Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 1251–1255, February 1991
Genetics

Requirements for bypass of UV-induced lesions in single-stranded DNA of bacteriophage φX174 in *Salmonella typhimurium*

(SOS/DNA polymerase III/RecA/MucAB/editing)

STEVEN C. SLATER AND RUSSELL MAURER

Department of Molecular Biology and Microbiology, Case Western Reserve University, School of Medicine, 2109 Adelbert Road, Cleveland, OH 44106-4901

Communicated by Evelyn M. Witkin, November 19, 1990

ABSTRACT **According to the current model for mutagenic bypass of UV-induced lesions, efficient bypass requires three proteins: activated RecA (RecA*) and either activated UmuD (UmuD′) and UmuC or their plasmid-encoded analogues, MucA′ and MucB. RecA* aids synthesis of UmuD′ and UmuC (and MucA′/MucB) at two levels: by inactivation of the LexA transcriptional repressor of these genes and by cleavage of UmuD (and MucA) to produce the active fragments, UmuD′ (MucA′). A third role for RecA is revealed when these two roles are otherwise satisfied in a suitably engineered strain. An often-suggested possible role for RecA in bypass is inhibition of editing by the ε subunit of DNA polymerase III. Here, by demonstrating that elimination of ε by deletion of its gene, *dnaQ*, does not relieve the requirement for the third function of RecA, we show that RecA must perform some function other than, or in addition to, inhibition of ε. We also show that elimination of ε does not relieve the requirement for either Muc protein. Moreover, we observed reactivation of irradiated φX174 in unirradiated cells expressing MucA′ and MucB. This finding makes it unlikely that the additional role of recA involves derepression of an unidentified gene or cleavage of an unidentified protein and makes it more likely that RecA participates directly in bypass.**

Ultraviolet (UV) light induces lesions in DNA capable of blocking replication *in vivo* and *in vitro* (1–6). In *Escherichia coli* and *Salmonella typhimurium*, a replication complex containing DNA polymerase III (Pol III) has two formal alternatives for continuing DNA synthesis past such a block. It may dissociate from the nascent strand and restart beyond the blocking lesion, or it may extend the nascent strand past the blocking lesion. The first of these alternatives produces DNA with daughter-strand gaps and double-strand breaks that are subsequently repaired by recombination in predominantly error-free fashion (7, 8). The second alternative, bypass synthesis, is mutagenic [SOS or Weigle mutagenesis (9)] due to the aberrant coding properties of UV-induced lesions. Genetic studies indicate that several proteins induced and/or activated in response to UV irradiation (RecA, UmuC, and UmuD or the analogous plasmid-encoded MucA and MucB) are required for most UV mutagenesis (10). These proteins are thought to assist synthesis across template lesions, resulting in mutagenesis and enhanced survival (SOS or Weigle repair) of UV-damaged cells or bacteriophage.

The status of the editing (ε) subunit of Pol III, the *dnaQ* product, has been proposed as a determinant of the fate of a DNA polymerase complex facing a UV lesion in the template strand (6, 11, 12). This hypothesis was initially developed to account for the accumulation of dNMPs during DNA synthesis using irradiated templates *in vitro* (5, 6). It was supposed that insertion of a nucleotide opposite a UV lesion would produce a nascent strand terminus with single-stranded character. The terminal nucleotide would be subject to rapid excision by ε, leading to futile cycles of addition and removal of a nucleotide opposite the lesion and the accumulation of dNMP. Thus, extension of the nascent strand past the template lesion would be aided by agents that inhibited ε or stripped it from the polymerase. RecA protein can partially inhibit the 3′–5′ exonuclease activity of ε *in vitro* (11–13) and therefore has been proposed as an inhibitor of ε during SOS-mediated DNA synthesis *in vivo*. Additionally, Foster and Sullivan (14) showed that UmuDC$^+$ function potentiates the spontaneous mutator effect of a partially defective ε. Thus, both RecA and UmuDC$^+$, the proteins implicated in targeted mutagenesis by UV, may have functional interactions with ε. Most recently, Jonczyk *et al.* (15) and Foster *et al.* (16) have reported that excess ε inhibits UV mutagenesis, in accord with the notion that ε is an antagonist of translesion synthesis.

On the other hand, several attempts to demonstrate an effect of editing on the likelihood of termination or on the precise site of termination of synthesis on UV-treated template strands have been unsuccessful (2, 3, 13, 17–19). Moreover, it is sometimes possible to observe UV mutagenesis *in vivo* in the absence of RecA and/or UmuC protein (20, 21). Finally, UV lesions in template DNA are bypassed at detectable frequencies *in vitro* (22). Together, these results suggest that neither RecA nor MucAB is strictly required for synthesis across some template lesions. Rather, these proteins may serve to facilitate a process of which the normal replication apparatus is intrinsically capable, despite having a functional ε subunit. Shwartz *et al.* (19) put forth the provocative suggestion that ε might even assist bypass synthesis by allowing Pol III to retreat from a lesion at which it is stuck and approach afresh.

In this study, we analyzed the effect of *dnaQ* on SOS repair of the single-stranded DNA of phage φX174. Productive infection by φX174 absolutely requires synthesis of a covalently closed, double-stranded DNA intermediate (23, 24), and there is no known dark-repair pathway that can eliminate UV damage in single-stranded DNA. Thus, plaque formation by irradiated phages involves obligatory synthesis across sites of template damage.

MATERIALS AND METHODS

Bacterial Strains and Procedures. *S. typhimurium* strains used are listed in Table 1. Null mutations in *dnaQ*, *recA*, or *uvrB* were introduced into strain RM821 (25) by P22-mediated generalized transduction. After all transduction steps were complete, a derivative of each strain susceptible to infection by φX174 was obtained by selection for resistance to phage Felix-O (26). Plasmids, if any, were introduced last by

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.

Abbreviations: Pol III, DNA polymerase III; IPTG, isopropyl β-D-thiogalactoside.

Table 1. *S. typhimurium* strains used

Strain	Relevant genotype			
	dnaQ	*recA*	*uvrB*	Plasmid
RM2575	+	+	−	None
RM2726	+	+	+	None
RM2728	−	+	+	None
RM3248	+	+	+	pFF441
RM3250	−	+	+	pFF441
RM3343	+	−	+	None
RM3579	+	−	+	pFF441
RM3580	−	−	+	pFF441
RM3703	−	+	+	pFF498
RM3704	−	+	+	pFF499
RM3711	+	+	+	pSE380

All strains shown are derived from RM821 (25) whose genotype is *thyA, deo, spq-2, zag-1256*::Tn*10dKm*. Strains designated *dnaQ*$^-$ carry *dnaQ201*::Tn*10dTc*, a replacement of nearly all of *dnaQ* by a tetracycline (Tc)-resistance element (25). Strains designated *recA*$^-$ carry a deletion from *recA* to *srl* (sorbitol utilization) designated *recA496* (obtained from E. Eisenstadt) and exhibit the expected UV-sensitivity. Strain RM2575 carries a *uvrB-chlA* deletion and is appropriately UV-sensitive.

electroporation. Most media and bacteriological procedures have been described (25).

Plasmid Construction. To obtain RecA/LexA-independent expression of MucB and the active fragment of MucA, the *mucAB*$^+$ *Eco*RI–*Sma* I fragment of pGW1700 (27) was cloned into phage M13mp19, and oligonucleotide-directed mutagenesis (28) was used to introduce an *Nco* I site (and therefore an in-frame ATG codon) immediately 5′ to codon 26 of *mucA*. The *Nco* I–*Sma* I fragment of the resulting phage was cloned into pSE380 (29), an expression vector that allows an open reading frame whose start codon overlaps an *Nco* I site to be fused precisely to an isopropyl β-D-thiogalactoside (IPTG)-inducible promoter and associated ribosome binding site. In this construct, pFF441, the modified *mucA′B* operon escapes RecA/LexA control and instead comes under heterologous control by LacI^q repressor encoded on pSE380. Two further constructs were made after first cloning a phage M13 replication origin into pFF441 to create pFF489. Plasmid pFF498 (*mucA′*, *mucB*$^-$) was derived by truncating the *mucA′B* operon of pFF489 at the *Bgl* II site, thus deleting 95% of *mucB*. Plasmid pFF499 (*mucA*$^-$, *mucB*$^+$) was derived by engineering *Nde* I sites at the *mucA′* and *mucB* initiation codons of pFF489 and then deleting the *mucA′ Nde* I fragment. All constructs were verified by DNA sequencing through the modified regions.

Phage Survival Assay. The assay used bacterial hosts of various genotypes to measure the plaque-forming titer of phage lysates that were irradiated to various degrees. In some cases, the bacterial strain was also irradiated. In general, host strains were grown to 2–3 × 10^8 cells per ml in Luria–Bertani (LB) broth plus appropriate drugs at 37°C. IPTG was added to a final concentration of 1 mM 30 min prior to plating. Phage lysates [1 × 10^9 plaque-forming units (pfu)/ml in phage suspension buffer (1)] were irradiated immediately before each assay by exposure to a 15-W GE germicidal lamp at a UV flux of 0.8 J/m^2 per sec. Bacteria, if they were to be irradiated, were collected from growth medium by centrifugation and resuspended at the original concentration in 10 mM Tris, pH 7.5/10 mM $MgSO_4$. The UV dose to the bacteria, 48 J/m^2, was sufficient to evoke strong Weigle reactivation of φX174 in *recA*$^+$ control strains (*dnaQ*$^+$ and *dnaQ*$^-$) carrying pKM101 (30). In experiments omitting irradiation of the bacteria, cultures were not washed. All bacterial samples were adjusted to 20 mM $CaCl_2$ before addition of phage lysate. Infection mixes (0.1 ml of culture + 0.1 ml of phage dilution) were incubated at room temperature for 5 min and then plated on LB + thymine plates in LB soft agar. Plaques were counted after overnight development in the dark at 37°C.

Each assay was conducted three times with independent bacterial cultures and different irradiated aliquots of phage lysate. The average of the three determinations is shown, with error bars indicating one standard deviation. The data are presented as fractional survival, which is the titer of the irradiated sample divided by the titer of an unirradiated sample on the same host strain. By calculating the data this way, small differences (<2-fold) in the plating efficiency of φX174 on the various strains were normalized. Repair efficiency, the fraction of lethal damage that is repaired under one set of conditions ("A") but not under a different set of conditions ("B") is calculated from the slopes of the survival curves, determined by linear regression analysis of the data, and is given by 1− (slope A/slope B) (21).

RESULTS

Repair of Irradiated φX174 in Unirradiated Cells Lacking MucAB. The data shown in Fig. 1 establish the background pattern of dark-repair of irradiated phage in unirradiated *S. typhimurium*. The decay of phage viable titer is exponential with dose to the phage, with an observed effective dose of 8.3 × 10^{-2} lethal hits per phage per J·m^{-2}. Since the DNA in the irradiated phage is single-stranded, it should not be susceptible to UvrABC-catalyzed repair, which requires the complementary strand as a template for repair. Consistent with this expectation, we found no difference between a *uvrB* mutant and *uvrB*$^+$ in survival of irradiated φX174. We also found no difference between *recA* mutant or *recA*$^+$ genotypes, as expected since the multiplicity of infection (based on the unirradiated phage titer) was <0.1 in all parts of the figure to minimize opportunities for recombinational repair. This result (and a similar lack of *recA*-dependent φX174 reactivation in irradiated cells; data not shown) also indicates that there is no constitutive or inducible reactivation pathway for φX174 that depends on RecA. Apparently, the endogenous *umuDC*$^+$ activity in *Salmonella* (31–33) is completely inactive in φX174 reactivation.

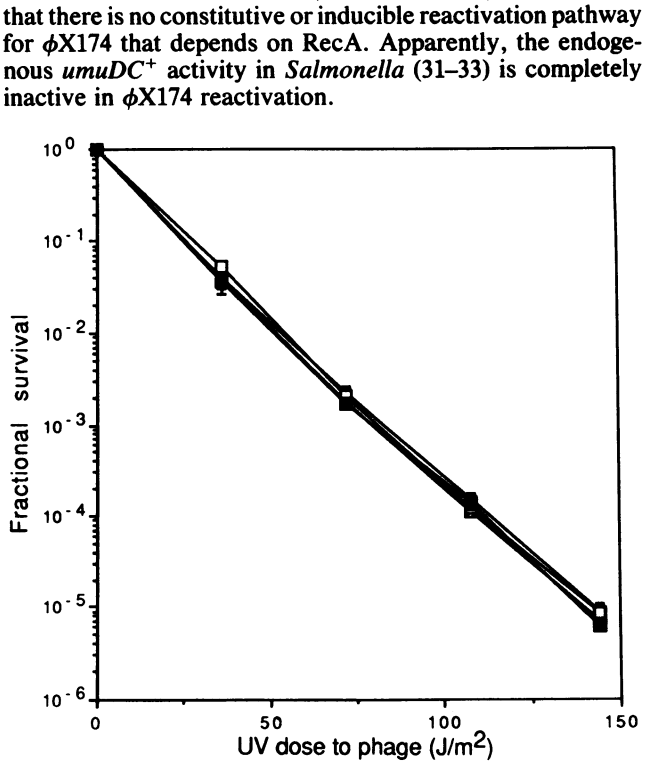

FIG. 1. Survival of UV-irradiated φX174 in *Salmonella* strains lacking MucA′B. ○, RM2575 (*uvrB*$^-$); △, RM2726 (*dnaQ*$^+$); □, RM2728 (*dnaQ*$^-$); ■, RM3343 (*recA*$^-$).

Tessman (21), arguing from *in vivo* data, and Livneh (22), arguing from *in vitro* data, have detected a limited capacity in *E. coli* for translesion synthesis in the absence of RecA and Umu proteins. Whether *S. typhimurium* has a similar capacity is difficult to establish from the data of Fig. 1. What is clear, however, is that any such capacity must be limited by something other than, or in addition to, an inhibitory effect of ε, because elimination of ε offered no improvement in the phage viable titer.

Effect of MucAB. RecA regulates MucAB expression (and *umuDC* similarly) at two levels: (*i*) transcriptional derepression of *mucAB* (*umuDC*) dependent on cleavage of the LexA repressor and (*ii*) cleavage of MucA (UmuD) to produce the active fragment MucA′ (umuD′) (34). When expression of *umuDC* was artificially uncoupled from RecA control, SOS mutagenesis still required RecA (35–37). To examine the effect of MucAB in the ϕX174 system, we circumvented the regulatory roles of RecA with plasmid pFF441, which expresses MucA′ and MucB from a heterologous promoter.

Fig. 2 shows the requirements for reactivation of ϕX174 in a *dnaQ*$^+$ strain. A strain harboring pSE380, a precursor of pFF441 that lacks *mucA′B*, is incapable of phage reactivation. In contrast, pFF441 leads to the repair of an estimated 29% of the lethal damage. Irradiation of the host bearing pFF441 produces little, if any, further increase of phage titer. Nevertheless, phage reactivation is strictly dependent on *recA*.

Fig. 3 shows that ϕX174 reactivation can occur in a *dnaQ* deletion mutant. As in the *dnaQ*$^+$ situation, reactivation depends on the presence of pFF441. The repair efficiency in the *dnaQ*$^-$ strain is estimated to be 35%, slightly greater than the 29% estimated for the *dnaQ*$^+$ strain. This difference is due primarily to better MucAB-dependent reactivation, rather than a lower level of background (MucAB-independent) repair in the *dnaQ*$^-$ strain.

The data in Fig. 4 show that none of the reactivation requirements defined in Fig. 2 can be satisfied alternatively by removal of ε. Reactivation is not observed in a *recA*$^-$, *dnaQ*$^-$ double mutant harboring pFF441, nor is it observed in a *recA*$^+$, *dnaQ*$^-$ mutant harboring derivatives of pFF441 that express either MucA′ alone or MucB alone. Thus, removal of Pol III editing capacity does not negate the requirement for RecA, MucA′, or MucB during template lesion bypass.

To confirm the functionality of MucA′ and MucB when expressed from separate messages, the IPTG-inducible *mucB* gene of pFF499 was cloned into pMS421 (38) creating pFF511. In the presence of IPTG, a strain harboring both pFF498 (*mucA′*) and pFF511 (*mucB*$^+$) reactivates irradiated ϕX174 as well as a strain harboring pFF441 (data not shown).

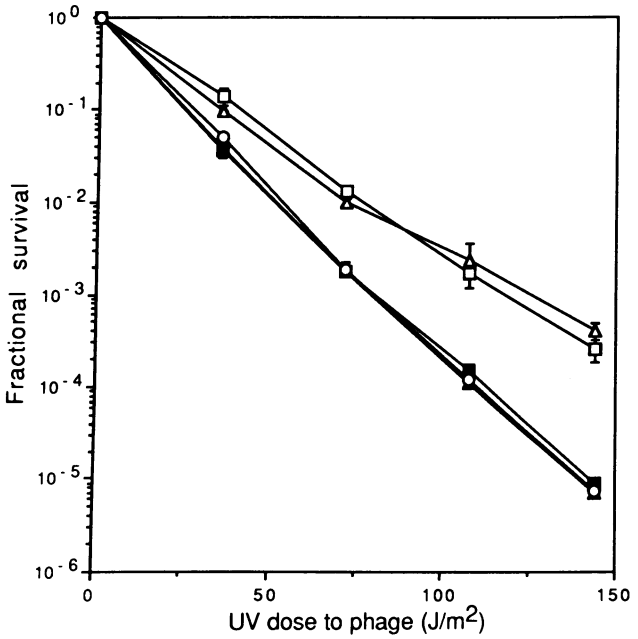

FIG. 2. MucA′B-dependent reactivation of UV-irradiated ϕX174 in *dnaQ*$^+$ *Salmonella* strains. ▲, RM2726 (no plasmid); △, RM3248 (pFF441), irradiated; □, RM3248 (pFF441), no irradiation; ■, RM3579 (*recA*$^-$, pFF441); ○, RM3711 (pSE380).

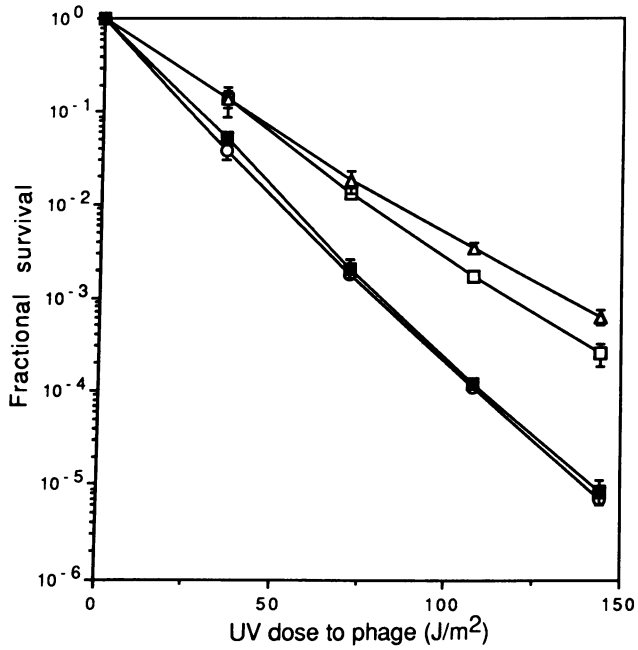

FIG. 3. Comparison of ϕX174 reactivation in *dnaQ*$^+$ vs. *dnaQ*$^-$ *Salmonella* strains. ○, RM2726 (*dnaQ*$^+$); ■, RM2728 (*dnaQ*$^-$); □, RM3248 (*dnaQ*$^+$, pFF441); △, RM3250 (*dnaQ*$^-$, pFF441).

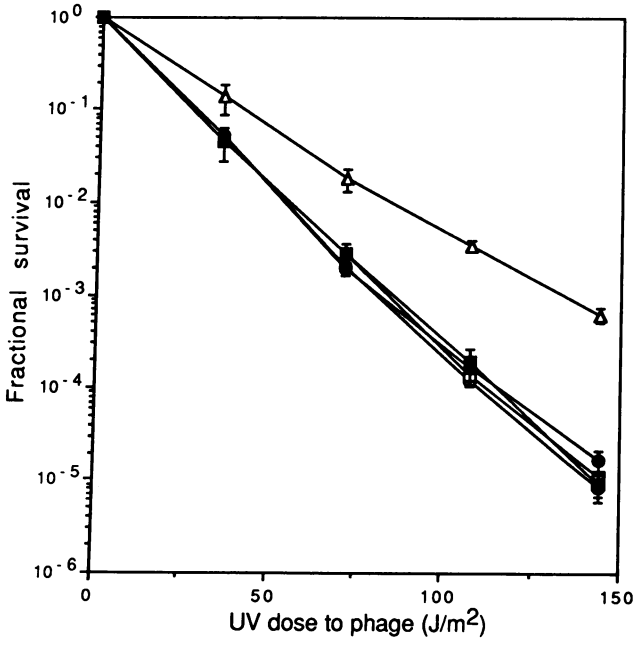

FIG. 4. Deletion of *dnaQ* does not negate the requirement for *recA*, *mucA′*, or *mucB* in reactivation of UV-irradiated ϕX174. ○, RM2728 (*dnaQ*$^-$); △, RM3250 (*dnaQ*$^-$, pFF441); □, RM3580 (*dnaQ*$^-$, *recA*$^-$, pFF441); ■, RM3703 (*dnaQ*$^-$, pFF498); ●, RM3704 (*dnaQ*$^-$, pFF499).

DISCUSSION

A DNA polymerase (probably Pol III), RecA, and either UmuDC or MucAB all cooperatively effect error-prone (SOS or Weigle) repair of UV-damaged DNA. However, the molecular interactions leading to DNA synthesis using a damaged template are unclear. A number of possible pathways have been proposed to account for SOS mutagenesis and enhanced survival. These include inhibition of the Pol III editing subunit ε, enhancement of Pol III tolerance for a distorted template, alteration of template conformation, and relaxation of Pol III stringency for correct base-pairing between the template and nascent strands (11, 12, 39, 40).

Much research on *in vitro* replication of irradiated DNA has focused on the possible role of editing, but with inconclusive results (see Introduction). A previous *in vivo* study of ε function relied on a dominant ε mutant (*mutD5*) with conditionally defective proofreading capability (41). Although this study found little effect of the ε mutation on UV mutagenesis, the use of an altered-function mutant meant that this study could not rigorously exclude ε as an inhibitor of Pol III progression past a template lesion. Additionally, the defective ε subunit was still present in these strains, so any requirement for ε [e.g., for binding RecA or MucAB or for enhancement of bypass as suggested by Shwartz *et al.* (19)] might have been missed.

Experimental System. We analyzed template lesion bypass in *S. typhimurium* strains differing by the presence or complete absence of the Pol III ε subunit. A previous study on such strains shows that their spontaneous mutation frequency responds appropriately to the status of *dnaQ* (25). Thus, we were able to analyze replication of UV-damaged DNA in strains differing strictly in the ability of Pol III to excise mispaired primer termini.

Deletion of *dnaQ* is detrimental to growth of *S. typhimurium* unless a suppressor mutation, designated *spq*, is present (25). All strains used in this study, whether $dnaQ^+$ or $dnaQ^-$, carry the *spq-2* mutation, which changes valine-832 to glycine in the α (polymerase) subunit of Pol III (42). *spq-2* Pol III is active *in vivo* and *in vitro* and appears to carry out the preponderance of cellular DNA synthesis under $dnaQ^-$ conditions but with an expanded role for DNA polymerase I (25). The DNA polymerase I requirement in $dnaQ^-$, *spq-2* strains does not include a requirement for its 3′–5′ exonuclease (E. Lancy, M. Lifsics, and R.M., unpublished data). The *spq-2* mutation by itself has no effect on φX174 reactivation or cellular UV tolerance in $dnaQ^+$ genotypes (data not shown).

Observations. The salient points of our data can be summarized as follows. First, template lesion bypass requires MucA′, MucB, and RecA, but not ε. Second, when MucA′, MucB, and RecA are provided, elimination of ε leads to a slight enhancement of phage reactivation at best. Third, when any one of MucA′, MucB, or RecA is missing, elimination of ε confers no enhancement of phage reactivation. Finally, preirradiation of the host cells is not necessary to achieve phage reactivation in strains constitutively expressing MucA′ and MucB.

Our results also bear on the suggestion (43) that the *uvpI* gene of pKM101 is required, along with *mucAB*, for enhanced cellular survival. This suggestion is inconsistent with our data because pFF441 does not contain *uvpI* but still enhances cellular survival (data not shown) and phage reactivation following UV irradiation.

Role of ε. DNA polymerase confronts a tripartite decision at a template lesion: extension, editing, or dissociation. Promotion of bypass must involve factors that favor extension at the expense of the other two possibilities. According to one view, inhibition of ε, by itself, must lead to more extension. This view is not supported by our results because elimination of editing did not confer any increase in bypass in the absence of MucA′B. On the other hand, there is a suggestion in our data that elimination of editing does increase bypass slightly when MucA′B and RecA are present. Elimination of editing did not alleviate the requirement for MucA′, MucB, or RecA when tested individually. Thus, if ε must be inhibited to allow bypass, our data imply that this cannot be the sole function of RecA (aside from proteolysis) or either Muc protein. This conclusion is tempered, for the case of MucA′ and MucB, by uncertainty about the ability of each of the proteins to carry out its intended function in the absence of the other. *In vitro*, the analogous UmuD′ and UmuC proteins are tightly associated (40, 44).

An alternative view more consistent with our data would be that idling at a template lesion cannot prevent eventual resolution of the block along one of the other two pathways. According to this model, inhibition of ε is unnecessary because idling is not the cause of a stalled replication fork, only a symptom. Factors that increase bypass, such as MucA′, MucB, RecA, and possibly other proteins, would do so by favoring extension specifically at the expense of dissociation. This effect could involve an increase in either the velocity of extension or the processivity (i.e., DNA affinity) of the DNA polymerase at the template lesion. These possibilities have also been suggested by others (45–47). This model is not necessarily at odds with the observation that excess ε inhibits UV mutagenesis, presumably by inhibition of lesion bypass (15, 16). That inhibitory effect, because it requires ε at levels apparently far in excess of Pol III, must depend on either mass action (implying exchange between enzyme-associated and free ε) or an *in vivo* activity of free ε. Inhibitory mechanisms incorporating these features have been proposed (15, 16, 48), but overproduction experiments do not address whether such inhibition would be physiologically significant at ordinary ε levels. Our observation that deletion of ε has little effect on bypass suggests that normal ε levels are not inhibitory, unless, alternatively, ε is antagonized during SOS in some way that our tests do not detect.

Role of RecA. In the experiment of Fig. 4, we satisfied the regulatory role of RecA by heterologous expression of MucA′B, and at the same time we fulfilled any involvement in ε inhibition by deleting *dnaQ*. Even so, lesion bypass was strictly dependent on RecA, showing that RecA has at least one other role. RecA was able to fulfill this role without irradiation of the cells, and irradiation produced little, if any, additional effect. Thus, unless RecA activation occurs by an indirect mechanism (such as interaction with the single-stranded viral DNA; ref. 49), the remaining role of RecA does not require activation. No reactivation was obtained in the absence of host irradiation when the parental $mucAB^+$ plasmid, pKM101 (30), was used in our system instead of pFF441 (data not shown). Thus, any indirect activation of RecA is not sufficient to stimulate functional MucAB expression requiring cleavage of LexA repressor and cleavage of precursor MucA. We infer that the remaining role of RecA does not depend on derepression of an unidentified gene or cleavage of an unidentified protein. Instead, RecA probably participates directly in lesion bypass. Several possible direct roles for RecA can be built around its known activities. For instance, RecA may bind the template lesion or adjoining single-stranded DNA, thus modifying the template. RecA could also serve as a localizing agent for MucA and/or MucB. Because binding at or near the lesion may activate RecA *in situ*, our studies do not address whether such activation is obligatory for lesion bypass (50, 51).

For the past decade, much research on the mechanism of bypass synthesis has dealt with the hypothesis that inhibition of ε may be the critical step in polymerase progression. Here, we have clearly demonstrated that elimination of Pol III editing capacity is insufficient to promote efficient template lesion bypass. In doing so, we have shown that RecA and

MucA'B do not require ε to enhance template lesion bypass and that these proteins must perform some function other than, or in addition to, inhibition of ε. Since elimination of editing is insufficient to promote template lesion bypass (this work and refs. 3, 11, and 13) and DNA polymerases are occasionally capable of polymerization opposite dimerized pyrimidines but are poorly capable of further DNA synthesis (13, 17, 18), translocation of stalled Pol III may be the critical step in lesion bypass.

We thank Graham Walker, Jürgen Brosius, Eric Eisenstadt, and C. Mark Smith for bacterial strains and plasmids and Miriam Lifsics for many helpful discussions. This research was supported by Public Health Service Grant GM39419 (to R.M.).

1. Callait-Fauquet, P., Defais, M. & Radman, M. (1977) *J. Mol. Biol.* **117,** 95–112.
2. Doubleday, O. P., Michel-Maenhaut, G., Brandenburger, A., Lecomte, P. & Radman, M. (1983) in *Chromosome Damage and Repair*, eds. Seeburg, E. & Kleppe, K. (Plenum, New York), pp. 447–459.
3. Livneh, Z. (1986) *J. Biol. Chem.* **261,** 9526–9533.
4. Moore, P. D., Bose, K. K., Rabkin, S. D. & Strauss, B. S. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 110–114.
5. Shwartz, H. & Livneh, Z. (1987) *J. Biol. Chem.* **262,** 10518–10523.
6. Villani, G., Boiteux, S. & Radman, M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3037–3041.
7. Howard-Flanders, P. & Boyce, R. P. (1966) *Radiat. Res. Suppl.* **6,** 156–184.
8. Rupp, W. D., Wilde, C. E., Reno, D. L. & Howard-Flanders, P. (1971) *J. Mol. Biol.* **61,** 25–44.
9. Weigle, J. J. (1953) *Proc. Natl. Acad. Sci. USA* **39,** 628–636.
10. Walker, G. C. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, eds. Neidhart, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington, DC), Vol. 2, pp. 1346–1357.
11. Fersht, A. R. & Knill-Jones, J. W. (1983) *J. Mol. Biol.* **165,** 669–682.
12. Lu, C., Scheuermann, R. H. & Echols, H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 619–623.
13. Bridges, B. A., Kelly, C., Hübscher, U. & Sedgwick, S. G. (1988) in *DNA Replication and Mutagenesis*, eds. Moses, R. E. & Summers, W. C. (Am. Soc. Microbiol., Washington, DC), pp. 277–283.
14. Foster, P. L. & Sullivan, A. D. (1988) *Mol. Gen. Genet.* **214,** 467–473.
15. Jonczyk, P., Fijalkowska, I. & Ciesla, Z. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9124–9127.
16. Foster, P. L., Sullivan, A. D. & Franklin, S. B. (1989) *J. Bacteriol.* **171,** 3144–3151.
17. Bridges, B. A., Woodgate, R., Ruiz-Rubio, M., Sharif, S. G., Sedgwick, S. G. & Hübscher, U. (1987) *Mutat. Res.* **181,** 219–226.
18. Moore, P. & Strauss, B. S. (1979) *Nature (London)* **278,** 664–666.
19. Shwartz, H., Shavitt, O. & Livneh, Z. (1988) *J. Biol. Chem.* **263,** 18277–18285.
20. Christensen, J. R., LeClerc, J. E., Tata, P. V., Christensen, R. B. & Lawrence, C. W. (1988) *J. Mol. Biol.* **203,** 635–641.
21. Tessman, I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6614–6618.
22. Livneh, Z. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4599–4603.
23. Eisenberg, S., Griffith, J. & Kornberg, A. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3198–3202.
24. Ikeda, J. E., Yudelevich, A. & Hurwitz, J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2669–2673.
25. Lancy, E., Lifsics, M. R., Kehres, D. & Maurer, R. (1989) *J. Bacteriol.* **171,** 5572–5580.
26. Wilkinson, R. G., Gemski, P. & Stocker, B. A. D. (1972) *J. Gen. Microbiol.* **70,** 527–554.
27. Perry, K. L. & Walker, G. (1982) *Nature (London)* **300,** 278–281.
28. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 488–492.
29. Brosius, J. (1989) *DNA* **8,** 759–777.
30. Mortelmans, K. E. & Stocker, B. A. D. (1976) *J. Bacteriol.* **128,** 271–282.
31. Herrera, G., Urios, A., Aleixandre, V. & Blanco, M. (1988) *Mutat. Res.* **198,** 9–13.
32. Smith, C. M. & Eisenstadt, E. (1989) *J. Bacteriol.* **171,** 3860–3865.
33. Thomas, S. M. & Sedgwick, S. G. (1989) *J. Bacteriol.* **171,** 5776–5782.
34. Marsh, L. & Walker, G. C. (1987) *J. Bacteriol.* **169,** 1818–1823.
35. Dutreix, M., Moreau, P. L., Bailone, A., Galibert, F., Battista, J. R., Walker, G. C. & Devoret, R. (1989) *J. Bacteriol.* **171,** 2415–2423.
36. Ennis, D. G., Ossanna, O. & Mount, D. W. (1989) *J. Bacteriol.* **171,** 2533–2541.
37. Nohmi, T., Battista, J. R., Dodson, L. A. & Walker, G. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1816–1820.
38. Graña, D., Gardella, T. & Susskind, M. M. (1988) *Genetics* **120,** 319–327.
39. Lu, C. & Echols, H. (1987) *J. Mol. Biol.* **196,** 497–504.
40. Woodgate, R., Rajagopalan, M., Lu, C. & Echols, H. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7301–7305.
41. Woodgate, R., Bridges, B. A., Herrera, G. & Blanco, M. (1987) *Mutat. Res.* **183,** 31–37.
42. Lancy, E., Lifsics, M. R., Munson, P. & Maurer, R. (1989) *J. Bacteriol.* **171,** 5581–5586.
43. Gigliani, F., Sporeno, E., Perri, S. & Battaglia, P. A. (1989) *Mol. Gen. Genet.* **218,** 18–24.
44. Freitag, N. & McEntee, K. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8363–8367.
45. Battista, J. R., Nohmi, T., Donnelly, C. E. & Walker, G. C. (1988) in *Mechanisms and Consequences of DNA Damage Processing*, eds. Freidberg, E. C. & Hanawalt, P. C. (Liss, New York), pp. 455–459.
46. Bridges, B. A., Bates, H. & Sharif, F. (1989) in *Proceedings of the 16th International Congress of Genetics*, ed. Moens, P. B. (Natl. Res. Council Canada, Ottawa), pp. 572–577.
47. Shavitt, O. & Livneh, Z. (1989) *J. Bacteriol.* **171,** 3530–3538.
48. Ciesla, Z., Jonczyk, P. & Fijalkowska, I. (1990) *Mol. Gen. Genet.* **221,** 251–255.
49. D'ari, R. & Huisman, O. (1982) *Biochimie* **64,** 623–627.
50. Sweasy, J. B., Witkin, E. M., Sinha, N. & Roegner-Maniscalo, V. (1990) *J. Bacteriol.* **172,** 3030–3036.
51. Sassanfar, M. & Roberts, J. W. (1990) *J. Mol. Biol.* **212,** 79–96.