellulose filter under suction. The filter was washed three times with 1 ml of cold reaction buffer and dried, and the retained ^{3}H-labeled DNA was determined by liquid scintillation counting. Filters were soaked for at least 30 min in 1 mM neutralized ATP prior to use. The second assay measured the RecA-mediated alteration in mobility of the ϕX174 DNA during agarose gel electrophoresis (30). The binding reaction was carried out as for the filter assay, after which 2 μl of loading buffer was added [0.25% bromphenol blue/0.25% xylene cyanol/40% (wt/vol) sucrose] and gel electrophoresis was carried out in 0.8% agarose using a Tris/borate buffer system (29, 30). The DNA was visualized by staining with ethidium bromide and photographed under UV light.

LexA Cleavage Assay. The RecA-mediated cleavage reaction for LexA was that reported by Little (27), except that the source of DNA was ϕX174 single-stranded or supercoiled, double-stranded circles. Reaction mixtures (10 μl) contained 2 μg of LexA, 20 ng of DNA, 0.4 μg of RecA, and 1 mM ATP[γ-S]. After incubation of the reaction mixture for 60 min, the products of the reaction were separated by acrylamide gel electrophoresis in $NaDodSO_4$ and the proteins were visualized by staining with Coomassie blue.

Exonuclease Assays. Editing exonuclease activity was measured by the release of [^{3}H]dCMP from a mispaired copolymer substrate $(dA)_{3000}\cdot[(dT)_{18}([^3H]dC)_{2.8}]_{10}$ (19, 23). The assay mixture (35 μl) contained 20 mM Tris·HCl (pH 7.7), 5 mM $MgCl_2$, 6 mM dithiothreitol, 7 mM NaCl, 5% (vol/vol) glycerol, 7% (wt/vol) polyethylene glycol 6000, bovine serum albumin (200 μg/ml), Ssb (17 μg/ml), 1 μg of copolymer substrate, 160 μM ATP[γ-S], 20 ng of ε protein or 50 ng of polIII holoenzyme, and RecA as noted.

RESULTS

Preferential Binding of RecA to UV-Irradiated DNA. To examine the binding of RecA to UV-irradiated, double-stranded DNA, we first used an assay that measured the retention of ^{3}H-labeled ϕX174 supercoils on a nitrocellulose filter. For DNA irradiated with UV light (400 J/m^2), there was preferential retention of the UV-damaged DNA on the filter in a binding reaction with purified RecA protein (Fig. 2). We estimate about six pyrimidine dimers per DNA molecule at this dosage of UV light, correcting data for single-stranded ϕX174 DNA to double-stranded (31, 32). The DNA-binding experiment was also carried out with a constant level of RecA (1 μM) and DNA subjected to a range of UV doses (Fig. 3). This experiment also indicates that the UV lesions favor the binding of RecA to double-stranded DNA. To check the double-stranded property of the irradiated DNA, we carried out agarose gel electrophoresis of the DNA samples. For each UV dose, the mobility of the DNA was unchanged from that of the nonirradiated supercoiled sample (data not shown). The very high level of retention of the ^{3}H-labeled DNA on the nitrocellulose filter was found only with ATP[γ-S] as the nucleotide cofactor; dATP gave a low level, and ATP only background levels (even with an added triphos-

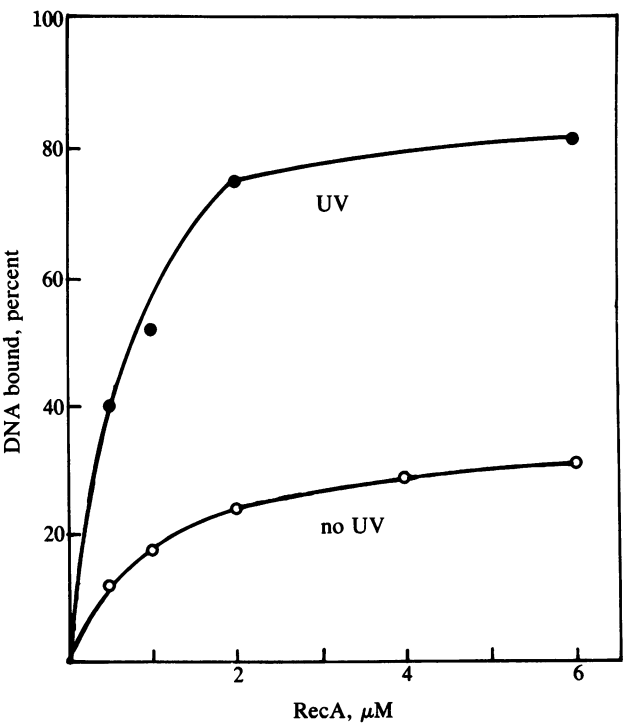

FIG. 2. Preferential binding of RecA to UV-irradiated DNA. Supercoiled ^{3}H-labeled ϕX174 DNA, either nonirradiated (○) or irradiated with UV light (400 J/m^2) (●), was incubated with various concentrations of RecA, with ATP[γ-S] as nucleotide cofactor. Samples were applied to a nitrocellulose filter and washed, and the radioactivity remaining on the filter was determined. A background of ^{3}H-labeled DNA retained in the absence of RecA has been subtracted; the background level was 5%.

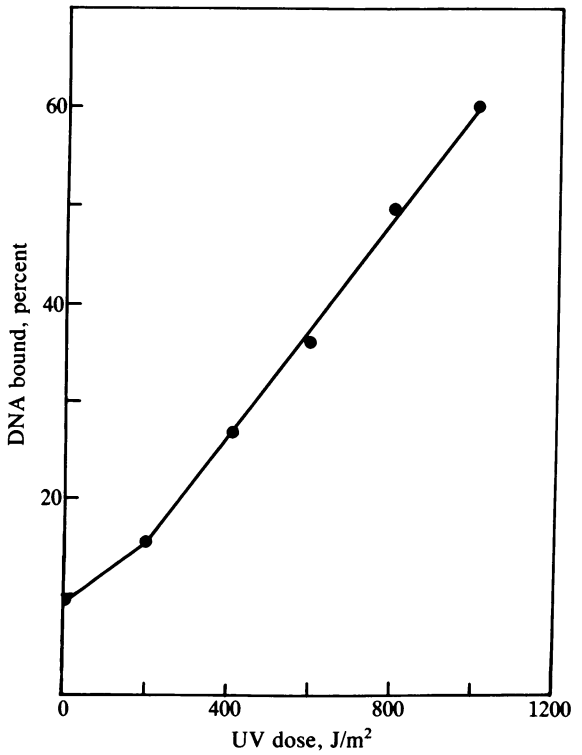

FIG. 3. Preferential binding of RecA to UV-irradiated DNA. RecA (1 μM) was incubated with ^{3}H-labeled DNA irradiated with various doses of UV light. Samples were applied to a nitrocellulose filter and processed as for Fig. 2.

phate-regenerating system). Previous work has shown binding of RecA to double-stranded DNA (see ref. 30 for a careful study). However, at neutral pH, this binding is relatively inefficient (Fig. 2 and ref. 30); the presence of pyrimidine dimers appears to enhance binding markedly.

To provide another measure of DNA-binding by RecA, we used agarose gel electrophoresis of RecA–DNA complexes, in which the migration of free DNA was compared to that of DNA with RecA (Fig. 4). To demonstrate that the DNA itself was unchanged in the binding reaction, the RecA was denatured by $NaDodSO_4$, and the mobility of the recovered DNA was compared to the input DNA. In Fig. 4, lanes 1–4 define the migration of the nonirradiated and irradiated supercoiled DNAs without protein; lanes 5–8 present the results of binding with 1 μM RecA, before and after denaturation of the protein, and lanes 9–12 show the effect of 2 μM RecA. For the irradiated DNAs of lanes 6 and 10, there is a much more pronounced effect of RecA on mobility than for the nonirradiated DNAs of lanes 5 and 9. Lanes 7, 8, 11, and 12 show that the DNA is itself unchanged by the binding assay.

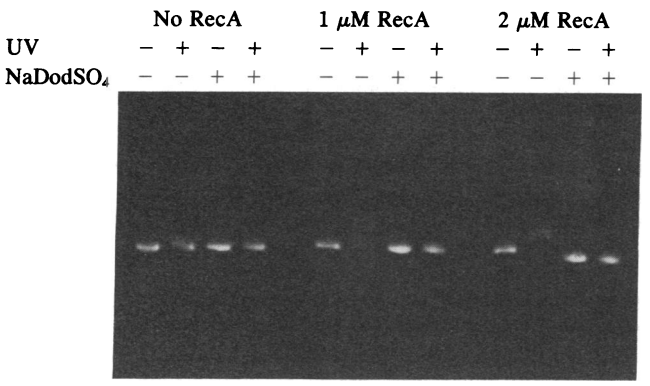

FIG. 4. Preferential binding of RecA to UV-irradiated DNA. RecA (1 μM or 2 μM) was incubated with supercoiled φX174 DNA with ATP[γ-S]. The products of the reaction were subjected to agarose gel electrophoresis, either directly or after the RecA was denatured by $NaDodSO_4$.

From the data presented in Figs. 2–4, we conclude that RecA binds more effectively to UV-irradiated, double-stranded DNA than to nonirradiated DNA. Thus, we presume that RecA has the capacity to recognize pyrimidine dimers. Preferential binding of RecA to dimers is not particularly surprising given that the dimers must distort B-type DNA and given the very high affinity of RecA for single-stranded DNA (17, 33). However, the demonstration of preferential binding to UV lesions has two important biological implications: (*i*) the primary localization of targeted mutagenesis may be achieved by localized binding of RecA to the site of damage; (*ii*) one "signal" for targeted mutagenesis is likely to be the lesion itself. The scope and limitations of these conclusions are considered in the *Discussion*.

Preferential Activation of Proteolytic Activity of RecA by UV-Irradiated DNA. In addition to its DNA-binding and strand-transfer activities, the RecA protein catalyzes cleavage of the LexA repressor (4, 27). If a signal for targeted mutagenesis is the DNA lesion, it seems plausible that the same signal might activate RecA for its regulatory role in the SOS response: cleavage of LexA. Therefore, we carried out a DNA-binding experiment analogous to that shown in Fig. 3, except that the RecA–DNA complex was used to catalyze cleavage of purified LexA (Fig. 5). The results of the cleavage assay indicate more efficient cleavage of LexA with UV-irradiated double-stranded DNA than with nonirradiated DNA (compare lane 4 with lanes 5–8). The effect of irradiation in the LexA reaction is less striking than with the direct DNA-binding experiments for RecA; however, the reaction is also more complex and the conditions used may not be optimal. Activation of RecA by UV-irradiated DNA is

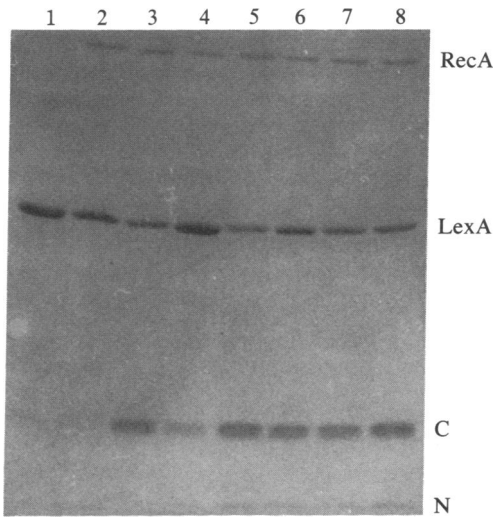

FIG. 5. Activation of RecA for LexA cleavage by double-stranded φX174 DNA. RecA was incubated with DNA and LexA protein, the products of the reaction separated by $NaDodSO_4$/15% PAGE, and the proteins visualized by staining with Coomassie blue. The migration positions of RecA, uncleaved LexA, and the LexA cleavage products C and N are indicated. The smaller of the two cleavage fragments is under-represented under the staining conditions used. Lanes: 1, LexA only; 2, RecA and LexA; 3, plus 10 ng of single-stranded φX174 DNA; 4, plus 20 ng of double-stranded φX174 DNA; 5–8, plus 20 ng of double-stranded DNA irradiated at 400, 600, 800, and 1000 J/m², respectively.

comparable to that provided by single-stranded DNA, equating a double-strand base pair with a single-strand base (compare lane 3 with lanes 5–8). Thus, activation of RecA for cleavage appears to require only efficient binding of RecA to DNA (single-stranded or double-stranded) and nucleotide cofactor. In the LexA cleavage reaction, dATP will also support preferential activation of RecA by UV-irradiated DNA (unpublished observation).

The capacity of UV-irradiated DNA to activate RecA indicates that in the SOS response *in vivo* the signal for cleavage of LexA by RecA may often be the lesion itself (see *Discussion*).

RecA-Mediated Inhibition of Exonuclease Activity of ε Protein. Previous work has shown that RecA inhibits the 3′ → 5′ exonuclease activity of polIII holoenzyme on a mispaired copolymer substrate (18). An attractive mechanism for this inhibition is an action of RecA on the ε subunit of polIII (Fig. 1). To examine this possibility, we studied the effect of RecA on the 3′ → 5′ exonuclease of the purified ε protein. To simulate replication conditions as closely as possible, we also added single-strand binding protein (Ssb). We found that RecA protein is an effective inhibitor of the editing exonuclease activity of ε (Fig. 6). The inhibitory activity of RecA requires a nucleotide cofactor. In addition, to ATP[γ-S], ATP or dATP will serve as a cofactor if a triphosphate-regenerating system is present. These studies will be reported in detail elsewhere. The capacity of RecA to inhibit the editing exonuclease of ε might be responsible for targeted mutagenesis along the lines of the mechanism proposed in Fig. 1. However, a more conclusive argument for this mechanism requires evidence of a direct interaction between RecA and ε. With our artificial mispaired system, the primary interaction is probably with DNA; the replication capacity of polIII holoenzyme is also partially inhibited (less than the exonuclease), and the amount of RecA required is stoichiometric with added DNA (unpublished data).

DISCUSSION

The Role of RecA in SOS-Induced Mutagenesis. RecA protein is required *in vivo* for targeted mutagenesis at pyrimidine dimers (13–15). The mutagenic process requires a mechanism for localization to the DNA lesion and for replication errors. We believe that RecA accomplishes both of these functions by two biochemical activities: preferential binding to pyrimidine dimers, and inhibition of exonucleolytic editing by the ε subunit of polIII holoenzyme. Because the error-prone polymerization events can bypass a replication-blocking lesion, recovery from UV damage will be enhanced by this RecA-mediated process ("Weigle reactivation") (1–5).

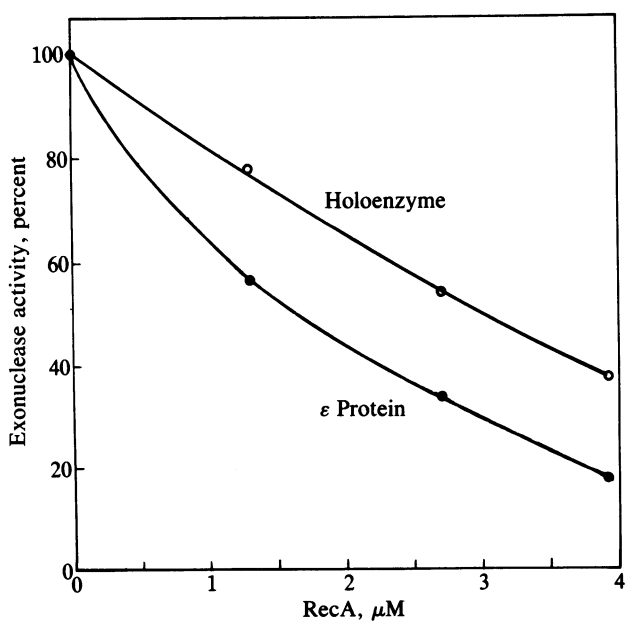

FIG. 6. Inhibition of editing exonuclease by RecA. The 3′ → 5′ exonuclease activity of either ε subunit (●) or polIII holoenzyme (○) was measured in the presence of RecA at the concentrations shown, using as a substrate the synthetic copolymer $(dA)_{3000}/[(dT)_{18}([^3H]dC)_2]_{10}$. The 3′ → 5′ exonuclease was determined by the remaining [^{3}H]dCMP in polymer form in the kinetic assay described (19, 23).

In addition to RecA, SOS-induced mutagenesis requires the UmuC/D proteins (5, 10–12). Experiments by Bridges and Woodgate have indicated that UmuC/D may have an ancillary role in mutagenesis because *umuC*$^-$ mutants exhibit UV mutagenesis after delayed removal of replication-blocking lesions by photoreversal (34, 43). These authors suggest that UmuC/D proteins function to allow DNA strand elongation past a blocking lesion once bases have been incorporated (or misincorporated) opposite the lesion by a process involving other protein(s). In our proposed mechanism (Fig. 1), we can think of three plausible biochemical roles for UmuC/D: (*i*) to facilitate tight binding by RecA at the rather small distortion introduced by the lesion; (*ii*) to facilitate binding of holoenzyme to the distorted primer–template region, preventing dissociation and permitting multiple rapid replication attempts; (*iii*) to help polIII holoenzyme clear the damaged site by freeing the enzyme from RecA or RecA from the DNA.

Another aspect of SOS-induced mutagenesis is the untargeted mutagenesis that occurs in DNA lacking a known lesion (1–5). The genetic requirements for this component of SOS-induced mutagenesis are controversial, probably because of its low level and the varied conditions employed for its study (5). In terms of our proposed mechanism, RecA might participate in untargeted mutagenesis by associating with double-stranded, nonirradiated DNA as a consequence of the large SOS-induced increase in cellular concentration of RecA (and perhaps of the appropriate cofactor—see below). A special case of untargeted mutagenesis is that mediated by the altered RecA produced by the *recA441* and *recA730* mutations (3, 5, 13–15). RecA441 bacteria are thermally inducible, and RecA730 cells are constitutive for mutagenesis and for cleavage of LexA protein. The phenotype of these mutations might derive from a highly enhanced capability of the mutant RecA to bind to double-stranded DNA lacking a lesion. Preliminary binding experiments with RecA441 support this idea (unpublished data).

The Signal for Mutagenesis and SOS Induction. *In vivo*, a high level of RecA protein and derepression of other LexA-regulated operons are not sufficient for SOS-induced mutagenesis (13–15). This finding indicates that one or more "activation signals" are required for the mutagenic activity of wild-type RecA. From our study, we infer that one signal for mutagenesis is the lesion itself. However, data from mutagenesis of bacteriophage λ indicate that DNA lesions enter the mutagenesis process at two levels: both the bacteriophage (*cis* effect) and host (*trans* effect) must be irradiated for maximal mutagenesis even if the SOS system is derepressed by mutation of LexA (15). Thus, we must understand the *trans* effect to achieve a consistent picture of the signal system.

All of the biochemical activities of RecA protein, including those described here, require DNA and a nucleoside triphosphate cofactor. Most studies have focused on an initial interaction of single-stranded DNA and ATP, dATP, or ATP[γ-S]. In this report, we have demonstrated that preferential binding of RecA to UV-irradiated DNA occurs with the cofactor ATP[γ-S], which appears to function as an especially effective nucleotide cofactor in DNA-binding and repressor cleavage reactions (e.g., refs. 30 and 35). Since ATP[γ-S] is unlikely to be the cofactor *in vivo*, the *trans*

activation of mutagenesis may involve the production of a high level of mononucleotide cofactor triggered by the block to bacterial DNA replication. This cofactor might be dATP (35) or a specialized signal nucleotide for the SOS response (4, 5).

The capacity of RecA to cleave LexA protein also depends on an activation signal (4, 5). From our study, we think it likely that, as for mutagenesis, one signal is often the lesion itself. Previous work has indicated that the signal system for SOS induction is different for different inducing treatments (e.g., ref. 36). For DNA damage that distorts the double-helix (e.g., pyrimidine dimer), the lesion itself may work. For example, no combination of bacterial mutants completely blocked UV induction (36). Moreover, substantial (though subnormal) SOS induction has been observed with UV-irradiated λ DNA under nonreplicating conditions (37). For other replication-blocking agents, such as nalidixic acid, the generation of single-stranded DNA by RecBC exonuclease might be required (36).

Implications of Targeted Mutagenesis: Antibody Variation and Genetic Variation. The concept that localized binding of a protein may target mutagenesis poses an interesting possibility for the component of antibody variation that is likely to be produced by somatic mutation (38, 39). In principle, a sequence-specific binding protein might target mutagenesis to any preferred site in a genome by causing localized loss of replication fidelity.

One of us (H.E.) has discussed in detail before the idea that an induced increase in mutation rate might be an advantage to a population in periods of severe environmental stress (17, 40). McClintock (41) has considered the same concept from the point of view of transposon mobility and chromosome restructuring. The mutagenic component of SOS induction can then be thought of as a quest for greater genetic variation in the bacterial population. Alternatively, targeted mutagenesis might be considered an unavoidable "side-effect" of a repair mechanism involving replicative bypass of a DNA lesion. The concept of an error-required repair mechanism as the sole role for induced mutagenesis seems unappealing because several error-free mechanisms are available (e.g., excision and recombination) and because the repair capability of the RecA–UmuC/D pathway is rather small (10–12). Although an additional repair pathway is undoubtedly useful, we think it likely that a major value of SOS-induced mutagenesis (targeted and untargeted) is enhanced genetic variation. In these terms, the targets for mutagenesis can be thought of as a counting system for the number of unrepaired lesions and therefore a measure of the level of environmental crisis.

We thank Arthur Kornberg, Robert Lehman, John Little, and Hisaji Maki for the gift of purified proteins and Don Ennis, Robert Lehman, David Mount, and Evelyn Witkin for unpublished information and helpful discussion. We thank also William Ricco for photography and Terri DeLuca for editorial help. This work was supported by a grant from the American Cancer Society (ACS MV-131B).

1. Weigle, J. J. (1953) *Proc. Natl. Acad. Sci. USA* **39**, 628–636.
2. Radman, M. (1975) in *Molecular Mechanisms for Repair of DNA*, eds. Hanawalt, P. & Setlow, R. B. (Plenum, New York), pp. 355–367.
3. Witkin, E. M. (1976) *Bacteriol. Rev.* **40**, 869–907.
4. Little, J. W. & Mount, D. M. (1982) *Cell* **29**, 11–22.
5. Walker, G. C. (1984) *Microbiol. Rev.* **48**, 60–93.
6. Witkin, E. M. & Wermundsen, I. E. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 881–886.
7. Miller, J. H. (1982) *Cell* **31**, 5–7.
8. Wood, R. D., Skopek, T. R. & Hutchinson, F. (1984) *J. Mol. Biol.* **173**, 293–305.
9. Miller, J. H. (1985) *J. Mol. Biol.* **182**, 45–68.
10. Kato, T. & Shinoura, Y. (1977) *Mol. Gen. Genet.* **156**, 121–131.
11. Steinborn, G. (1978) *Mol. Gen. Genet.* **165**, 87–93.
12. Bagg, A., Kenyon, C. J. & Walker, G. C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5749–5753.
13. Blanco, M., Herrera, G., Collado, P., Rebollo, J. & Botella, L. M. (1982) *Biochimie* **64**, 633–636.
14. Witkin, E. M. & Kogama, T. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7539–7543.
15. Ennis, D. G., Fisher, B., Edmiston, S. & Mount, D. W. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3325–3329.
16. Villani, G., Boiteux, S. & Radman, M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3037–3041.
17. Echols, H. (1982) *Biochimie* **64**, 571–575.
18. Fersht, A. R. & Knill-Jones, J. W. (1983) *J. Mol. Biol.* **165**, 669–682.
19. Scheuermann, R. H. & Echols, H. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7747–7751.
20. Spanos, A., Sedgwick, S. J., Yarranton, G. T., Hubscher, U. & Banks, G. R. (1981) *Nucleic Acids Res.* **9**, 1825–1839.
21. Kornberg, A. (1982) *DNA Replication* (Freeman, San Francisco), Suppl., pp. 531–535.
22. McHenry, C. S. (1984) *Mol. Cell. Biochem.* **66**, 71–85.
23. Echols, H., Lu, C. & Burgers, P. M. J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2189–2192.
24. Cox, M. M., McEntee, K. & Lehman, I. R. (1981) *J. Biol. Chem.* **256**, 4676–4678.
25. McHenry, C. & Kornberg, A. (1977) *J. Biol. Chem.* **252**, 6478–6484.
26. Weiner, J. H., Bertsch, L. L. & Kornberg, A. (1975) *J. Biol. Chem.* **250**, 1972–1980.
27. Little, J. W. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1375–1379.
28. Pagano, J. S. & Hutchison, C. A. (1971) in *Methods in Virology*, eds. Maramorosch, K. & Kaprowski, H. (Academic, New York), Vol. 5, pp. 79–123.
29. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 86–96, 150–172.
30. McEntee, K., Weinstock, G. M. & Lehman, I. R. (1981) *J. Biol. Chem.* **256**, 8829–8844.
31. Wacker, A., Dellweg, H. & Jackerts, D. (1964) *J. Mol. Biol.* **4**, 410–412.
32. Livneh, Z. & Lehman, I. R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3171–3175.
33. Pearlman, D. A., Holbrook, S. R., Pirkle, D. H. & Kim, S.-H. (1985) *Science* **227**, 1304–1308.
34. Bridges, B. A. & Woodgate, R. (1984) *Mol. Gen. Genet.* **196**, 364–366.
35. Phizicky, E. M. & Roberts, J. W. (1981) *Cell* **25**, 259–267.
36. McPartland, A., Green, L. & Echols, H. (1980) *Cell* **20**, 731–737.
37. D'Ari, R. & Huisman, O. (1982) *Biochimie* **64**, 623–627.
38. Baltimore, D. (1981) *Cell* **26**, 295–296.
39. Manser, T., Wysocki, L. J., Gridley, T., Near, R. I. & Gefter, M. L. (1985) *Immunol. Today* **6**, 94–101.
40. Echols, H. (1981) *Cell* **25**, 1–2.
41. McClintock, B. (1984) *Science* **226**, 792–801.
42. Maki, H. & Kornberg, A. (1985) *J. Biol. Chem.* **260**, 12982–12986.
43. Bridges, B. A. & Woodgate, R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4193–4197.