*Proc. Natl. Acad. Sci. USA*
Vol. 90, pp. 8169–8173, September 1993
Biochemistry

# Targeting of the UmuD, UmuD′, and MucA′ mutagenesis proteins to DNA by RecA protein

(affinity chromatography/chemiluminescent immunoassay/DNA mobility-shift assay/nucleoprotein complexes)

EKATERINA G. FRANK, JANET HAUSER, ARTHUR S. LEVINE, AND ROGER WOODGATE*

Section on DNA Replication, Repair, and Mutagenesis, Building 6, Room 1A13, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892

*Communicated by Evelyn M. Witkin, June 1, 1993*

**ABSTRACT** **In addition to its critical role in genetic recombination, the *Escherichia coli* RecA protein plays a pivotal role in SOS-induced mutagenesis. This role can be separated genetically into three steps: (*i*) derepression of the SOS regulon by mediating the posttranslational cleavage of the LexA repressor, (*ii*) activation of UmuD′-like proteins by mediating cleavage of the UmuD-like proteins, and (*iii*) a direct step, possibly to interact with and to target the Umu-like mutagenesis proteins to lesions in DNA. We have analyzed RecA's third role biochemically using protein affinity chromatography and an agarose-based DNA mobility-shift assay. RecA730 protein from a crude cell extract was specifically retained on UmuD and UmuD′ protein affinity columns, suggesting that these proteins physically interact. Normally, neither UmuD nor UmuD′ shows any affinity for DNA. In the presence of RecA protein, however, UmuD and UmuD′ were targeted to DNA. RecA1730 protein, which is defective for UmuD′ but proficient for MucA′-promoted mutagenesis, showed a dramatically reduced capacity to target UmuD′ to DNA but was able to target a significant portion of MucA′ to DNA. These data support the suggestion that the direct role of RecA protein in SOS-induced mutagenesis is to interact with and target the Umu-like mutagenesis proteins to DNA.**

Living organisms are continually exposed to a variety of synthetic and natural agents that damage their DNA. To maintain the integrity of their genomes, many organisms have equipped themselves with an array of DNA repair enzymes. For example, exposure of *Escherichia coli* to replication-inhibiting agents results in the induction of the so-called "SOS" response, during which ≈20 cellular genes are induced to deal with damaged DNA (1, 2). Although most of the damage is processed via error-free repair pathways, some lesions are processed via an error-prone pathway (3, 4). Genetic and physiological experiments have identified several proteins that are essential for the error-prone pathway, including DNA polymerase III holoenzyme, RecA protein, and the UmuD and UmuC mutagenesis proteins (5–9). The latter three proteins are all members of the SOS regulon and are expressed at elevated levels after DNA damage. As part of the mutagenic process, UmuD is posttranslationally processed to a shorter but mutagenically active form termed UmuD′ (10–12). Genetic experiments suggest that these proteins act together to facilitate error-prone translesion DNA synthesis (13). In support of this hypothesis, Rajagopalan *et al.* (14) have recently shown that the highly purified UmuD′, UmuC, and RecA proteins help DNA polymerase III holoenzyme to synthesize past a single abasic lesion *in vitro*.

RecA's role in the mutagenic process is complex. It is involved in at least three genetically separable steps. The first two appear regulatory and indirect, in that, first, RecA mediates the cleavage of the LexA transcriptional repressor protein, leading to the induction of all LexA-regulated proteins, and, second, it mediates cleavage of the mutagenically inactive UmuD protein to generate the mutagenically active UmuD′ protein. Even when these processes are obviated genetically, RecA appears to have a third "direct" role (6, 11, 15). Although many hypotheses have been proposed for this direct role, the most attractive is that it is to interact with, and correctly position, the Umu mutagenesis proteins at lesions in the DNA (15–17). We have directly tested this hypothesis using protein affinity chromatography and an agarose-based DNA mobility-shift assay. We find that RecA does indeed physically interact with the UmuD, UmuD′, and the functionally homologous MucA′ mutagenesis proteins and that this interaction appears to target the Umu-like mutagenesis proteins to DNA.

## MATERIALS AND METHODS

**Reagents.** Restriction enzymes, T4 DNA ligase, double-stranded (ds) $\phi$X174 replicative form I DNA, and single-stranded (ss) $\phi$X174 virion DNA were purchased from New England Biolabs; pET11d was from Novagen; DEAE-Sephacel was from Pharmacia; hydroxylapatite and Affi-Gel 15 were from Bio-Rad; phosphocellulose P-11 was from Whatman; isopropyl $\beta$-D-thiogalactopyranoside (IPTG) was from United States Biochemical; adenosine 5′-[$\gamma$-thio]-triphosphate (ATP[$\gamma$-S]) was from Calbiochem; BA85 nitrocellulose was from Schleicher & Schuell; Immobilon-P membrane was from Millipore; and the chemiluminescent immunodetection kit was from Tropix (Bedford, MA).

**Overproduction of UmuD′ and MucA′ Mutagenesis Proteins.** We have constructed two vectors that overproduce the UmuD′ mutagenesis protein. The first places a genetically engineered *umuD′* gene under the control of the thermally inducible $\lambda P_L$ promoter. This overproducing vector (pEC42) was constructed by ligating three DNA fragments: (*i*) a 1-kilobase-pair (kb) *Pst* I–*Eco*RI fragment containing the $\lambda P_L$ promoter from pRC23 (18), (*ii*) a 4-kb *Cla* I–*Pst* I fragment from pRW30 (19) that carries the entire *umuC* gene and the 3′ end of the *umuD* gene, and (*iii*) an ≈55-base-pair (bp) *Eco*RI–*Cla* I fragment constructed from two synthetic oligonucleotides, which, when annealed together, generated the 5′ end of the recombinant *umuD′* gene. In addition, the oligonucleotides were synthesized so that an *Nco* I restriction enzyme site was introduced at the start codon of the *umuD′* gene. This enabled us to construct the second UmuD′ overproducing plasmid (pEC48). This plasmid was constructed by ligating a 520-bp partial *Nco* I–*Bgl* II fragment containing the entire *umuD′* gene from pEC42 into *Nco* I–*Bam*HI-digested

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: IPTG, isopropyl $\beta$-D-thiogalactopyranoside; BSA, bovine serum albumin; ss, single-stranded; ds, double-stranded; ATP[$\gamma$-S], adenosine 5′-[$\gamma$-thio]triphosphate.

*To whom reprint requests should be addressed.

pET11d, which places the recombinant *umuD'* gene under the control of an IPTG-inducible T7 RNA polymerase promoter (20).

A MucA' overproducing vector was constructed by ligating an *Nco* I–*Bam*HI fragment containing the *mucA'* and *mucB* genes from pFF441 (21) into the similarly digested pET11d plasmid. Like pEC48, this vector, termed pJH19, places the *mucA'* and *mucB* genes under the direct control of an IPTG-inducible T7 RNA polymerase promoter (20).

**Purification of Mutagenesis Proteins.** UmuD' was purified either from RW36/pEC42 that had been thermally induced (22) or from BL21(DE3)/pEC48 that had been induced with IPTG (20). Purification was similar to that previously described (23) except that the soluble proteins were precipitated with 277 mg of ammonium sulfate per ml (wt/vol).

UmuD protein was purified from RW36/pSB13 (10, 22) based upon the previously described procedure (23) but with the same modifications as noted above for UmuD'.

MucA' was purified from IPTG-induced BL21(DE3)/pLysS/pJH19 cells. Purification of MucA' was identical to that for the functionally homologous UmuD' protein described above except that the soluble MucA' protein was precipitated with 243 mg of ammonium sulfate per ml (wt/vol).

RecA1730 was purified from GY7648/pGY8243 (17) using a slight modification of the procedure previously described (17). Briefly, soluble proteins were precipitated with 243 mg of ammonium sulfate per ml (wt/vol) and applied to a DEAE-Sephacel column. Proteins were eluted with a linear 50–300 mM NaCl gradient and RecA1730-containing fractions were applied to a phosphocellulose P-11 column. Flow-through fractions, which contained most of the RecA1730 protein, were pooled and subsequently applied to a hydroxylapatite column. Proteins were eluted with a linear 20–350 mM sodium phosphate gradient. Fractions containing the highest purity of RecA1730 were stored at −70°C until required.

Wild-type RecA protein was purchased from United States Biochemical.

**Protein Affinity Chromatography.** UmuD and UmuD' mutagenesis proteins or bovine serum albumin (BSA, fraction V, Sigma) were crosslinked to Affi-Gel 15 affinity support beads as described (23). Under these conditions, ≈4–5 mg of protein was crosslinked per ml of support matrix. All three proteins have acidic isoelectric points (pI ≈4.4–5.3). The BSA affinity column therefore serves as a control to identify proteins retained through ionic rather than specific protein–protein interactions. A soluble *E. coli* whole cell extract was obtained from the K-12 strain DE274 [*recA730 lexA*(Def)] (24). This strain was chosen because it is known to express all known RecA functions, including the previously undefined "third" role, constitutively (15). Cells were grown to mid-logarithmic phase (≈2–4 × $10^8$ per ml), harvested, resuspended in RS buffer (Tris·HCl, pH 7.8/10% sucrose), and lysed by sonication. Soluble proteins were precipitated with 611 mg of ammonium sulfate per ml (wt/vol) and collected by centrifugation at 134,000 × *g* for 1 hr. Precipitated proteins were resuspended and dialyzed overnight against R buffer (20 mM Tris·HCl, pH 7.5/50 mM KCl/1 mM EDTA/1 mM dithiothreitol/10% glycerol). The cell extract was applied to either the UmuD, UmuD', or BSA protein affinity columns equilibrated in R buffer. Columns were then washed with 8–10 column volumes of R buffer to remove any loosely associated proteins. Proteins that were retained on the column were eluted with 8–10 column volumes of R buffer containing 150 mM, 250 mM, and 500 mM KCl, respectively. Eluted proteins were separated by SDS/polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, probed with polyclonal RecA antiserum, and visualized using a chemiluminescent assay as described (25).

**DNA Gel Mobility-Shift Assays.** Standard reaction mixtures (10 μl) usually contained 40 ng of ss φX174 DNA, 1.9 μg of RecA, 300 ng of UmuD, UmuD', or MucA', 20 mM Tris·HCl (pH 7.5), 50–400 mM NaCl (as indicated), 10 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, and 0.5 mM ATP[γ-S]. Nucleoprotein complexes were formed by incubating the reaction mixtures at 37°C for 20 min. Protein–DNA complexes were chemically crosslinked at room temperature for 10 min in the presence of glutaraldehyde (final concentration, 0.01%). Complexes were separated by electrophoresis in a 0.9% agarose gel. Where indicated, DNA–protein complexes were visualized after staining with ethidium bromide. The relative mobility of the UmuD, UmuD', and MucA' proteins was analyzed by transferring the DNA–protein complex to an Immobilon-P membrane. Membranes were incubated with a 1:20,000 dilution of either polyclonal UmuD/D' or MucA antiserum, and proteins were visualized by the chemiluminescent assay as described (22).

## RESULTS

**Protein Affinity Chromatography.** Affinity chromatography is a powerful tool to study protein–protein interactions. For example, using a UmuD/D' protein affinity column, we have previously demonstrated an interaction between the UmuD/D' and UmuC mutagenesis proteins (23). In this work, we have used a similar approach except that we have used soluble whole cell extracts instead of fractions enriched for specific proteins. Most cellular proteins were not retained on any of the three protein affinity columns (unpublished data). A few proteins do, however, appear to be specifically retained on the UmuD and UmuD' columns but not the control BSA column; we have yet to identify all of these proteins.

An interaction between RecA and UmuD has been inferred from RecA's ability to mediate UmuD cleavage (10, 12). We explored this possibility further by probing the affinity column fractions with polyclonal antiserum to RecA protein. Although most of the RecA730 protein was observed in the flow-through fraction (data not shown), some of the RecA730 protein appeared to bind specifically to the UmuD and UmuD' columns to a greater extent than to the BSA column (Fig. 1). Densitometric analysis revealed that between 6- and 7-fold more RecA730 protein was retained on either the UmuD or UmuD' column than on the control BSA column. Interestingly, the bound RecA protein eluted from the UmuD column with a higher salt concentration than from the UmuD' column, suggesting that the interaction between RecA and UmuD is tighter than that of RecA and UmuD'. We believe that the RecA–UmuD/D' interaction is specific since another larger protein (also present in the extract), which crossreacts with the RecA antiserum, appeared to have a completely different specificity to that of RecA. Unlike RecA, the

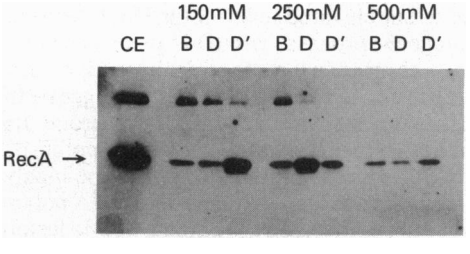


FIG. 1. Detection of RecA protein retained on the BSA, UmuD, and UmuD' protein affinity columns. Fractions were prepared and proteins visualized as described in the text. CE is the soluble whole cell extract from DE274 [*recA730 lexA*(Def)] that was applied to each of three affinity columns: B, BSA; D, UmuD; D', UmuD'. The concentration of KCl that was required to elute the retained proteins from the respective columns is indicated: 150 mM, 250 mM, 500 mM.

unidentified protein appears to have a greater affinity for the BSA column than for either the UmuD or UmuD′ column (Fig. 1).

**DNA Mobility-Shift Assays.** UmuD and UmuD′ proteins have acidic isoelectric points and do not show any significant ability to bind DNA (23). In contrast, RecA protein binds tightly to ssDNA to form spectacular spiral filaments (26). Since the affinity chromatography demonstrated that RecA interacts with UmuD and UmuD′ proteins, we considered the possibility that this interaction might target the Umu proteins to DNA. The formation of RecA–DNA complexes can be conveniently monitored using an agarose-based DNA mobility shift assay (27). An example of this type of assay is shown in Fig. 2. When stained with ethidium bromide, 40 ng of ss $\phi$X174 DNA is clearly visible. Addition of UmuD′ protein does not affect the relative position of this band, confirming that UmuD′ has no detectable affinity for DNA. In contrast, when wild-type RecA is added to the DNA, a RecA–DNA complex forms, and, due to its large size, it has limited mobility in the gel (Fig. 2 *Left*). Further addition of UmuD′ to the reaction mixture gives essentially the same result. In the last experiment, where several proteins are involved, the ethidium-stained gel does not provide any information as to the proteins that form the DNA–protein complex. However, this information can be obtained if the complex is transferred to a support membrane that is subsequently probed with the appropriate antibodies (Fig. 2 *Right*). Under these conditions, one can clearly see that a portion of the UmuD′ protein migrates at a position that is consistent with the formation of a RecA–DNA–UmuD′ complex. Because no discrete product is formed, it has been difficult to estimate the stoichiometry of the RecA–DNA–UmuD′ interaction. Clearly, not all of the UmuD′ protein has an altered mobility. Since in the initial reaction mix, RecA is present in ≈5-fold excess over UmuD′ dimer molecules, the best estimate is that the RecA to UmuD′ stoichiometry is >5:1. This may reflect specific targeting of the UmuD′ protein to particular regions of the DNA or may be simply due to an inability to trap the complex under our assay conditions. Generally, the formation of a slower migrating complex appears to be dependent upon the presence of RecA protein and DNA. The most efficient

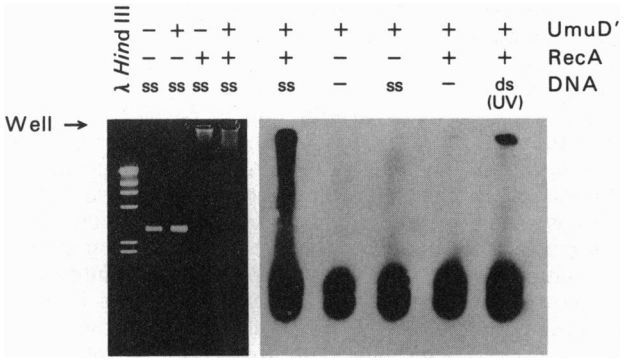


FIG. 2. Analysis of nucleoprotein complex formation using an agarose-based DNA mobility-shift assay. Nucleoprotein complexes were formed as described in the text. After electrophoretic separation in 0.9% agarose, one portion of the gel (*Left*) was visualized after staining with ethidium bromide. The other portion of the gel (*Right*) was transferred to a support membrane and visualized with a chemiluminescent immunoassay. Both parts of the gel were photographed at the same magnification and, consequently, the positions of particular bands in either the ethidium-stained or the transferred portions of the gel are relative to each other. Because of their large size, the nucleoprotein complexes have limited mobility in the agarose gel and are therefore retained at the top of the gel. In contrast, the free protein migrates much more quickly. Individual components in each reaction are indicated above their respective lanes.

complex formation was in the presence of ssDNA, although some complexes were formed with dsDNA that had been irradiated with UV light (600 J·m$^{-2}$) and to a lesser extent with dsDNA (data not shown). In some of the darker film exposures, a background smear, or a slower migrating band, was visible in the absence of either DNA or RecA (data not shown). We have estimated that this accounts for <5% of the total UmuD′ protein present in the reaction mix and possibly reflects nonspecific interactions.

Specific complex formation was not limited to UmuD′; UmuD and the functionally homologous MucA′ proteins form similar complexes (Figs. 3 and 4). The UmuD (D′)–RecA–DNA complex formation was also dependent upon the concentration of NaCl present in the reaction mixtures. Formation of the UmuD–RecA–DNA complex appeared to be slightly more resistant to increasing NaCl concentration than the UmuD′–RecA–DNA complex (compare Figs. 3 and 5 *Upper*). In contrast to UmuD and UmuD′, which generally appeared to form a complex on RecA-coated DNA, MucA′ appeared to interact with RecA in a DNA-independent manner, as judged by the altered mobility of MucA′ in the presence of RecA alone (Fig. 4 *Upper*, tracks 1 and 2). Upon the addition of DNA, however, this MucA′–RecA complex was specifically targeted to DNA (Fig. 4 *Upper*, tracks 3–7). Unlike the Umu–RecA–DNA complex, the MucA′–RecA–DNA complex appeared to be relatively unaffected by the NaCl concentration in the reaction mixtures (Fig. 4 *Upper*). Based upon their sensitivities to NaCl concentration, it would appear that an interaction between MucA′–RecA–DNA > UmuD–RecA–DNA > UmuD′–RecA–DNA. Even though these complexes were resistant to moderate ionic strength, they were not resistant to the physical forces of gel electrophoresis and could only be visualized if the proteins were chemically crosslinked with glutaraldehyde before electrophoresis (unpublished data). Presumably, in the cell, these protein–DNA complexes would not be subject to such physical forces and/or additional ancillary factors such as the UmuC/MucB proteins might serve to stabilize the interactions.

When we substituted RecA1730, a mutant that is partially defective for nucleoprotein filament formation (17), for wild-type RecA, very little UmuD′ was targeted to DNA (Fig. 5 *Lower*, compare tracks 2 and 3). One interpretation of this result is that formation of a nucleoprotein filament is a critical requirement for UmuD′–RecA–DNA complex formation, although other defects in RecA1730 activity cannot be excluded. In contrast to UmuD′, MucA′ appeared to be targeted to DNA by RecA1730 with moderate efficiency (Fig. 4 *Lower*, compare tracks 2 and 3). These results possibly reflect a tighter interaction between these proteins or perhaps

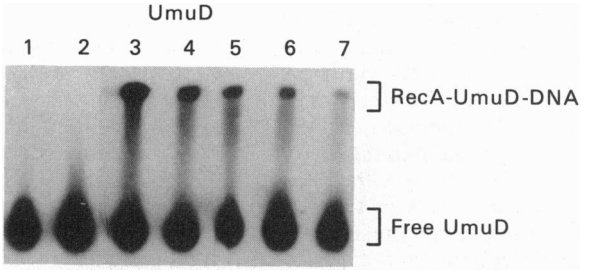


FIG. 3. Influence of NaCl on the formation of UmuD–RecA–DNA nucleoprotein complexes. Complexes were separated and the UmuD protein was detected by the chemiluminescent assay. The numbers above the respective tracks refer to individual reaction conditions: 1, UmuD alone; 2, UmuD plus RecA; 3–7, UmuD plus RecA and ss $\phi$X174 DNA. Tracks 1–3, in the presence of 50 mM NaCl; 4, 100 mM NaCl; 5, 200 mM NaCl; 6, 300 mM NaCl; and 7, 400 mM NaCl.

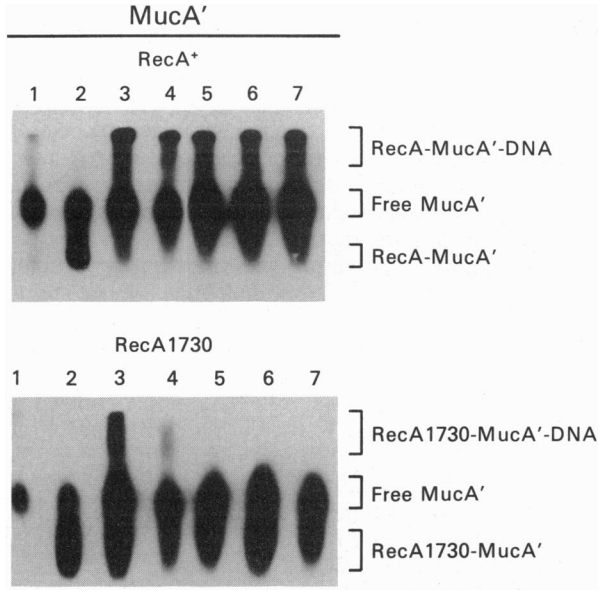


FIG. 4. Ability of wild-type RecA protein or the mutant RecA1730 protein to target MucA' to DNA. (*Upper*) MucA'–RecA$^+$–DNA nucleoprotein complexes. (*Lower*) MucA'–RecA1730–DNA nucleoprotein complexes. As with Fig. 3, in both experiments the numbers above the respective tracks refer to individual reaction conditions: 1, MucA' alone; 2, MucA' plus RecA$^+$ or RecA1730; 3–7, MucA' plus RecA$^+$ or RecA1730 and ss $\phi$X174 DNA. Tracks 1–3, in the presence of 50 mM NaCl; 4, 100 mM NaCl; 5, 200 mM NaCl; 6, 300 mM NaCl; and 7, 400 mM NaCl.

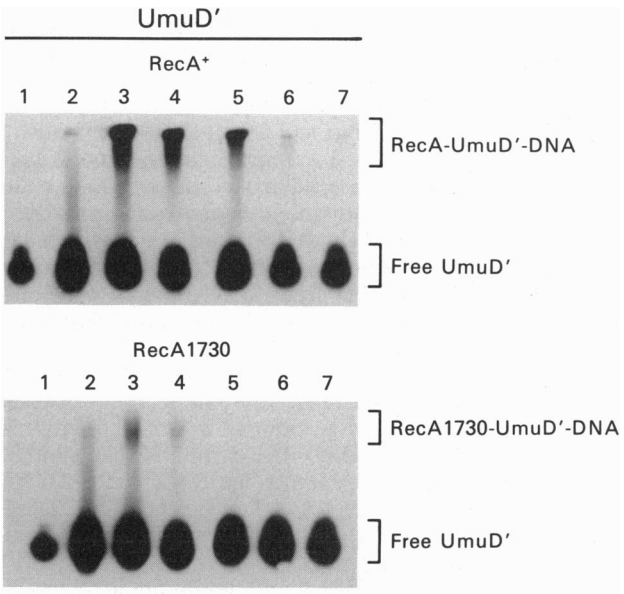


FIG. 5. Ability of wild-type RecA protein or the mutant RecA1730 protein to target UmuD' to DNA. (*Upper*) UmuD'–RecA$^+$–DNA nucleoprotein complexes. (*Lower*) UmuD'–RecA1730–DNA nucleoprotein complexes. As with Figs. 3 and 4, in both experiments the numbers above the respective tracks refer to individual reaction conditions: 1, UmuD' alone; 2, UmuD' plus RecA$^+$ or RecA1730; 3–7, UmuD' plus RecA$^+$ or RecA1730 and ss $\phi$X174 DNA. Tracks 1–3, in the presence of 50 mM NaCl; 4, 100 mM NaCl; 5, 200 mM NaCl; 6, 300 mM NaCl; and 7, 400 mM NaCl.

MucA'–RecA–DNA complex formation is less sensitive to the filament structure of RecA than UmuD'.

## DISCUSSION

**Interaction of RecA with UmuD and UmuD' Mutagenesis Proteins.** The fact that RecA mediates the posttranslational cleavage of UmuD and that *recA718* mutants require active UmuDC proteins for the resumption of DNA synthesis after DNA damage implies a direct interaction between the RecA and UmuDC proteins (10–12, 28). Indeed, Freitag and McEntee (29) have previously demonstrated that radiolabeled UmuC in crude cell extracts is specifically retained on a RecA protein affinity column. UmuD' protein was, however, retained only in the presence of UmuC, suggesting that it interacted with RecA via UmuC (29). We have also used protein affinity chromatography to study these protein–protein interactions. In contrast to the results of Freitag and McEntee, we found that RecA730 protein in crude cell extracts was retained on the UmuD and UmuD' columns. Since the extract was prepared from a *umuC*$^+$ strain, we considered the possibility that RecA was interacting with UmuD and UmuD' via UmuC protein. Using the same approach as that for RecA, the column fractions were probed with UmuC antibodies. However, we were unable to detect any UmuC protein in the column fractions (data not shown). These results imply either that UmuD and UmuD' interact directly with RecA or that the interaction is through some unidentified factor present in the crude extract. Our subsequent DNA band-shift experiments with the purified proteins suggest that the RecA–UmuD(D') interaction is DNA dependent (Figs. 2 and 3). It is likely therefore that the coupling factor present in the crude extract is in fact cellular DNA.

**Targeting of the Umu-Like Mutagenesis Proteins to DNA.** The significance of the RecA–UmuD(D') interaction was demonstrated using an agarose-based DNA mobility-shift assay. Neither UmuD nor UmuD' usually has any affinity for DNA. In the presence of RecA protein, however, a significant portion of the UmuD and UmuD' proteins migrated at a position that was consistent with an interaction with the RecA–DNA complex. The extent of the complex formation was dependent upon the type of DNA used (ssDNA > UV-irradiated dsDNA > dsDNA) and correlates with the ability of RecA to bind to these DNAs (27). This interaction therefore provides a mechanism by which the UmuD/D' proteins are targeted to DNA. Interestingly, the UmuD–RecA–DNA complex appeared to be slightly more resistant to increasing ionic strength compared to the UmuD'–RecA–DNA complex. This finding is consistent with the affinity chromatography data that suggested that a UmuD–RecA interaction is tighter than a UmuD'–RecA interaction. Although we have not performed any direct competition experiments, it is conceivable that under physiological conditions the mutagenically inactive UmuD protein might compete with the mutagenically active UmuD' protein for potential binding sites on RecA protein. If this were indeed the case, our findings might offer an explanation for the observations of Battista *et al.* (30), who found that overproduction of a noncleavable UmuD protein is antimutagenic.

The UmuDC proteins of *E. coli* belong to a family of mutagenesis proteins that are structurally and functionally related (3). To determine if the RecA–DNA–UmuD' interaction might provide a general mechanism for SOS mutagenesis, we analyzed the ability of RecA to target the functionally related MucA' protein to DNA. In contrast to the UmuD'–RecA interaction, which appeared to be largely DNA dependent, some MucA' appeared to interact with RecA in a DNA-independent manner (Fig. 4). In the presence of DNA, however, the MucA'–RecA complex was specifically targeted to DNA. Unlike the UmuD'–RecA–DNA complex, which appeared to dissociate at moderate salt concentrations (200–300 mM NaCl) (Fig. 3), the MucA'–RecA–DNA complex was largely unaffected by high salt conditions (400 mM NaCl) (Fig. 4), suggesting that MucA' has a higher affinity for the RecA nucleoprotein filament than UmuD'. Indeed, preliminary results from competition experiments

suggest that MucA′ does have greater affinity for binding sites on the RecA–DNA filament than UmuD′ (unpublished data). The MucAB proteins are usually more proficient at promoting mutagenesis functions than the related UmuDC proteins (31). We have recently speculated that this difference can be attributed to more efficient posttranslational processing of MucA protein compared to UmuD (22). However, based upon the above observations, it appears that the high affinity of MucA′ for RecA also contributes to the enhanced mutagenic potential of the MucAB proteins.

Is this interaction the third role of RecA in SOS mutagenesis? To address this question directly, we have utilized a mutant of RecA, RecA1730, that is selectively defective for the third role in the presence of the UmuD′C proteins but is partially proficient for mutagenesis in the presence of the functionally homologous MucA′B proteins (16). Comparison of wild-type RecA and RecA1730 proteins revealed that RecA1730 targeted UmuD′ to DNA much less efficiently than RecA$^+$ (Fig. 5 *Lower*, compare tracks 2 and 3). RecA1730 was able, however, to efficiently target MucA′ to DNA (Fig. 4 *Lower*, compare tracks 2 and 3). Our findings are therefore consistent with the hypothesis that targeting of the Umu-like protein complex to DNA is the direct role of RecA in SOS mutagenesis (15–17).

Several lines of evidence suggest that the third role of RecA in SOS mutagenesis requires the formation of a nucleoprotein filament or the so-called "activated state." Sweasy *et al.* (15) found that the ability of certain *recA* mutants to promote the third role correlated with their ability to become "activated." Furthermore, recent x-ray crystallographic studies suggest that the potential binding site for the LexA/UmuD/MucA proteins is a pocket formed between two RecA monomers (32). RecA1730 has a mutation in this pocket that apparently affects its ability to form nucleoprotein filaments (6, 17) and, as we have demonstrated here, it is also unable to target UmuD′ to DNA. Two of the studies that have suggested that the third role of RecA does not necessarily require activation used the MucA′B proteins (21, 22). The data presented here suggest that MucA′ has a higher affinity for RecA than UmuD′. It is possible that the limited amount of activated RecA protein that is always present in the cell is sufficient to promote the third role in conjunction with MucA′B or when the UmuD′C proteins are expressed at artificially high levels (33) but not when the UmuD′C proteins are expressed at more physiological levels. Although it has been possible to separate RecA's roles in SOS mutagenesis genetically, it seems that in reality it only has one function: the formation of nucleoprotein filaments that the repressor and mutagenesis proteins might have evolved to recognize as indicators of cellular stress (3).

Based upon previous data, as well as that presented here, it is possible to hypothesize upon the timing of events that lead to SOS mutagenesis. A prerequisite would be the formation of the RecA nucleoprotein filament, possibly at regions of ss-DNA generated when DNA polymerase III encounters a lesion (34) or perhaps by RecA binding directly to the damaged DNA (27, 35). The LexA and UmuD proteins recognize and bind to this structure, leading to their posttranslational cleavage, and, in the case of UmuD, its mutagenic activation. At this point we envisage that one of two possibilities might occur. Because UmuD′ binds less tightly to RecA than the mutagenically inactive UmuD protein, it could dissociate from the RecA filament and, as a consequence, no mutagenesis would occur. Alternatively, UmuD′ may remain bound to the filament long enough for its cognate partner, the UmuC mutagenesis protein, to bind to the UmuD′–RecA–DNA complex, to form a "mutasome" (23). In this way, all of the mutagenesis proteins would be correctly positioned for their interaction with DNA polymerase III holoenzyme and their subsequent role in translesion DNA synthesis (14). Although the work of Freitag and McEntee (29) suggests that UmuC and RecA directly interact, it seems unlikely that a UmuC–RecA interaction would be the first step in the mutagenic process. If this were the case, one might expect UmuC to influence UmuD cleavage. The rate of UmuD cleavage *in vivo* is, however, unaffected by the presence or absence of UmuC (36). In addition, UmuD is far more abundant and stable than UmuC (25, 37). Given these observations, we favor the hypothesis that the first step in the mutagenic process is the binding of UmuD(D′) to RecA followed by the addition of UmuC. Further biochemical studies with the purified mutagenesis proteins should address this hypothesis directly.

We dedicate this paper to Hatch Echols, who died recently. Without his guidance, inspiration, and pioneering work over the years much of the data presented here would not have been possible.

We are extremely grateful to Adriana Bailone and Raymond Devoret for sending us the RecA1730 overproducing strain; Hatch Echols for the UmuD overproducing strain and antibodies to UmuD/D′; Martin Wojciechowski for antibodies to RecA protein; Konstantin Chumakov for synthesizing oligonucleotides; Steven Slater and Russell Maurer for plasmid pFF441; Joseph Shiloach for fermentor services; Don Ennis for comments on the manuscript; and Bryn Bridges and Steve West for helpful suggestions.

1. Walker, G. C. (1984) *Microbiol. Rev.* **48**, 60–93.
2. Witkin, E. M. (1976) *Bacteriol. Rev.* **40**, 869–907.
3. Woodgate, R. & Sedgwick, S. G. (1992) *Mol. Microbiol.* **6**, 2213–2218.
4. Echols, H. & Goodman, M. F. (1990) *Mutat. Res.* **236**, 301–311.
5. Bridges, B. A., Mottershead, R. P. & Sedgwick, S. G. (1976) *Mol. Gen. Genet.* **144**, 53–58.
6. Dutreix, M., Moreau, P. L., Bailone, A., Galibert, F., Battista, J. R., Walker, G. C. & Devoret, R. (1989) *J. Bacteriol.* **171**, 2415–2423.
7. Hagensee, M. E., Timme, T., Bryan, S. K. & Moses, R. E. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4195–4199.
8. Kato, T. & Shinoura, Y. (1977) *Mol. Gen. Genet.* **156**, 121–131.
9. Steinborn, G. (1978) *Mol. Gen. Genet.* **165**, 87–93.
10. Burckhardt, S. E., Woodgate, R., Scheuermann, R. H. & Echols, H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1811–1815.
11. Nohmi, T., Battista, J. R., Dodson, L. A. & Walker, G. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1816–1820.
12. Shinagawa, H., Iwasaki, H., Kato, T. & Nakata, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1806–1810.
13. Bridges, B. A. & Woodgate, R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4193–4197.
14. Rajagopalan, M., Lu, C., Woodgate, R., O'Donnell, M., Goodman, M. F. & Echols, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10777–10781.
15. Sweasy, J. B., Witkin, E. M., Sinha, N. & Roegner-Maniscalco, V. (1990) *J. Bacteriol.* **172**, 3030–3036.
16. Bailone, A., Sommer, S., Knezevic, J., Dutreix, M. & Devoret, R. (1991) *Biochimie* **73**, 479–484.
17. Dutreix, M., Burnett, B., Bailone, A., Radding, C. M. & Devoret, R. (1992) *Mol. Gen. Genet.* **232**, 489–497.
18. Crowl, R., Seamans, C., Lomedico, P. & McAndrew, S. (1985) *Gene* **38**, 31–38.
19. Woodgate, R. (1992) *Mutat. Res.* **281**, 221–225.
20. Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorf, J. W. (1990) *Methods Enzymol.* **185**, 60–89.
21. Slater, S. C. & Maurer, R. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1251–1255.
22. Hauser, J., Levine, A. S., Ennis, D. G., Chumakov, K. M. & Woodgate, R. (1992) *J. Bacteriol.* **174**, 6844–6851.
23. Woodgate, R., Rajagopalan, M., Lu, C. & Echols, H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7301–7305.
24. Ennis, D. G., Fisher, B., Edmiston, S. & Mount, D. W. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3325–3329.
25. Woodgate, R. & Ennis, D. G. (1991) *Mol. Gen. Genet.* **229**, 10–16.
26. Heuser, J. & Griffith, J. (1989) *J. Mol. Biol.* **210**, 473–484.
27. Lu, C. & Echols, H. (1987) *J. Mol. Biol.* **196**, 497–504.
28. Witkin, E. M., Roegner-Maniscalco, V., Sweasy, J. B. & McCall, J. O. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6804–6809.
29. Freitag, N. & McEntee, K. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8363–8367.
30. Battista, J. R., Ohta, T., Nohmi, T., Sun, W. & Walker, G. C. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7190–7194.
31. Blanco, M., Herrera, G. & Aleixandre, V. (1986) *Mol. Gen. Genet.* **205**, 234–239.
32. Story, R. M., Weber, I. T. & Steitz, T. A. (1992) *Nature (London)* **355**, 318–325.
33. Bates, H. & Bridges, B. A. (1991) *Biochimie* **73**, 485–490.
34. Sassanfar, M. & Roberts, J. W. (1990) *J. Mol. Biol.* **212**, 79–96.
35. Rosenberg, M. & Echols, H. (1990) *J. Biol. Chem.* **265**, 20641–20645.
36. Koch, W. H., Ennis, D. G., Levine, A. S. & Woodgate, R. (1992) *Mol. Gen. Genet.* **233**, 443–448.
37. Donnelly, C. E. & Walker, G. C. (1992) *J. Bacteriol.* **174**, 3133–3139.