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Modulation of RecA Nucleoprotein Function by the Mutagenic UmuD′C Protein Complex*

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The RecA, UmuC, and UmuD′ proteins are essential for error-prone, replicative bypass of DNA lesions. Normally, RecA protein mediates homologous pairing of DNA. We show that purified Umu(D′)$_2$C blocks this recombination function. Biosensor measurements establish that the mutagenic complex binds to the RecA nucleoprotein filament with a stoichiometry of one Umu(D′)$_2$C complex for every two RecA monomers. Furthermore, Umu(D′)$_2$C competitively inhibits LexA repressor cleavage but not ATPase activity, implying that Umu(D′)$_2$C binds in or proximal to the helical groove of the RecA nucleoprotein filament. This binding reduces joint molecule formation and even more severely impedes DNA heteroduplex formation by RecA protein, ultimately blocking all DNA pairing activity and thereby abridging participation in recombination function. Thus, Umu(D′)$_2$C restricts the activities of the RecA nucleoprotein filament and presumably, in this manner, recruits it for mutagenic repair function. This modulation by Umu(D′)$_2$C is envisioned as a key event in the transition from a normal mode of genomic maintenance by "error-free" recombinational repair, to one of "error-prone" DNA replication.

DNA lesions that impede replication pose a severe threat to cellular survival. Much of our basic understanding of the ways that DNA damage is repaired or endured comes from bacterial systems. Exposure of *Escherichia coli* to factors that damage DNA or interfere with DNA replication induces the SOS regulon (1, 2). This global stress response initially induces enzymes that function in the high fidelity modes of excision and recombinational repair; however, when damage is too extensive to be corrected by such conventional means, a mutagenic process is activated that permits replicative synthesis past lesions but in an error-prone manner (3). The products of the *umuC* and *umuD* genes are essential to this translesion synthesis (4, 5). Although DNA polymerase III itself is capable of some misincorporation (6), the UmuC and UmuD′ proteins facilitate continued extension from the mispaired base (7), and very recent data suggest that UmuC and UmuD′ proteins directly affect nucleotide mis-insertion (8) as well.

Expression from the *umuDC* operon is induced when RecA protein is activated by single-stranded regions within damaged DNA to stimulate autodigestion of the LexA protein, the transcriptional repressor of SOS genes (3). In addition to this regulatory function, "activated" RecA protein is also responsible for proteolytic processing of the inactive UmuD protein to yield the mature UmuD′ product, which is essential for mutagenesis (9–11). Homodimers of the UmuD′ polypeptide associate with UmuC protein to form a mutagenically active Umu(D′)$_2$C complex that compels DNA polymerase III to perform translesion synthesis (12). The observation that a *recA* deficient strain expressing UmuD′ and all other SOS-controlled proteins at constitutive levels remains nonmutable revealed that, besides mediating derepression of the *umu* genes and maturation of the UmuD protein, activated RecA protein has a more direct role in the mutagenic process (11, 13, 14).

The current prevailing model suggests that RecA protein targets a limiting number of Umu(D′)$_2$C complexes to lesions within DNA, thereby ensuring productive encounters with DNA polymerase III (14–17). The mutagenic complex serves as a specialized elongation factor that assists polymerase in the bypass process (8, 18–20). Evaluation of this proposal was previously hindered due to the inherent insolubility of the UmuC protein. Recently, this imitation was overcome by the efficient purification of soluble intact Umu(D′)$_2$C complex without the need for denaturing conditions (21) and by the subsequent reconstitution *in vitro* of lesion bypass that is dependent on the Umu(D′)$_2$C mutagenic complex and RecA protein (8).

In addition to its role in SOS induction and mutagenesis, RecA protein promotes the homologous pairing and exchange of DNA, a process that is fundamental to genetic recombination (22). To promote DNA strand exchange, RecA protein assembles on ssDNA[1] to form a nucleoprotein filament known as the presynaptic complex; this filament then seeks homologous double-stranded DNA (dsDNA). Although the roles of RecA protein in genetic recombination and in the induction of cellular responses to DNA damage require remarkably different activities, they share a common prerequisite: assembly of an active nucleoprotein complex. Previous studies established that Umu(D′)$_2$C overexpression antagonizes both conjugal recombination *in vivo* (23, 24) and homologous DNA pairing assayed on membranes using crude bacterial extracts (25). Devoret and colleagues (23, 24) hypothesized that the Umu(D′)$_2$C proteins might bind the tip of a growing RecA protein filament, thereby both blocking RecA protein function and delivering Umu(D′)$_2$C to the DNA lesion. Thus, it is likely that the mutagenic-targeting function of RecA protein involves the binding of Umu(D′)$_2$C

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[1] The abbreviations used are: ssDNA, single-stranded DNA; dsDNA double-stranded DNA; ATPγS, adenosine 5′-[thio] triphosphate; SSB, single-stranded DNA binding.

to the functional form of RecA protein and the redirection of the function of this filament to assist with mutagenic lesion bypass. To further our understanding of the initial steps in mutagenic bypass, the interaction between the purified Umu(D′)$_2$C complex and RecA protein was examined. Here we establish that the Umu(D′)$_2$C complex can bind along the entire length of the RecA nucleoprotein filament.

EXPERIMENTAL PROCEDURES

Proteins—RecA protein was purified using a preparative protocol based on spermidine acetate precipitation (26) in combination with polyethyleneimine precipitation (27). SSB protein was prepared as described (28). Concentrations of RecA and SSB proteins were determined using extinction coefficients at 280 nm of 2.7 and 3.0×10^4 M^{-1} cm^{-1}, respectively. The Umu(D′)$_2$C complex was purified as previously reported (21) with modifications to reduce minor contaminating ATPase, nuclease, and DNA polymerase activities (8).

Biosensor Binding Experiments—RecA nucleoprotein filaments were assembled on a 5′-biotinylated oligomer (48 nucleotides in length derived from an M13mp7 sequence) that was immobilized through avidin to the carboxymethylated dextran surface of an IAsys cuvette. The two different saturated RecA-ssDNA complexes containing 1× and 2× amount of RecA protein were prepared in reaction buffer comprised of 25 mM Tris acetate, pH 7.5, 0.1 mM dithiothreitol, 500 μM ATPγS, and either 1 or 10 mM magnesium acetate, respectively; these two different magnesium acetate concentrations enable formation of stoichiometric RecA protein-ssDNA complexes that differ by 2-fold in RecA protein content (29). After each addition of Umu(D′)$_2$C, the cuvette was washed with reaction buffer containing 2 M NaCl and again with reaction buffer to eliminate nonspecific interactions.

LexA Repressor Cleavage Assays—Assays were conducted as described previously (30). Reactions contained 1 mM ATP, 6 μM M13mp7 ssDNA, and 1 μM RecA protein and were initiated by the simultaneous addition of 5, 10, or 20 μM of the LexA repressor and Umu(D′)$_2$C.

DNA Strand Exchange Assays—The agarose gel assay for DNA strand exchange was conducted as described previously (31). Reactions contained 5 μM M13mp7 ssDNA, 3 μM RecA protein, 0.45 μM SSB, protein and 9 μM M13mp7 dsDNA that was linearized with *Eco*RI restriction endonuclease and radiolabeled at the 5′ end. Reactions were initiated by the simultaneous addition of the dsDNA and Umu(D′)$_2$C. Quantitation was done using a Molecular Dynamics Storm system.

RESULTS

The RecA Nucleoprotein Filament Is a Scaffold for Stoichiometric Binding of the Umu(D′)$_2$C Complex—The binding of Umu(D′)$_2$C complex to a RecA nucleoprotein filament was measured directly using a biosensor instrument by monitoring the change in refractive index due to the accompanying increase in mass. In Fig. 1, two nucleoprotein filaments saturating at different concentrations of RecA protein (designated 1× and 2×) were each titrated with mutagenic complex; the filaments were formed using ATPγS, resulting in a filament that is irreversibly bound to the ssDNA and, hence, nondissociable. In both reactions, the change in refractive index (mass) increased linearly with addition of Umu(D′)$_2$C complex until it approached an apparent plateau; the change in mass upon saturation of the nucleoprotein filament is proportional to the amount of initially bound RecA protein. Thus, the Umu(D′)$_2$C complex binds to the RecA nucleoprotein filament with a defined stoichiometry. Given that the molecular mass of the Umu(D′)$_2$C complex (~ 71.4 kDa) is 1.8-fold greater than the RecA protein (~ 38.7 kDa), if each mutagenic complex interacted with a single RecA monomer, the refractive index increase upon saturation by Umu(D′)$_2$C would be 1.8 times that of the RecA nucleoprotein filament; if it interacted with only the tip of each filament, the increase would be negligible. Fig. 1 demonstrates that, at the equivalence point, Umu(D′)$_2$C complex adds about 0.9-fold more mass than present at the start (the gradual, further increase is nonspecific binding to the surface,[2] suggesting a stoichiometry of 2 RecA protein monomers/Umu(D′)$_2$C complex. This binding was further confirmed by gel electrophoretic, band-shift experiments.[3]

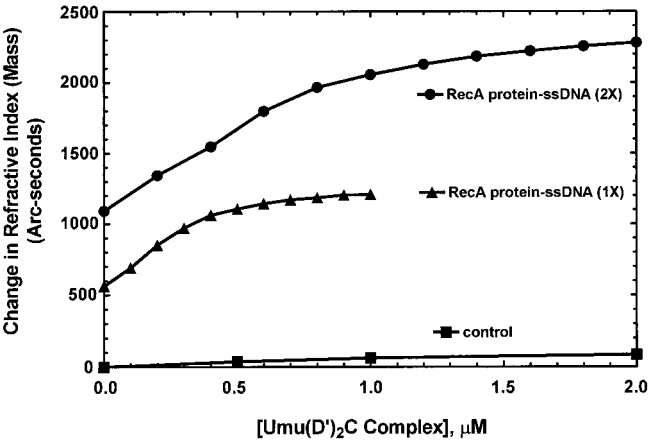

FIG. 1. **Stoichiometric binding of the Umu(D′)$_2$C complex to the RecA nucleoprotein filament.** Two different saturated ATPγS-RecA-ssDNA nucleoprotein complexes were prepared (see "Experimental Procedures"), containing amounts of bound RecA protein that differed by 2-fold (labeled *1X* and *2X*). The control represents the level of nonspecific binding to the dextran surface.

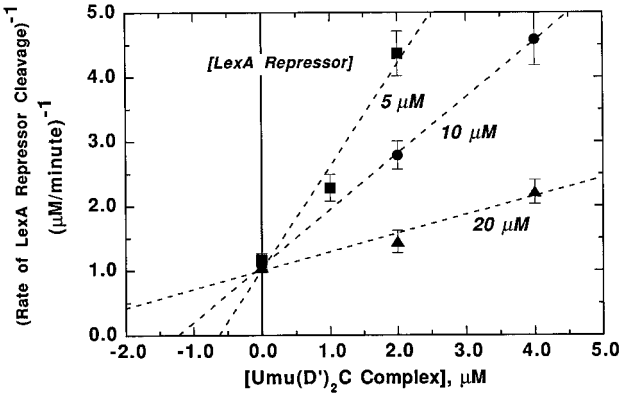

FIG. 2. **Competitive inhibition of LexA repressor proteolysis by the Umu(D′)$_2$C complex implies that Umu(D′) $_2$C binds to the large groove of the RecA nucleoprotein filament.** The linear functions fitting the data sets at different repressor concentrations define an apparent K_i that is less than 0.5 μM.

The Umu(D′)$_2$C Complex Likely Binds within the Effector-binding Groove of the RecA Nucleoprotein Filament—Given the stoichiometry of the Umu(D′)$_2$C-RecA protein interaction, it is reasonable to expect that the most likely binding site would be the deep groove of the RecA nucleoprotein filament. This groove is large and is the site for binding of other cellular effectors such as LexA protein (32). If the Umu(D′)$_2$C complex binds to the same site, then its binding to the RecA nucleoprotein filament would be mutually exclusive with LexA repressor binding. To potentially map the interaction site of Umu(D′)$_2$C binding, the effect of the mutagenic complex on repressor cleavage was assayed. The rate of LexA repressor proteolysis stimulated by RecA protein decreased with increasing amounts of Umu(D′)$_2$C complex (Fig. 2). Moreover, at a constant amount of the mutagenic complex, increasing concentrations of LexA repressor result in higher rates of proteolytic digestion. These characteristics suggest that the Umu(D′)$_2$C complex acts competitively to inhibit RecA protein-promoted cleavage of LexA repressor (with a K_i of less than 0.5 μM). The simplest inter-

[2] W. M. Rehrauer, I. Bruck, R. Woodgate, M. F. Goodman, and S. C. Kowalczykowski, unpublished observations.

[3] E. G. Frank, N. Cheng, I. Bruck, E. H. Egelman, M. F. Goodman, A. C. Steven, and R. Woodgate, unpublished observations.

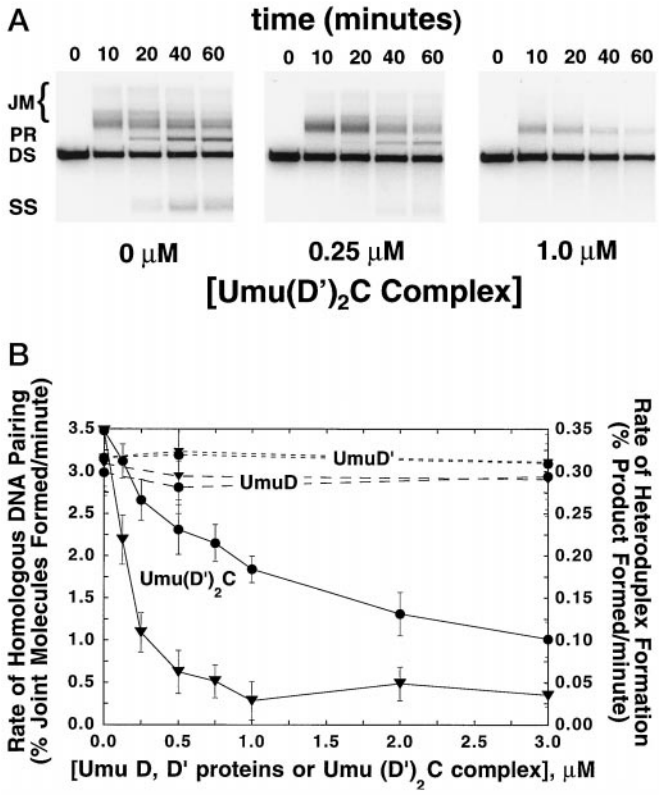

FIG. 3. **The mutagenic Umu(D′)$_2$C complex inhibits DNA strand exchange promoted by RecA protein.** *A*, time courses for RecA protein-promoted DNA strand exchange in the absence or presence of 0.25 and 1.0 μM of the Umu(D′)$_2$C complex. Electrophoretic separation of linear M13mp7 dsDNA (DS) and circular M13mp7 ssDNA (SS) from both homologously paired joint molecules (JM) and the DNA heteroduplex product gapped circular M13mp7 dsDNA (PR). *B*, rates of homologous DNA pairing and the complete DNA heteroduplex extension plotted as a function of Umu(D′)$_2$C concentration. ●, homologous DNA pairing; ▼, complete heteroduplex.

pretation is that the mutagenic complex binds to the RecA nucleoprotein filament at the same or at an overlapping site as LexA repressor, although an alternative interpretation is that allosteric changes restrict binding of LexA repressor. The former conclusion is supported by direct visualization of the RecA protein-Umu(D′)$_2$C interaction *via* electron microscopy.[3]

Association of Umu(D′)$_2$C with the RecA Nucleoprotein Complex Prevents Its Participation in DNA Strand Exchange—The binding of Umu(D′)$_2$C complex to this site, the deep groove of the RecA filament, should occlude an interaction between the filament and homologous dsDNA, a situation similar to that established for the uncleavable LexA protein (33). To test this supposition, the effect of the Umu(D′)$_2$C complex on the recombination function of RecA protein was tested. The mutagenic complex indeed inhibits DNA strand exchange between homologous circular ssDNA and linear dsDNA; increasing amounts of the Umu(D′)$_2$C complex cause a greater decrease in activity (Fig. 3*A*). No decrease in activity is seen in parallel reactions using either UmuD or UmuD′ proteins (Fig. 3*B*), suggesting that the interaction between UmuD or UmuD′ and the RecA nucleoprotein filament is much weaker than that of the Umu(D′)$_2$C complex.

Quantitative analysis of this *in vitro* reaction reveals that the stages of initial joint molecule formation and DNA heteroduplex extension display different sensitivities to the mutagenic complex (Fig. 3*B*). Nascent joint molecule formation slows appreciably over the range of 1.0–2.0 μM of Umu(D′)$_2$C, concentrations that are roughly equivalent to the amount of RecA protein (1.7 μM) present as part of the presynaptic complex. In comparison, the complete exchange of DNA strands to yield a circular gapped heteroduplex product is far more susceptible, approaching maximum inhibition at levels that are approximately 4-fold lower (0.30 μM Umu(D′)$_2$C). The strong inhibition of DNA heteroduplex extension, maximal at Umu(D′)$_2$C concentrations of 0.25–5.0 μM, shows that RecA protein recombination function is impeded at even subsaturating amounts of Umu(D′)$_2$C.

Inhibition of DNA strand exchange by Umu(D′)$_2$C varies directly with the amount of RecA protein and ssDNA (data not shown). The inhibition of homologous pairing and exchange of DNA strands is not dependent on SSB protein,[2] a characteristic that both distinguishes the effects of Umu(D′)$_2$C proteins from those of LexA repressor (30) and is in precise agreement with the physiological consequences of SSB protein overproduction *in vivo* (34). Furthermore, Umu(D′)$_2$C affects the ssDNA-dependent ATP hydrolysis activity of the RecA protein minimally.[2] Collectively, these data argue that a specific interaction between the Umu(D′)$_2$C complex and the RecA protein nucleoprotein filament is responsible for abrogating recombination function and, consequently, providing DNA polymerase III with a scaffold decorated with Umu(D′)$_2$C that can be used for mutagenic bypass.

DISCUSSION

We have established that the mature mutagenesis complex Umu(D′)$_2$C binds directly to the activated form of RecA protein, the nucleoprotein filament that assembles on ssDNA, and that the binding site of this complex is likely to be the deep groove between turns of the RecA nucleoprotein helix. We conclude, therefore, that the Umu(D′)$_2$C complexes modify the activity of the RecA nucleoprotein filament to which they are bound, preventing the filament from participating in both LexA repressor cleavage and normal recombination function.

RecA Protein and Its Role in Homologous Recombination, SOS Induction, and SOS Mutagenesis—Although at first glance the participation of RecA protein in each of these three biological processes appears diverse, it is clear that all three processes have the common prerequisite for RecA protein in its so-called activated form. This activated form is the extended nucleoprotein filament that assembles on ssDNA in the presence of ATP or a suitable NTP analog. A major role of RecA protein is to mediate homologous recombinational repair of dsDNA breaks that arise from ever-present cellular metabolites and from interrupted DNA replication during normal cell growth (35). When the cell is exposed to DNA-damaging agents or when it acquires mutations that increase the normal cellular amount of ssDNA, the SOS response is induced and SOS mutagenesis occurs. Thus, in addition to its recombination function, the RecA nucleoprotein filament protein assumes new, cellular functions that are crucial for survival when excessive DNA damage is encountered. However, as we have shown here and previously (30, 33), these functions represent mutually exclusive activities of the filament.

The Umu(D′)$_2$C Binding Site on RecA—The site for dsDNA binding is most likely to be within the deep helical groove of the RecA nucleoprotein filament (36, 37). Previous studies demonstrated that LexA repressor binds to the same site (32) and that such binding inhibits DNA strand exchange (30, 33). We established that the mature mutagenesis complex Umu(D′)$_2$C behaves similarly, binding to the RecA presynaptic filament and saturating it at a stoichiometry of one Umu(D′)$_2$C complex for every two RecA protein monomers. Furthermore, we established that the binding of Umu(D′)$_2$C to the filament inhibits both LexA repressor cleavage and DNA strand exchange but not ATPase activity; thus blockage of these function results from the binding of Umu(D′)$_2$C to nucleoprotein helix rather

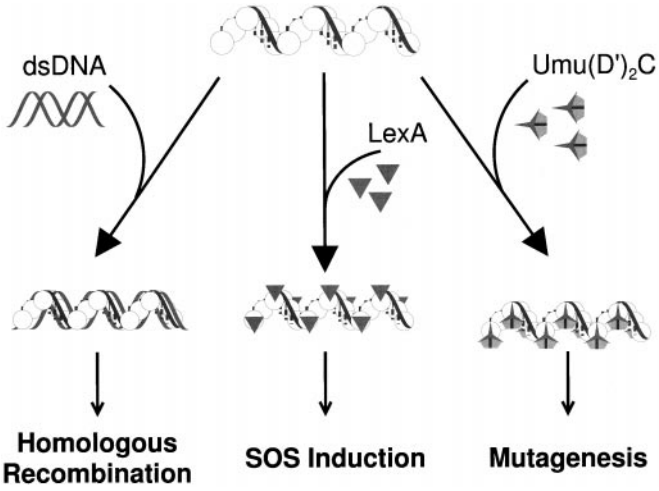

FIG. 4. **Participation of the RecA nucleoprotein filament in the parallel but competing processes of homologous recombination, SOS induction, and mutagenic repair.**

than from displacement of RecA protein from the ssDNA by $Umu(D')_2C$. In agreement, recent data suggest that the precursor of UmuD′ protein, the unprocessed UmuD protein, may also occupy the same site on RecA protein as do dsDNA, LexA repressor, and the $Umu(D')_2C$ complex (38, 39).

Although our studies provide essential insight into the mechanism of SOS-induced mutagenesis, they do not, however, fully explain a critical feature of the so-called "direct role" of RecA protein in this process; it remains unclear how the $Umu(D')_2C$ complex is actually targeted to a DNA lesion so that it can productively encounter the stalled DNA polymerase III required for translesion DNA synthesis. Indeed, if $Umu(D')_2C$ bound randomly and with equal affinity to all sites on the RecA nucleoprotein filament, it is unlikely that the normally limiting amounts of the $Umu(D')_2C$ complex that are produced under physiological conditions *in vivo* (40, 41) would be sufficient to permit appropriate positioning of a $Umu(D')_2C$ complex at a DNA lesion. Such targeting clearly occurs, however, as efficient Umu-dependent translesion synthesis is only observed *in vitro* in the presence of activated RecA (8). One appealing hypothesis is that only the $Umu(D')_2C$ proteins that are bound to the very end of the filament participate in SOS mutagenesis (24), whereas those bound elsewhere along the filament simply inhibit recombination and LexA cleavage. Another possibility is that the RecA nucleoprotein filament assembled on DNA-containing a lesion is somehow different from that formed on undamaged ssDNA; the DNA damage might introduce a distortion into the filament, permitting preferential recognition by the $Umu(D')_2C$ complex and thereby targeting it to a particular location in the nucleoprotein filament, namely, the actual site of the DNA damage.

Multifunctional Roles of RecA Protein—The RecA protein nucleofilament is clearly an adaptable structure that can participate in recombination, SOS induction, or mutagenesis but each in a mutually exclusive manner (Fig. 4). These processes represent branched, competing pathways that are all potentially occurring simultaneously, depending only on the cellular levels of homologous DNA, LexA protein, or $Umu(D')_2C$ complex, respectively, and on their relative affinities for the filament. Thus, when physiological conditions are altered to produce high levels of the $Umu(D')_2C$ complex, recombination is inhibited. Therefore, filament-binding proteins such as $Umu(D')_2C$ can determine the function of the RecA nucleoprotein complex and redirect a filament toward mutagenic repair.

This concept leads to an encompassing view that not every RecA protein monomer need be recruited for mutagenesis, but rather only a few of the filaments are appropriated for mutagenic repair. Both the *in vivo* and *in vitro* data support the idea that a given filament can only perform one of these functions at any given time and that $Umu(D')_2C$ is one of the factors that modifies the function of a subpopulation of nucleofilaments. With RecA protein in excess, the task of $Umu(D')_2C$ is to appropriate an amount of that protein and use it in the mutagenic process, rather than to allow the RecA protein to participate in the (competitive) recombination process.

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