The DinI and RecX Proteins Are Competing Modulators of RecA Function*

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The DinI and RecX proteins of Escherichia coli both modulate the function of RecA protein, but have very different effects. DinI protein stabilizes RecA filaments, preventing disassembly but permitting assembly. RecX protein blocks RecA filament extension, which can lead to net filament disassembly. We demonstrate that both proteins can interact with the RecA filament, and propose that each can replace the other. The DinI/RecX displacement reactions are slow, requiring multiple minutes even when a large excess of the challenging protein is present. The effects of RecX protein on RecA filaments are manifest at lower modulator concentrations than the effects of DinI protein. Together, the DinI and RecX proteins constitute a new regulatory network. The two proteins compete directly as mainly positive (DinI) and negative (RecX) modulators of RecA function.

RecA is the central recombination protein in Escherichia coli and other bacteria, playing an important role in the processes of recombinational DNA repair (reviewed in Refs. 1 and 2). Additionally, RecA regulates the induction of the SOS response by facilitating the autocatalytic cleavage of the LexA repressor protein (3). In these important roles, RecA protein functions as a nucleoprotein filament.

RecA protein filaments are assembled in a process featuring discrete nucleation and end-dependent extension phases (1, 4). Disassembly is also end-dependent, occurring at the opposite end to that at which assembly occurs. Both assembly and disassembly thus proceed 5’ to 3’ on the single-stranded DNA (ssDNA)1 (5–8). Assembly can take place with ATP analogues that are not hydrolyzed by RecA protein, whereas disassembly requires ATP hydrolysis. The inherent rate of extension of RecA filaments during assembly must be faster than end-dependent disassembly or long filaments would never form.

RecA filament assembly and disassembly must be directed in the cell to ensure that RecA activities initiate properly both temporally and spatially. One modulator of these dynamic processes is the SSB protein. The nucleation step of RecA filament assembly, but not the extension phase, is inhibited by SSB (1, 9). RecF, RecO, and RecR proteins play a role in the loading of RecA protein onto SSB-coated ssDNA (6, 10–13). Two additional RecA filament modulators have been identified, RecX and DinI, which are particularly relevant to the SOS response.

The RecX protein is an inhibitor of RecA-mediated DNA strand exchange, coprotease, and DNA-dependent ATP hydrolysis activities (14, 15). In vitro, the RecX protein effectively blocks RecA filament extension, probably employing a capping mechanism (15). RecX also binds along the length of the RecA filament (16). By blocking assembly while not affecting disassembly, a net disassembly reaction is observed in the presence of RecX (15). The interaction of RecX with RecA is affected by the RecA C terminus, as well as by the overall functional state of the RecA filament (17). The recX gene is immediately downstream of the recA gene. The two genes are co-transcribed, although the RecX protein is synthesized at barely detectable levels during vegetative growth (14, 18). Both RecA and RecX are induced during the SOS response leading to the suggestion that RecX mainly acts during SOS (14).

In contrast, the DinI protein stabilizes the RecA filament, reducing or preventing disassembly under most conditions (19). At the same time, DinI has a positive or neutral effect on most RecA activities (19, 20). One exception is the RecA-mediated cleavage of the UmuD protein, which is inhibited by DinI even though the cleavage of LexA protein is not (21). DinI protein acts mainly as a positive modulator of RecA function. Destabilization of RecA filaments and inhibition of RecA function can be observed when DinI is present at a 20–100-fold excess relative to RecA (19, 22), although it is not clear that this effect is physiologically relevant (19). The DinI protein is not detectable during normal growth of the bacterial cells, but like RecX is induced at the beginning of the SOS response (22).

Separately, the RecX and DinI proteins have quite different and opposing effects on RecA filament dynamics. In this study, we set out to determine the effects of the two proteins on RecA filaments when they are added together.

**EXPERIMENTAL PROCEDURES**

Enzymes and Biochemicals—Escherichia coli RecA and SSB proteins were purified as described (23, 24). The concentration of purified RecA and SSB proteins were determined from the absorbance at 280 nm using the extinction coefficients 2.23 × 10^4 M^-1 cm^-1 (25) and 2.83 × 10^4 M^-1 cm^-1 (26), respectively. Native RecX protein was purified as described (17). RecX protein is stored in 20 mM Tris-HCl (80% cation, pH 7.5), 1 mM dithiothreitol, 0.1 mM EDTA, 100 mM potassium glutamate, and 50% (w/v) glycerol. Preparations over 98% pure and free of detectable nuclease activities. The concentration of purified protein was determined from the absorbance at 280 nm using the extinction coefficient 2.57 × 10^4 M^-1 cm^-1 (17).

Unless otherwise noted, reagents were purchased from Fisher, PStI, NdeI, and EcoRI restriction endonucleases were purchased from New England Biolabs. Lysenzyme, streptomycin sulfate, erythromycin, and all components of ATP regeneration and coupling systems were from Sigma. Hydroxyapatite Bio-Gel HRT resin was from Bio-Rad. Source 15Q and Sephacryl S-100 resins were purchased from Amersharm Biosciences.

Buffers and Media—P buffer contained 20 mM potassium phosphate
Cloning and Overexpressing the DinI Protein—The dinI gene was isolated from the *E. coli* genome (strain MG1655, a gift from George Weinstock (27)) by polymerase chain reaction using DNA primers that added a NdeI restriction site upstream and an EcoRI restriction site downstream of the *dinI* gene. These primers were used to amplify a DNA fragment of the appropriate size (265 bp) by the polymerase chain reaction. The *dinI* gene fragment was digested with NdeI and EcoRI and then ligated into cloning vector pET21A (Novagen), which was digested with NdeI and EcoRI. This plasmid containing the native *dinI* gene under the control of the T7 RNA polymerase promoter was designated pEAW334. The integrity of the entire *dinI* gene in this construct was verified by direct sequencing.

Cloning vector plasmids pET21A and pET21B were transformed with plasmid pEAW334. Ten liters of culture were grown in LB broth to an *A*<sub>600</sub> of 0.5. DinI protein expression was induced by the addition of isopropyl 1-thio-β-D-galactopyranoside to 0.4 mM. Following a 3-h incubation at 37 °C, ~7 g of cells were harvested by centrifugation, flash frozen in liquid N<sub>2</sub>, and stored at −80 °C.

Purification the DinI Protein—The DinI protein was purified by extraction of the pellet (17 g) with 100 ml of Buffer A (25 mM Tris-HCl (80% cation, pH 7.5), 0.1 mM EDTA, and 10% (w/v) glycerol). Cells were lysed by a 60-min incubation with lysozyme (in 250 mM Tris-HCl, 80% cation) at 2.5 mg/ml, followed by the addition of EDTA to 7 mM, sonication, and centrifugation. The lysate was precipitated by incubation of streptomycin sulfate to 5% for 60 min followed by centrifugation. The resulting lysate was precipitated with solid ammonium sulfate to 30% saturation and centrifuged. The DinI protein was precipitated from the supernatant with solid ammonium sulfate to 80% saturation and centrifuged. The pellet was resuspended in Buffer P, dialyzed *versus* P buffer, and loaded onto an hydroxypatite column. The column was washed with 2 column volumes of Buffer P and the protein was eluted with a linear gradient from 20 to 500 mM phosphate over 10 column volumes. Fractions were pooled, and dialyzed *versus* R buffer plus 50 mM potassium chloride. The protein was then loaded onto a Source 15Q column. The column was washed with 2 column volumes of R buffer plus 50 mM potassium chloride and the protein was eluted with a linear gradient of 50–500 mM potassium chloride. Peak fractions were pooled as before, diluted 2-fold with R buffer, and reloaded onto the same Source 15Q column. The protein was then eluted in R buffer plus 500 mM potassium chloride to concentrate the protein. The eluted protein (8 ml) was immediately loaded onto a HR 26/60 Sephacryl S-100 gel filtration column equilibrated in R plus 500 mM potassium chloride and washed with 1 column volume of the same. Peak fractions were pooled and dialyzed into R buffer, flash frozen in liquid N<sub>2</sub>, and stored at −80 °C. The concentration of the DinI protein was determined using the extinction coefficient of 1.41 × 10<sup>4</sup> M<sup>−1</sup> cm<sup>−1</sup> at 280 nm (19). Preparations were greater than 98% pure and contained no exonuclease activities. We have recently detected a very low level endonuclease activity in this and earlier (19) DinI preparations that appears to be specific for supercoiled DNA. Incubation of 20 μM DinI protein (from the preparation used in these experiments) with 20 μM supercoiled plasmid DNA for 90 min at 37 °C results in the linearization of just over 1% of the DNA (data not shown). The activity does not co-elute with DinI protein during gel filtration chromatography, and is thus unlikely to be an activity of DinI. We have detected no effect of this activity on any of the DNAs used in the current experiments.

DNA Substrates—Bacteriophage *dX174* circular ssDNA and replicative form I supercoiled circular duplex DNA were purchased from New England Biolabs and Invitrogen, respectively. Full-length linear duplex DNA (dsDNA) was generated by the digestion of *dX174* replicative form I DNA (5386 bp) with the PstI endonuclease. Circular single-stranded DNA from bacteriophage M13mp8 (7229 nucleotides) was prepared as described (29). Concentrations of ssDNA and double-stranded DNA were determined by absorbance at 260 nm, using 36 and 50 μg ml<sup>−1</sup> A<sub>260</sub> extinction coefficients and conversion factors. DNA concentrations are given in micromolar nucleotide concentrations.

**ATPase Assay**—A coupled spectrophotometric enzyme assay (7, 30) was used to measure DNA-dependent ATPase activities of the RecA protein as described (23). Reactions were carried out at 37 °C in Buffer A (25 mM Tris-OAc (80% cation, pH 7.4), 1 mM dithiorthreitol, 3 mM potassium glutamate, 10 mM Mg(OAc)<sub>2</sub>, and 5% (w/v) glycerol), an ATP regeneration system (10 units/ml pyruvate kinase and 3.5 mM phosphoenolpyruvate), a coupling system (1.5 mM NADH and 10 units/ml lactate dehydrogenase), and 2 μM M13mp8 circular ssDNA. The aforementioned components were incubated with 1.2 μM RecA protein for 10 min. The reaction was initiated by addition of SSB protein (to 0.2 μM), ATP (to 3 mM). Figure legends note the time of addition of the indicated concentrations of DinI and/or RecX proteins and any modifications of the basic protocol.

DNA Three-strand Exchange Reaction—Three-strand exchange reactions were carried out at 37 °C in Buffer A. An ATP regeneration system (10 units/ml creatine phosphokinase and 12 mM phosphocreatine) was also included. The following are final concentrations. RecA protein (3 μM) and DinI protein (10.5 μM) were preincubated with 9 μM *dX174* circular ssDNA for 7 min. SSB protein (0.9 mM) and ATP (3 mM) were added, followed by another 7-min incubation. RecX protein (90 μM) was added to the reactions and incubated for 7 min before the reactions were initiated by the addition of *dX174* linear dsDNA to 9 μM. Reactions were incubated for 60 min, then stopped and analyzed by gel electrophoresis as described (15).

**LexA Cleavage Assay**—Reactions were carried out at 37 °C in Buffer A (except the Mg(OAc)<sub>2</sub>, which was 3 mM and an ATP regeneration system (12 mM phosphocreatine and 10 units/ml creatine phosphokinase). The following are final concentrations. RecA protein (3 μM) and DinI protein (1 or 3 μM) were preincubated with 9 μM *dX174* circular ssDNA for 7 min. ATP (3 mM) was added, followed by another 7-min incubation. RecX was added to 1 μM and incubated for 20 min. LexA protein (3 μM, a gift from John Little) was added to start the reaction. Reactions were incubated for 15 min, then stopped and analyzed as described (15).

**UV Survival Assays**—Strains used were MG1655 and the isogenic derivative, EAW8, which was created by A Red Recombinase (31) integration of our *dinI*::kan disruption from pEAW350 (kan<sup>46</sup> gene inserted into the *dinI* gene of pEAW334 at the BsaAI restriction site) into MG1655. PCR amplification and sequencing confirmed that the *dinI::kan* replaced the *dinI* gene of MG1655. Overnight cultures of strains MG1655 and EAW8 carrying pGCR4 or pGCC4/RecX (14) were diluted into LB-erythromycin-isopropyl 1-thio-β-D-galactopyranoside (final erythromycin and isopropyl 1-thio-β-D-galactopyranoside concentrations are 250 μg/ml and 1 mM, respectively) and grown to early stationary phase (*A*<sub>600</sub> = 1.0), serially diluted, and spot-plated onto LB-erythromycin-isopropyl 1-thio-β-D-galactopyranoside, and allowed to dry for 1 h. Plates were exposed to 0, 25, 50, and 75 J/m<sup>2</sup> UV light (UV Stratalinker 1800, Stratagene), and the survival of the RecX-expressing strain was compared with each vector control strain.

**RESULTS**

**Experimental Design**—Using purified proteins, we explored the combined effects of the DinI and RecX proteins on RecA function. The RecA-mediated ATPase activity was used as the primary assay, providing a real-time measure of the status of the overall RecA filament. As previously demonstrated, changes in ATP hydrolysis seen when DinI or RecX are added to RecA filaments reflect changes in the absolute amount of DNA-bound RecA protein as opposed to changes in the intrinsic ATPase activity of the filaments (15, 19). The effects of DinI and RecX on other RecA functions are also examined, and we make a start in the exploration of RecA and DinI function in vivo.

The DinI and RecX Proteins Have Opposing Effects on RecA Filament Assembly and Disassembly—The primary effects of DinI and RecX proteins are shown in Fig. 1B. When DinI (8.4 μM) was added with RecA (1.2 μM) and circular ssDNA (2 μM) a slight increase in the rate of ATP hydrolysis was observed. This reflects the DinI-mediated stabilization of the filaments and suppression of any RecA filament disassembly occurring at steady state (19). When RecX (60 nM) is added instead of DinI, a slow decline in the ATP hydrolysis rate was measured as the RecA filaments disassembled. However, if the DinI protein was preincubated with RecA before the addition of the RecX protein, no decrease in ATP hydrolysis was observed. This suggests that DinI is protecting the RecA filament from the effects of RecX. This experiment was carried out under conditions that include a 7-fold excess of DinI protein relative to RecA. Previously we determined that this ratio is required for maximal
stabilization of RecA protein filaments on ssDNA (19). The effects of DinI and RecX in this experiment can be explained by a simple model. RecX protein does not promote disassembly, but simply allows it to occur without the compensating effects of RecA filament extension. If DinI binds to the filament and prevents the disassembly, the effects of RecX are countered.

We next wanted to determine whether RecX protein could overcome the effects of a DinI concentration (8.4 μM) sufficient to provide optimal stabilization of these same RecA filaments. We titrated RecX protein into reactions containing fully DinI-stabilized filaments (Fig. 2B). RecX concentrations up to 300 nM had small effects on filament stability as monitored by the ATPase activity. The major effects of RecX were observed as the RecX concentration was increased to 400 nM. The effect of RecX protein on ATPase activity reached a maximum at RecX concentrations of 700 nM and above. At no RecX concentration was the stabilizing effect of DinI eliminated immediately. At the highest RecX concentrations tested, the decline in RecA-mediated ATP hydrolysis required about three times as long as the decline triggered by only 60 nM RecX in the absence of DinI. Nevertheless, DinI was eventually replaced if enough RecX was added, as indicated by the net disassembly of the RecA filaments. This suggests that RecX is not simply preventing RecA filament extension. RecX, at sufficiently high concentrations, is also replacing the DinI protein bound along the length of the RecA filament so as to eliminate the stabilizing effects of DinI and permit filament disassembly. The results are most economically explained by the dissociation of DinI and its replacement by RecX. Because the maximum effects of RecX in the presence of DinI are still much reduced relative to the effects of RecX in the absence of DinI, there must be a slow step in the replacement reaction that does not depend on RecX concentration. This slow step could be DinI dissociation. If the binding of DinI and RecX are mutually exclusive, the effects of RecX could not be manifested until DinI dissociated, and was then replaced by RecX.

The DinI protein, when present, was preincubated with RecA in all experiments shown in Figs. 1 and 2. To determine whether competition is truly occurring between RecX and DinI, we changed the order of addition, adding DinI protein 5, 15, or 30 min after the RecX protein (Fig. 3A). In the first 5 min after RecX addition, RecA filaments are actively disassembling. Adding DinI protein at this time completely reversed the RecA filament extension. RecX, at sufficiently high concentrations, is also replacing the DinI protein bound along the length of the RecA filament so as to eliminate the stabilizing effects of DinI and permit filament disassembly. The results are most economically explained by the dissociation of DinI and its replacement by RecX. Because the maximum effects of RecX in the presence of DinI are still much reduced relative to the effects of RecX in the absence of DinI, there must be a slow step in the replacement reaction that does not depend on RecX concentration. This slow step could be DinI dissociation. If the binding of DinI and RecX are mutually exclusive, the effects of RecX could not be manifested until DinI dissociated, and was then replaced by RecX.

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filament disassembly process. The ATPase activity recovered over the course of several minutes. This suggests that DinI is not only stabilizing the RecA filaments that remain on the DNA at the moment DinI is added, but also relieving the block to RecA filament extension imposed by RecX.

When DinI is added later in the reaction (15 or 30 min after RecX), a recovery of RecA-mediated ATP hydrolysis was again observed (Fig. 3A). However, the recovery was progressively slower as the time of addition of DinI was increased. Under conditions used for the experiments of Figs. 1 and 2, RecA filaments disassemble almost completely over a 20-min period (15). After 10 min, only about half the DNA molecules still have RecA filament segments bound to them (15). SSB protein was present in this experiment, binding to the vacated ssDNA and inhibiting the nucleation of new RecA filaments on that DNA. Therefore, the slower recovery provoked by the addition of DinI at 15 and 30 min after the RecX protein (Fig. 3A) could involve a DinI-mediated enhancement of new RecA filament nucleation on the SSB-bound DNA. Indeed the DinI protein slightly reduced the lag associated with nucleation of RecA onto ssDNA pre-bound with SSB protein (Fig. 3B). This limited enhancement of RecA binding by DinI generally accounts for the slow recovery seen in Fig. 3A when DinI was added at 30 min, but not the more robust recovery seen when DinI was added at 15 min. We therefore propose that DinI is acting on RecA filament remnants that remain after 15 min of RecX-provoked RecA filament disassembly, but which are absent 30 min after RecX addition. DinI is replacing the RecX protein on whatever filament remnants are present, allowing filament extension to proceed again and suppressing filament disassembly.

The DinI-RecX Antagonism Is Manifested in Other RecA Protein-mediated Reactions—The RecX protein inhibits both the LexA cleavage and DNA strand exchange activities of the RecA protein (14, 15, 17). We tested the effect of DinI protein under conditions in which RecX inhibition of these RecA activities (Fig. 4). The requirements for these two activities are quite different. LexA cleavage can be promoted by short fragments of RecA filaments, and is thus less sensitive to RecX. In contrast,
a complete DNA strand exchange reaction requires an intact RecA filament that covers the DNA substrate. A short break in the filament may preclude the completion of the reaction, rendering this reaction especially sensitive to RecX.

A substantial inhibition of LexA cleavage requires an extended (20 min) incubation with RecX prior to addition of the LexA, to allow for nearly complete disassembly of the RecA filaments (15). Therefore, we added a relatively high concentration of RecX (1 μM) to RecA filaments and waited a full 20 min to add the LexA protein. As shown in Fig. 4A, this treatment resulted in substantial (albeit not complete) inhibition of LexA cleavage. The further addition of 3 μM DinI completely attenuated the inhibition of LexA cleavage imposed by the RecX protein, although 1 μM DinI protein did not. We wished to independently determine the state of the RecA filaments at the time of LexA addition in this experiment, and therefore carried out an ATPase assay (Fig. 4B) under the same conditions (except that we did not add the LexA protein). At the point where the LexA protein would be added (20 min following the RecX protein) the filament disassembly process was largely complete when up to 1 μM DinI was present. When 3 μM DinI was present, the disassembly process was not complete, accounting for the RecA-facilitated LexA cleavage observed in Fig. 4A.

We also examined the effects of these modulator proteins on RecA-mediated DNA strand exchange (Fig. 4C). In these experiments, the addition of 10.5 μM DinI protein had no effect on the yield of strand exchange products. Addition of 90 nM RecX protein, in contrast, was sufficient to nearly eliminate product formation. The addition of the DinI protein with RecX attenuated the effects of RecX significantly, although there was still considerable inhibition. Although the sensitivity of the reactions to RecX varies, there is one clear pattern: the DinI and RecX proteins have opposing activities in their regulation of RecA function.

**Evidence for Functional RecX-DinI Antagonism in Vivo**—Overexpressing the RecX protein results in a large decrease in UV resistance relative to cells carrying the empty overexpression vector alone, although whether this effect is because of inhibition of co-protease or recombinase abilities, or both is not known (14). We wished to determine whether the inhibitory effects of RecX protein in these cells were attenuated in any way by the presence of DinI protein. To test the effect of a dinI knockout on cells overexpressing recx, we measured the UV resistance of strains MG1655 and its isogenic derivative EAW8 (dinI::kan) carrying either plasmid pGCC4/recX or the parental vector, pGCC4. The relative survival of strain MG1655 carrying pGCC4/recX versus pGCC4 alone was significantly higher than that of strain EAW8 (Fig. 5), confirming that DinI can partially counteract the inhibitory effects of RecX on UV resistance, and showing that this regulatory circuit also is operational in vivo.

**DISCUSSION**

We conclude that the RecX and DinI proteins are each part of a regulatory network acting on the RecA recombinase. Expression of both RecX and DinI is induced by DNA damaging agents, and the network thus has particular relevance to the role of RecA protein in the induction and course of the SOS response. Most important, the two proteins have antagonistic rather than complementary or synergistic roles in RecA regulation. Both proteins appear to affect primarily the assembly and disassembly of RecA filaments, as opposed to the function of the filaments once assembled.

A model for DinI and RecX action is presented in Fig. 6. Both proteins bind to RecA filaments, but their effects reveal a kind of yin and yang relationship. The RecX protein blocks RecA protein filament extension, most likely capping the filament end where extension occurs (15). The end-dependent disassembly of RecA filaments continues unimpeded when RecX is present. Thus, RecX produces a net disassembly of RecA filaments. RecX protein also binds along the length of the filament, at least when present at levels stoichiometric to the RecA protein (16). However, RecX protein has an inhibitory effect at very low, substoichiometric levels relative to RecA (14, 15). There is no evidence that the binding of RecX along the filament, to the extent it might occur when RecX is present at low levels, interferes with the function of any RecA filament segments still remaining as filament disassembly proceeds (15). The loss of RecA function when RecX is present occurs gradually and coincides with the disassembly of the RecA filaments.

The effect of DinI protein is nearly the converse of the RecX effect. DinI stabilizes RecA filaments, allowing extension to occur but suppressing the end-dependent disassembly reaction (19). DinI binds to RecA filaments in a 1:1 complex with the RecA protein monomers in the filament (20, 32). The optimal level of stabilization is seen when DinI is in 5–7-fold excess relative to the bound RecA (much higher levels of DinI are destabilizing (19, 22)). Given the reported 1:1 binding stoichiometry, the need for excess DinI implies that the binding of DinI to RecA filaments is relatively weak. Under the conditions in which it stabilizes RecA
filaments, DinI protein has little to no effect on most RecA activities (19), except UmuD cleavage (21).

The opposing effects and the competition between the two proteins is readily seen in experiments in which RecA filaments are studied in the presence of both the RecX and DinI proteins. In these experiments, RecX protein consistently exhibits a measurable effect on RecA filaments at lower modulator concentrations than is seen with the DinI protein. This suggests that the RecX-RecA interaction is more robust than the DinI-RecA interaction. The stabilization effect of DinI protein predominates only when DinI is present at concentrations about 20-fold or more greater than the RecX concentration (Fig. 2). A replacement of RecX by DinI and vice versa can be seen in experiments in which RecA filaments being acted on by one of the proteins are challenged by high levels of the other (Figs. 2 and 3). In summary, the RecA-DinI and RecA-RecX filaments have quite distinct properties, and the RecA filaments can interchange between the two forms as the two modulator proteins replace each other (Fig. 6).

The DinI-RecX replacement reactions are not fast. When DinI-stabilized filaments are challenged with RecX protein, the disassembly of RecA filaments takes about three times longer than is seen when RecX protein challenges a RecA filament in the absence of DinI. This is the case regardless of how much RecX protein is added (Fig. 2B). This implies that RecX binding to RecA filaments is limited by a slow step that is not affected by RecX concentration. This could reflect a relatively slow dissociation of bound DinI, as a prerequisite to RecX binding. Similarly, addition of high levels of DinI to RecA filaments disassembling in the presence of RecX (Fig. 3A) results in recovery of the filaments. The recovery requires several minutes even when DinI is added at relatively early times in the disassembly process. Both effects are readily explained by a simple model in which slow dissociation of one protein is followed by binding of the other protein, assuming that the two proteins bind in a mutually exclusive manner. We cannot preclude the possibility that the two proteins interact in some manner in the reactions that lead to replacement of one protein by the other. However, there is no detectable interaction between DinI and RecX in yeast two-hybrid studies.2

The competition between RecX and DinI provides a potential explanation for the binding of RecX along the length of the RecA filament in addition to the assembling end. To promote the recovery of filaments that are disassembling in the presence of RecX protein, DinI must presumably replace the RecX that is bound at the end where RecA filament extension occurs. The short delay observed in the recovery reaction may reflect the dissociation of that specific RecX complex. Because the effects of DinI protein require amounts of DinI that are stoichiometric with RecA (19), we presume that DinI binds along the RecA filament. To overcome the effects of DinI, RecX would have to not only cap the RecA filament to prevent extension, but also displace the DinI protein so as to permit filament disassembly.

The competition between DinI and RecX has measurable effects on the function of RecA in vivo. The overexpression of RecX protein leads to a significant decline in the capacity of E. coli cells to repair UV damage. The deleterious effects of RecX overexpression are ameliorated by the DinI protein (Fig. 5). Thus, the competition between DinI and RecX can be monitored in vivo as well as in vitro. This result may telegraph a potentially product path for a detailed exploration of the regulation of RecA protein in vivo.

The DinI function is somewhat analogous to the role of UvsY protein in the stabilization of UvX protein filaments in the

2 E. Stohl, K. Burkle, and H. Seifert, unpublished data.
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