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# The DinI Protein Stabilizes RecA Protein Filaments*

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**When DinI is present at concentrations that are stoichiometric with those of RecA or somewhat greater, DinI has a substantial stabilizing effect on RecA filaments bound to DNA. Exchange of RecA between free and bound forms was almost entirely suppressed, and highly stable filaments were documented with several different experimental methods. DinI-mediated stabilization did not affect RecA-mediated ATP hydrolysis and LexA co-protease activities. Initiation of DNA strand exchange was affected in a DNA structure-dependent manner, whereas ongoing strand exchange was not affected. Destabilization of RecA filaments occurred as reported in earlier work but only when DinI protein was present at very high concentrations, generally super-stoichiometric, relative to the RecA protein concentration. DinI did not facilitate RecA filament formation but stabilized the filaments only after they were formed. The interaction between the RecA protein and DinI was modulated by the C terminus of RecA. We discuss these results in the context of a new hypothesis for the role of DinI in the regulation of recombination and the SOS response.**

The RecA protein plays a principle role in the processes of homologous recombinational DNA repair (reviewed in Refs. 1–3). In addition to its direct involvement in the repair of stalled replication forks, RecA regulates multiple repair pathways via its role in the induction of the SOS response (4), and is required for translesion DNA synthesis by DNA polymerase V (5). RecA nucleates onto regions of single-stranded DNA (ssDNA)[1] that develop as a result of DNA damage and forms a helical nucleoprotein filament. This ATP-dependent activated RecA filament interacts with and promotes the autocatalytic cleavage of the LexA repressor protein, thereby inducing the genes of the SOS regulon (6). Whereas the mechanism of SOS induction has been well studied, the means by which the SOS response is terminated is not clear.

The biochemical data available for the *Escherichia coli* RecA protein and the reactions that it catalyzes (ATP hydrolysis and DNA strand exchange) are abundant (1, 7), but we are just beginning to understand the complex layers of regulation to which RecA is subject. The C-terminal 25 amino acid residues play a role in autoregulation of RecA function. Removal of just 17 C-terminal amino acid residues (RecAΔC17) has a dramatic and positive effect on the capacity of RecA protein to bind to duplex DNA (8), to displace single-stranded binding protein (SSB) when binding ssDNA (9), and to carry out DNA strand exchange in the absence of free $Mg^{2+}$ (10). The complete C-terminal domain is larger, consisting of residues 270–352. The last invariant residue among 64 bacterial RecA species is Gly-301, and the sequence alignments indicate very little conservation in residues from 301 to the C-terminal residues. Most but not all bacterial RecA proteins, including the RecA of *E. coli*, contain a high concentration of negatively charged residues at their far C termini (7).

RecA protein function is also regulated by other proteins in bacteria. The RecFOR proteins modulate the assembly and disassembly of RecA filaments on DNA (11–14). The RecX protein inhibits the ATPase and DNA strand exchange activities of RecA (15). Finally, the DinI protein also inhibits RecA function, and this protein has a postulated role in the termination of the SOS response (16–18). The interaction between DinI and RecA and the functional consequences of that interaction are the subject of the present report.

The *dinI* gene was first identified by David Mount and colleagues (19) as a chromosomal locus containing a high affinity operator for the LexA repressor. Subsequently, the *dinI* gene was isolated as a multicopy suppressor of the cold-sensitive SOS induction phenotype of the *dinD68* mutation (20). The gene encodes a small protein with 80 amino acids and an $M_r$ of 8818 if the N-terminal Met residue is removed. Over-expression of the *dinI* gene from a multicopy plasmid conferred UV sensitivity and suppressed induction of the SOS response by mitomycin C (21). Purified DinI protein has been shown to interact with both active and inactive forms of RecA (16, 17).

By comparing relative binding affinities for the RecA filament, Yasuda *et al.* (17) determined that LexA bound tighter than DinI, which in turn bound tighter than UmuD. Recently, Yoshimasu *et al.* (18) found that DinI binds RecA at the RecA core domain. Specific interactions have previously been detected with a DNA-binding segment of the core of RecA called loop 2 (16). Shibata and co-workers (18) suggest that DinI binds the RecA nucleoprotein filament in the major filament groove, in the same site or region that binds UmuD (22). This groove is the "gateway" for double-stranded DNA (dsDNA) entry (23) to the presynaptic filament (24). This region of RecA is the region that we have suggested is blocked by the C terminus at low magnesium ion concentrations (10).

The actual effect of DinI on RecA filaments has been the subject of some controversy. Some of us (16) previously showed that added DinI protein disrupts RecA filaments, accounting for several inhibitory effects of DinI. Another study (17) indi-

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[1] The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; nt, nucleotide(s); DTT, dithiothreitol; SSB, single-stranded DNA-binding protein; ATPγS, adenosine 5′-*O*-(thiotriphosphate); ExoI, exonuclease I.

cated that RecA filaments remain intact after DinI addition. In this report, we further explore the DinI-RecA interaction. DinI protein destabilizes RecA filaments, but only at very high DinI concentrations. At lower, more nearly stoichiometric concentrations, DinI has a very substantial stabilizing effect on RecA filaments and does not significantly inhibit many RecA functions. We further demonstrate that the RecA C terminus modulates the interaction between DinI and RecA.

## EXPERIMENTAL PROCEDURES

*Enzymes and Biochemicals*—The *E. coli* wild-type RecA and RecAΔC17 mutant proteins were purified as described (8). The RecA K72R mutant protein was purified as described (25). The concentrations of the purified RecA proteins were determined from the absorbance at 280 nm using the extinction coefficient of $2.23 \times 10^4$ M$^{-1}$ cm$^{-1}$ (26). The *E. coli* DinI protein was purified as described (16), and its concentration was determined using the extinction coefficient of $1.44 \times 10^4$ M$^{-1}$ cm$^{-1}$ at 280 nm calculated using the ProtParam tool (from the ExPASy web site). The *E. coli* SSB protein was purified as described (25). The concentration of the purified SSB protein was determined from the absorbance at 280 nm using the extinction coefficient of $2.83 \times 10^4$ M$^{-1}$ cm$^{-1}$ (27). Unless otherwise noted, all reagents were purchased from Fisher. ATPγS and creatine phosphokinase were purchased from Roche Molecular Biochemicals. Dithiothreitol (DTT) was obtained from Research Organics. Lactate dehydrogenase, pyruvate kinase, phosphoenolpyruvate, phosphocreatine, ATP, NADH, and bromphenol blue were purchased from Sigma. The restriction endonuclease PstI was obtained from New England Biolabs, and XhoI was from Amersham Biosciences.

*DNA Substrates*—All DNA concentrations are given in terms of total nucleotides. Poly(dT) was purchased from Amersham Biosciences, and the approximate average length is 229 nt. The concentration of poly(dT) was determined by the absorbance at 260 nm using the extinction coefficient of 8.73 mM$^{-1}$ cm$^{-1}$. Bacteriophage φX174 circular single-stranded DNA (virion) was purchased from New England Biolabs. M13mp8 bacteriophage circular ssDNA (7229 nt) was prepared as described (8). φX174 RF I supercoiled circular duplex DNA was purchased from Invitrogen. Unless otherwise stated, full-length linear duplex DNA was generated by the digestion of φX174 RF I DNA (5386 bp) with the XhoI restriction endonuclease using the conditions provided by the enzyme supplier. The digested DNA was extracted as described (8). The concentrations of ssDNA and dsDNA were determined by absorbance at 260 nm, using 36 and 50 μg ml$^{-1}$ $A_{260}^{-1}$, respectively, as conversion factors.

*ATP Hydrolysis Assays*—A coupled spectrophotometric enzyme assay (28, 29) was used to measure the DNA-dependent ATPase activities of the wild-type RecA protein. The assays were carried out on a Varian Cary 300 dual beam spectrophotometer with a temperature controller and a 12-position cell changer. The NADH extinction coefficient at 380 nm of 1.21 mM$^{-1}$ cm$^{-1}$ was used to calculate the rate of ATP hydrolysis. The reactions were carried out at 37 °C in 25 mM Tris-OAc (80% cation), 1 mM DTT, 3 mM potassium glutamate, 10 mM $Mg(OAc)_2$, 5% (w/v) glycerol, an ATP regeneration system (10 units/ml pyruvate kinase and 2 mM phosphoenolpyruvate), a coupling system (3 mM NADH and 10 units/ml lactate dehydrogenase), and the concentrations of DNA, RecA, protein and, where applicable, DinI protein indicated in the figure legends. For assays including circular ssDNA, the aforementioned components were preincubated for 10 min, and the assay was initiated by the addition of the SSB protein (to 0.3 μM) and ATP (to 3 mM). Unless otherwise noted, assays using poly(dT) DNA cofactor did not contain SSB protein. In this case all components except for wild-type RecA protein were included in the preincubation and the assay was initiated with the addition of RecA.

*Analysis of the Stability of the Presynaptic Filament Formed on a 55-Mer Oligonucleotide*—The oligonucleotide 55T (synthesized on an Applied Biosystems 380A synthesizer and purified by denaturing PAGE) has the sequence 5′-TGT GGA ATG CTA CAG GCG TTG TAG TTT GTA CTG GTG ACG AAA CTC AGT GTT ACG G-3′. The presynaptic filament was formed by preparing a master mix of 500 nM 55T, $^{32}$P phosphorylated at its 5′ terminus, and 600 nM RecA in the presence of 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, 3 mM $MgCl_2$, 0.4 mM DTT, and 1 mM ATPγS for 30 min at 37 °C. 18 μl of the presynaptic filament mix was transferred to a fresh tube containing 2 μl of DinI in DinI storage buffer (10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 10% (w/v) glycerol) and incubated for 30 min at 37 °C. The final concentrations of DinI were 0, 3.5, 5.3, 8, 12, and 18 μM. After incubation, each reaction mixture was split in half. One-half was mixed with 2.5 μl of 5× DNA gel loading solution (Quality Biological) and subjected to electrophoresis in a 8% (19:1) polyacrylamide gel prepared in a 100 × 100 × 1-mm Invitrogen cassette in Tris borate-EDTA (TBE, purchased from KD Medical) buffer supplemented with 3 mM $MgCl_2$. Electrophoresis was conducted at room temperature at a constant 125 V for 50 min. Under these electrophoretic conditions, the absence of RecA-DNA complexes in the absence of DinI may reflect destabilization due to gel warming. The second half of the reaction was treated with 500 units/ml exonuclease I (purchased from United States Biochemical) for 15 min at 37 °C. ExoI reactions were stopped by the addition of 5 μl (per 10 μl of reaction) of a solution containing 60 mM EDTA, 6% SDS, 30% glycerol, and 0.1% bromphenol blue. Five μl of the samples were electrophoresed in 0.4-mm 20% SequaGel (National Diagnostics) denaturing gels, and the marker dyes were all allowed to migrate for 17 cm. Images of the radioactive gels were obtained with a Fuji BAS 2500 phosphorimaging system.

*Electron Microscopy of Poly(dT)-RecA Nucleoprotein Filaments*—A modified Alcian method was used to visualize RecA filaments. Activated grids were prepared as described previously (8). Samples for electron microscopy were prepared by preincubating the indicated amount of RecA and poly(dT) ssDNA, 25 mM Tris-OAc (80% cation) buffer, 1 mM DTT, 5% (w/v) glycerol, 3 mM potassium glutamate, and 10 mM $Mg(OAc)_2$ for 10 min at 37 °C. An ATP regeneration system of 12 mM phosphocreatine and 10 units/ml creatine phosphokinase was also included in the preincubation. ATP was added to 3 mM, followed by another 10-min incubation. In reactions that contain DinI protein, the DinI protein was added following a 5-min incubation after the addition of ATP followed by another 5-min incubation. Where indicated, ATPγS was added followed by another 3-min incubation. The reaction mixture described above was diluted as indicated with 200 mM ammonium acetate, 2 mM Hepes buffer (pH 7.5), 10% glycerol, and 8 μl of the diluted sample was adsorbed to the activated carbon film for 3 min. The grid was then touched to a drop of the above described buffer followed by floating on a drop of the same buffer for 30 s. The sample was stained by touching to a drop of 5% uranyl acetate followed by floating on a fresh drop of the same solution. Finally the grid was washed by touching to a drop of water followed by immersion in two 10-ml beakers of water and one beaker of ethyl alcohol. After the sample was dried, it was rotary shadowed with platinum. This protocol is designed for visualization of complete reaction mixtures, and no attempt was made to remove unreacted material. Although this approach should yield results that give a true insight into reaction components, it does lead to samples with a high background of unreacted proteins.

*DNA Three-strand Exchange Reactions*—All of the following reagent concentrations represent final concentrations after the addition of all reaction components. DNA three-strand exchange reactions were carried out in 25 mM Tris-OAc buffer (80% cation), 1 mM dithiothreitol, 5% (w/v) glycerol, 3 mM potassium glutamate, and the indicated concentration of $Mg(OAc)_2$. Reactions also contained an ATP regeneration system consisting of 10 units/ml pyruvate kinase and 2 mM phosphoenolpyruvate. All incubations were carried out at 37 °C. The wild-type or ΔC17 RecA proteins (3 μM) were preincubated with 10 μM φX174 circular ssDNA for 10 min. SSB protein (1 μM) and ATP (3 mM) were then added followed by another 10-min incubation. In reactions that contained DinI protein, the DinI protein was added following a 5-min incubation after the addition of SSB and ATP followed by another 5-min incubation. The reactions were initiated by the addition of φX174 linear dsDNA to 10 μM and incubated for 60 min. The reaction was stopped by the addition of 5 μl (per 10 μl of reaction) of a solution containing 60 mM EDTA, 6% SDS, 25% (w/v) glycerol, and 0.2% bromphenol blue. Samples were electrophoresed at 10–20 mA in 0.8% agarose gels with 1 × TAE (40 mM Tris-OAc, 80% cation, and 1 mM EDTA), stained with ethidium bromide, and exposed to ultraviolet light. Gel images were captured with a digital CCD camera using GelExpert software (Nucleotech). When indicated the DNA bands were quantitated with the TotalLab software package (version 1.10) from Phoretix.

*Surface Plasmon Resonance Measurements*—Experiments were performed on a Biacore 3000 instrument (BIAcore) at 25 °C. The flow buffer was 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10 mM $MgCl_2$, 0.4 mM DTT, and 0.005% surfactant P20 (purchased from BIAcore). The wild-type DinI protein was immobilized on a CM5 sensor chip to give a signal of 570 response units using an amine coupling kit from BIAcore. A control (empty) surface was created in the same batch run. The same chip was used for all the measurements described in this work. The wild-type RecA protein was injected at concentrations of 0, 64, 128, 256, 512, and 1024 nM; for the ΔC17 RecA mutant we used concentrations of 0, 32, 64, 128, 256, and 512 nM. The contact time was 5 min at a flow rate of 50 μl/min. After injection, the complex was allowed to dissociate

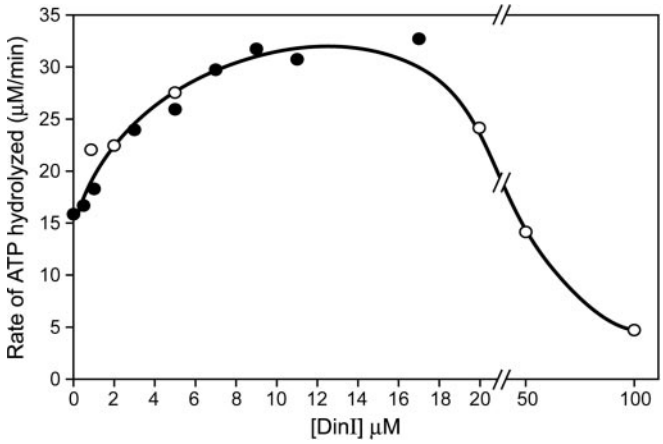



FIG. 1. **The rate of ATP hydrolyzed by the wild-type RecA protein as a function of DinI protein.** RecA-mediated ATP hydrolysis was monitored as described under "Experimental Procedures." The indicated amount of DinI protein was preincubated for 10 min with all reaction components (including 3 $\mu$M poly(dT) linear ssDNA) except RecA, and the reaction was initiated with the addition of RecA protein to 1 $\mu$M. The *open* and *closed circles* represent reactions carried out on different days.

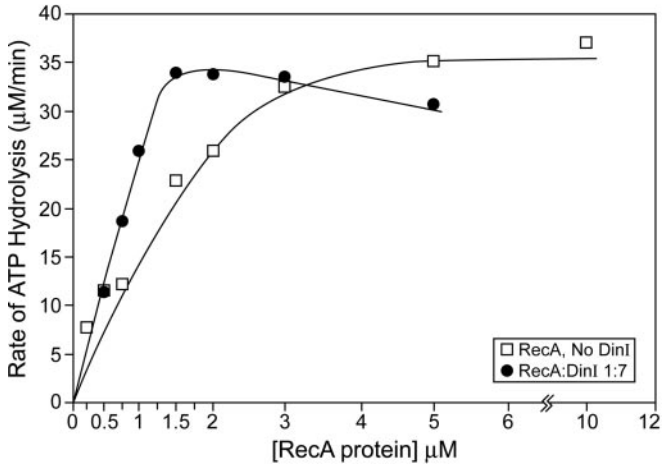



FIG. 2. **The rate of ATP hydrolyzed as a function of wild-type RecA protein concentration in the presence (*closed circles*) or absence (*open squares*) of DinI protein.** The reactions included 3 $\mu$M poly(dT) and the concentrations of RecA protein indicated. The reactions represented by *closed circles* included DinI protein at a constant molar ratio of RecA to DinI protein of 1:7.

for 6 min. The surfaces were regenerated by two consecutive injections, 50 $\mu$l each, of 3 M NaCl in the flow buffer. After subtraction of the control surface signals, we used BIAevaluation software, version 4.0.1, to fit experimental curves to the Langmuir model, which describes the simplest 1:1 binding of the analyte to the ligand. Despite the fact that the DinI-RecA interaction is more complex than assumed by this model, we obtained the best agreement of our experimental data with theoretically simulated curves using this particular fitting mode. Therefore, rate and equilibrium constants of the DinI-RecA interactions generated in this manner should be considered as "apparent" and should be used only for purposes of comparing the wild-type and mutant RecA proteins.

## RESULTS

There were two areas of investigation: the effects of DinI on RecA filament stability and the role of the RecA C terminus in the DinI-RecA interaction.

### *DinI Stabilizes RecA-DNA Nucleoprotein Filaments*

To examine the effects of DinI on RecA filaments, we first explored the DNA-dependent RecA mediated ATPase activity. In many respects, the RecA ATPase is a sensitive barometer of RecA filament status, and it has often been used as a real-time indicator of the level of RecA protein binding to DNA (3, 7, 11, 28, 30, 31). We thus titrated the DinI protein into a reaction in which RecA protein was bound to ssDNA and hydrolyzing ATP. We carried out the ATPase assays using poly(dT) ssDNA as a cofactor to avoid the use of SSB protein while at the same time avoiding any complications due to secondary structure in the DNA. On such linear ssDNA molecules, the observed ATP hydrolysis reflects a steady state in which RecA filaments are constantly assembling and disassembling in an end-dependent fashion (3, 7, 11, 30, 32). At constant protein concentrations of RecA protein (1 $\mu$M) and ssDNA (3 $\mu$M), increasing the amount of DinI protein initially enhanced the rate of ATP hydrolysis by the wild-type RecA protein (Fig. 1). No measurable ATP hydrolysis was observed when RecA was omitted from the reaction (data not shown), so the added ATP hydrolysis did not come from DinI or a contaminant in the DinI preparation. Without DinI protein, the RecA protein hydrolyzes ATP at a rate of 16 $\mu$M/min. This rate is lower than that observed on circular ssDNA because the steady state RecA filament disassembly and re-assembly on the poly(dT) leaves the DNA only partly bound by RecA at any given moment (11, 30, 33). The added DinI protein initially appears to stabilize the RecA filament on linear ssDNA. The maximum observed rate of hydrolysis by the RecA protein, seen with DinI concentrations from about 7 to 18 $\mu$M, was very similar to the rates observed when the same concentration of RecA protein is bound to circular ssDNA (~30 $\mu$M/min), and were consistent with those expected when RecA protein has saturated the available DNA binding sites (3, 7). The result suggested that DinI is inhibiting the end-dependent disassembly of RecA filaments, facilitating the assembly process, or both. As additional DinI protein was added to the reaction, the ATPase rates began to decline. At 100 $\mu$M DinI, the rate of ATP hydrolysis had declined by more than 80%. The highest DinI concentrations used were comparable with those used in our previous study (16) documenting a disruption of RecA filaments. We now suggest that the interaction of DinI with RecA filaments is more complex, disrupting the filaments only at very high concentrations. The lower concentrations of DinI appear to have the opposite effect, substantially stabilizing RecA filaments on ssDNA. Because a stabilization effect had not been observed previously, we set out to confirm and characterize it in several ways.

Whenever the indirect ATPase assay is utilized to monitor RecA binding to DNA, it is always possible that some reagent (in this case DinI) is affecting the inherent ATPase activity rather than actually increasing the level of binding. To distinguish between these possibilities, a RecA protein titration was carried out in the presence and absence of the DinI protein (Fig. 2). Without DinI, a nearly 5-fold excess of RecA protein (beyond the one monomer per three nucleotides that represents the amount of RecA that theoretically can be bound to ssDNA) is required to reach a maximal rate of ATP hydrolysis (Fig. 2, *open squares*). This is again because of the continuous assembly and disassembly of RecA filaments on the linear ssDNA. At stoichiometric concentrations of RecA, there is enough disassembly occurring to prevent saturation of the DNA. As RecA concentrations are increased, the rate of nucleation and filament assembly increases, and the net occupation of DNA binding sites increases. The maximum rate observed, signaling saturation, was just over 30 $\mu$M min$^{-1}$. In the presence of DinI, the rate of ATP hydrolysis reached the same maximum, but only a 1.5-fold excess of RecA is required. This indicates that the rate of RecA-mediated ATP hydrolysis is identical with or without DinI at the point of DNA saturation with RecA, suggesting that the intrinsic ATP hydrolytic activity of RecA is unaffected by DinI. However, in the presence of DinI, the DNA is saturated at lower RecA concentrations, consistent with a

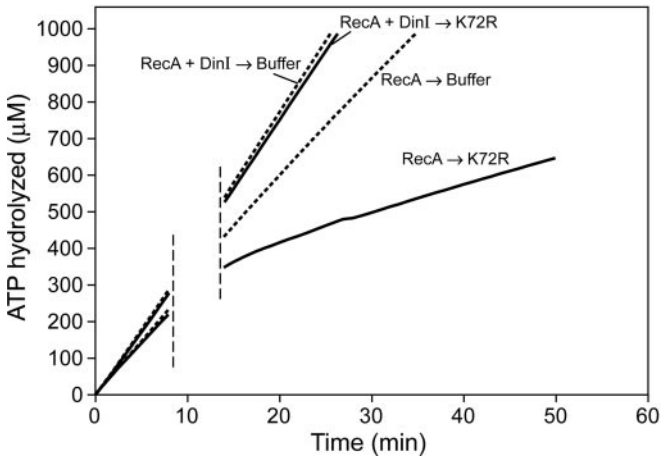



FIG. 3. **The effect of the RecA K72R mutant protein on the rate of ATP hydrolyzed by the wild-type RecA protein in the presence and absence of the DinI protein.** Reactions included 3 μM poly(dT), 3 μM wild-type RecA, and where indicated, 21 μM DinI protein. After 10 min, the data collection was paused for the addition of the RecA K72R mutant protein (to 3 μM, *solid lines*) or the equivalent volume of RecA K72R storage buffer (20 mM Tris-HCl (80% cation), 1 mM dithiothreitol, 0.1 mM EDTA, and 10% (w/v) glycerol; *dashed lines*), and data collection was resumed. The time of the addition is *bordered* by *dashed vertical lines*.

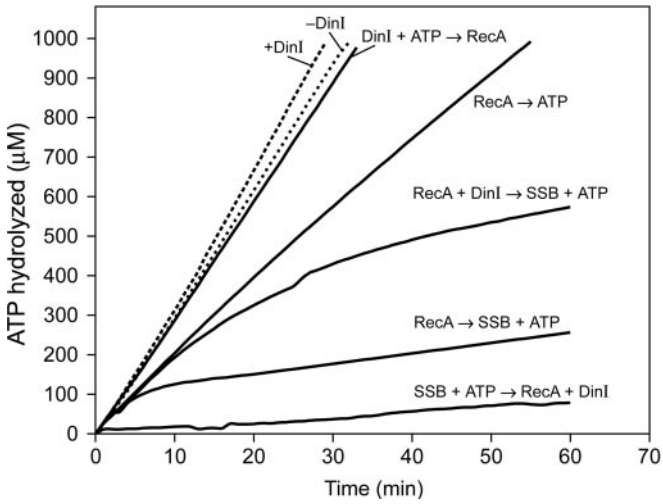



FIG. 4. **The effect of the DinI protein on RecA filament stability.** Suppression of end-dependent and SSB-facilitated disassembly of RecA filaments by DinI is shown. Experiments marked with *dashed lines* included 3 μM M13mp8 circular ssDNA, 3 μM RecA, 0.3 μM SSB, 3 mM ATP, and where indicated 21 μM DinI protein. Reactions were initiated by the addition of ATP and SSB. Experiments marked with *solid lines* included 3 μM poly(dT), 1 μM RecA, 3 mM ATP, and where indicated 7 μM DinI and/or 0.42 μM SSB protein. Each trace (*solid lines*) representing experiments carried out with poly(dT) is labeled to indicate the order of addition of all reagents other than the buffers and DNAs. One set of reagents was preincubated with the DNA for 10 min, and the assay was initiated with the addition of the second set, with the two sets separated by an *arrow*.

filament stabilizing effect of DinI (Fig. 2, *closed circles*). Half-saturation is observed at ~0.7 and 1.25 μM RecA protein in the presence and absence of DinI protein in this experiment. We independently determined that DinI does not enhance or otherwise affect the very limited ATPase activity of RecA observed in the absence of DNA (data not shown).

*RecA Filaments Become Less Dynamic in the Presence of DinI Protein*—RecA filament dynamics can be assayed by utilizing the K72R mutant RecA protein (34, 35). RecA K72R contains a point mutation in the Walker A box and can bind DNA but does not hydrolyze ATP (36). The equilibrium between bound and free RecA protein can be probed by challenging a reaction mixture containing RecA filaments on DNA with this mutant protein and then monitoring the decline in ATP hydrolysis as the wild-type protein dissociates and is replaced in the filament by the mutant protein (25, 34). When challenged by the K72R mutant in the presence of poly(dT), the rate of wild-type RecA protein-mediated ATP hydrolysis drops significantly (Fig. 3) indicating that the RecA is frequently dissociating from the DNA and the RecA K72R protein is binding to the vacated binding sites. However, when the K72R protein is added in the presence of the DinI protein (21 μM), there is no drop in the ATP hydrolysis rate (Fig. 3). This clearly indicates a reduction in RecA filament dissociation in the presence of DinI protein. The addition of bovine serum albumin at 0.19 or 0.38 μg/μl (either equal to or twice the bulk protein concentration by weight provided by 21 μM DinI protein) had no detectable effect on RecA filament dissociation in an identical experiment challenging RecA filaments with RecA K72R protein (data not shown), indicating that the stabilization afforded by DinI is not because of molecular crowding or some other nonspecific effect of DinI protein.

The effects of the SSB protein can also be used to monitor filament dynamics. When the SSB protein is prebound to ssDNA, the RecA protein has a limited capacity to displace it, and nucleation is inhibited (37, 38). Furthermore, on linear ssDNA SSB replaces RecA protein during the end-dependent disassembly of RecA filaments, and this leads to a decline in ATP hydrolysis (Refs. 11 and 30 and Fig. 4). When SSB is prebound to the DNA, DinI does not increase the capacity of RecA to displace SSB. The inhibition of RecA binding by prebound SSB results in low rates of RecA-mediated ATP hydrolysis even with DinI present (Fig. 4). The rates observed were identical to those observed when DinI is not present (data not shown; Ref. 9). However, when DinI and RecA are preincubated with the DNA before the addition of SSB, RecA remains bound to the DNA ~20 min longer than in the absence of DinI protein. This again suggests that DinI is stabilizing the RecA filament and, in particular, suppressing the end-dependent filament disassembly process. In experiments without SSB protein, the order of addition of the DinI protein had no effect on the rate of ATP hydrolyzed by the RecA protein (data not shown). When M13mp8 circular ssDNA is utilized as a cofactor, the rate of ATP hydrolyzed by the RecA protein is not significantly affected by the addition of the DinI protein (Fig. 4, *dashed lines*), again indicating that the intrinsic rate of ATP hydrolysis is not affected by the DinI protein.

*Stabilization of a Nucleoprotein Filament Formed on a 55-Mer Oligonucleotide*—Because an increase of the ATP hydrolysis rate in the presence of DinI suggests stabilization of DNA-RecA filaments, we used an independent approach to assess the stabilization effect directly. DinI was added to the nucleoprotein filament preformed on a 55-mer oligonucleotide of mixed sequence, 55T, and the ternary complexes were subjected to either gel electrophoresis under destabilizing conditions or treatment with exonuclease I. The stabilization effect of DinI is seen clearly in Fig. 5*A*. The 55T-RecA complex formed in the presence of ATPγS remains in the well upon electrophoresis in an 8% polyacrylamide gel. Decay of the complex causes the "smearing effect" evident in the figure. The addition of DinI to the DNA-RecA complex results in DinI concentration-dependent accumulation of the radioactively labeled oligonucleotide in the wells (Fig. 5*A*, *lanes 3*–*7*). This reflects the stabilizing effect of DinI on the RecA-DNA nucleoprotein complex.

Radding and colleagues (39) demonstrated that DNA is protected against the 3′-5′ exonuclease activity of ExoI in ssDNA-RecA complexes formed in the presence of ATPγS. Protein-free oligonucleotide 55T, treated by ExoI under our experimental

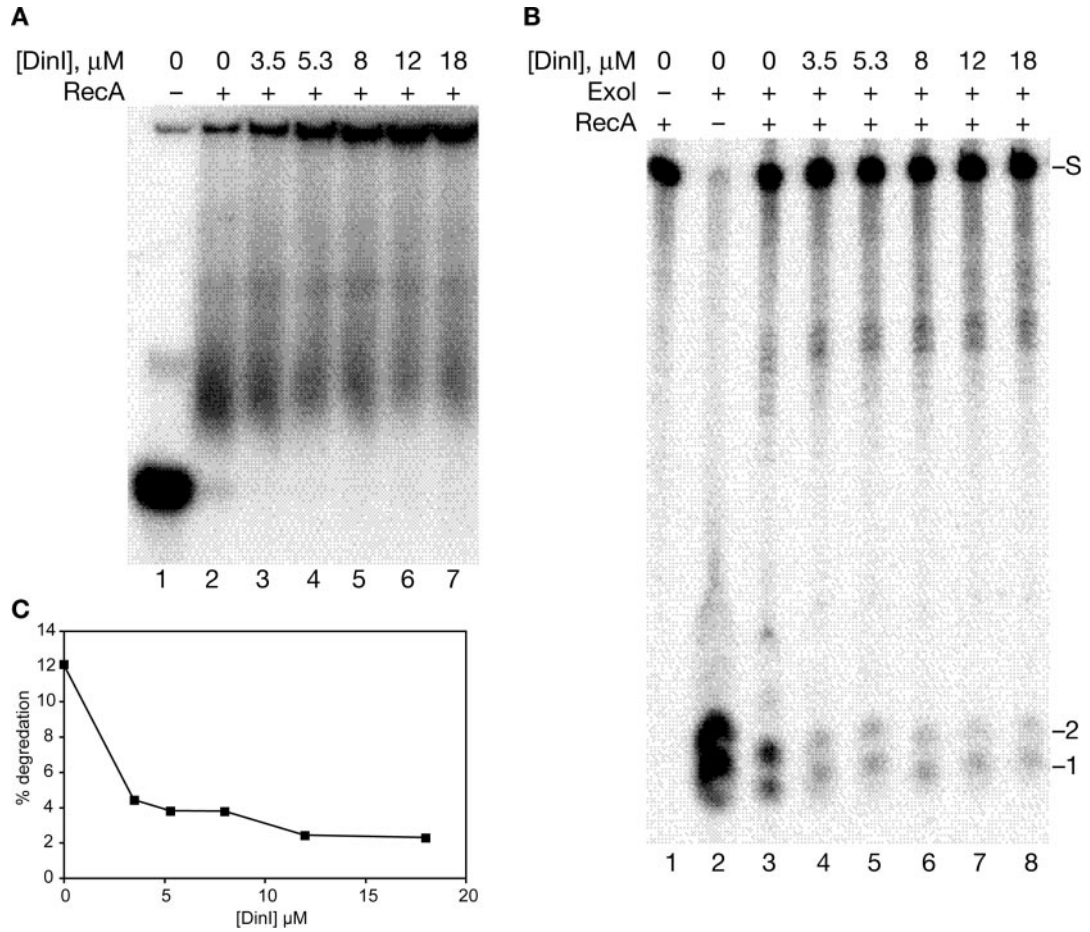



FIG. 5. **Monitoring DNA-RecA nucleoprotein filament stability by gel shifts and exonuclease I treatment.** Complexes of a 55-mer oligonucleotide, RecA, and varying amounts of DinI were formed as described under "Experimental Procedures." The same complex was either electrophoresed in nondenaturing polyacrylamide gel (*A*) or treated with ExoI followed by electrophoresis under denaturing conditions (*B*). The concentrations of DinI are given at the *top*. In *B*, the labels *S, 1*, and *2* correspond to the substrate oligonucleotide, mononucleotides, and dinucleotides, respectively. *C*, quantitation of *lanes 3–8* from *panel B* (those experiments containing RecA protein and varying amounts of DinI prior to exonuclease I treatment).

conditions, was completely degraded by the processive nucleolytic activity, yielding very short DNA fragments (presumably mono- and dinucleotides) (Fig. 5*B*, *lane 2*). As expected, the addition of RecA and ATPγS resulted in protection of most of the 55T from digestion, although about 12% of the radioactivity was shifted to the bottom of the gel (Fig. 5*B*, *lane 3*). The addition of DinI stabilized the 55T-RecA complex, and as a result degradation of the oligonucleotide by ExoI decreased further from 12% to just over 2% (Fig. 5, *B*, *lanes 4–8*, and *C*).

*RecA Nucleoprotein Filaments on Poly(dT) Are More Continuous and Abundant in the Presence of the DinI Protein*—To visualize the effects of the DinI protein in stabilizing RecA filaments on linear ssDNA, filaments were analyzed by electron microscopy. Several experimental parameters affect these experiments. RecA filaments are quite dynamic, and historically, the slowly hydrolyzed ATP analog ATPγS is added to RecA/DNA complexes before they are adsorbed to the electron microscopy grid for analysis to stabilize the filaments (11). In addition, stable filaments of RecA protein (with ATPγS) on linear DNA can associate end-to-end to create filaments that appear longer than unit length (40).

For the first few experiments, ATPγS was not added. At relatively low protein and DNA concentrations roughly equivalent to those used in the ATPase assays described above (1 μM RecA, 3 μM poly(dT) and 8 μM DinI where indicated), RecA filaments observed in the absence of DinI protein were few in number and significantly shorter overall when compared with filaments formed with the DinI protein (Fig. 6, *A* and *B*). From these experiments, random grid fields were selected and the filaments were counted. Experiments represented by Fig. 6*A* (no DinI) averaged 2.1 filaments/grid field (47 fields counted). When DinI was included (Fig. 6*B*) the average number of filaments per grid field increased to 8.1 (17 fields counted). In this analysis, all filaments long enough to be clearly identified as RecA were included. As already noted, RecA bound to oligos can polymerize *in vitro* to create an elongated filament containing multiple DNA oligos in tandem. Although the difference in filament numbers per field are significant, it is important to note that the length of filaments formed in the presence of DinI protein is also at least fives times greater than those formed in the absence of DinI (Fig. 6, *A* and *B*). Of 101 molecules counted from the experiment in Fig. 6*B*, 61% were linear and 39% were circles. Fig. 6, *C* and *D*, is representative of filaments observed when the concentrations of RecA and poly(dT) are increased to a level approximating the strand exchange conditions used later in this study (6.7 μM RecA, 20 μM poly(dT), and 50 μM DinI where indicated). Notably, the filaments observed on poly(dT) in the absence of DinI are highly discontinuous (Fig. 6*C*). Furthermore, as detailed above, without the stabilizing effect of ATPγS, there are fewer filaments overall than when DinI is included in the assay (Fig. 6*D*). Of 175 molecules counted on the grid from which Fig. 6*D* is derived, 83% were linear, 3% were circles, and 14% were

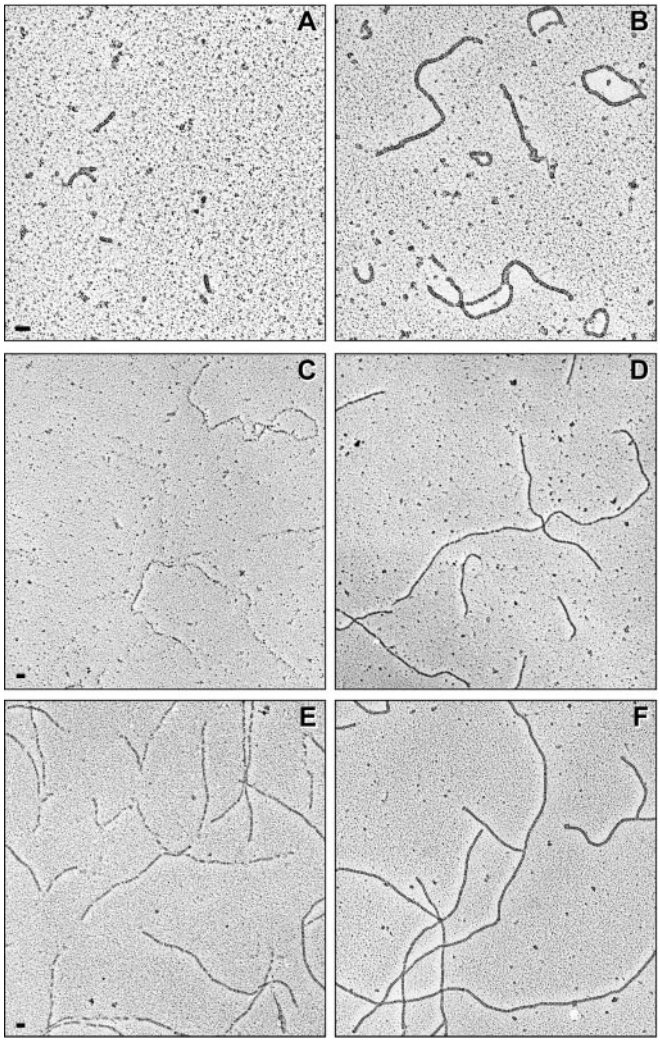



FIG. 6. **The effect of DinI protein on RecA filaments formed on poly(dT) single-stranded DNA.** Electron micrographs show RecA nucleoprotein filaments formed in the absence (*A*, *C*, and *E*) or presence (*B*, *D*, and *F*) of the DinI protein. The reactions were carried out with 3 mM ATP. Filaments shown in *A–D* were not fixed or stabilized with ATPγS before spreading (see "Experimental Procedures"), whereas reactions shown in *E* and *F* were fixed by the addition of 3 mM ATPγS. *A* represents filaments formed at low concentrations of RecA (1 μM), poly(dT) ssDNA (3 μM; average length 229 nt), and 8 μM DinI is included for *B*. Reaction mixtures were diluted 5-fold before adhesion to the electron microscopy grid. *C* and *E* represent filaments formed at higher concentrations of RecA (6.7 μM) and DNA (20 μM), and 50 μM DinI is included for *D* and *F*. Reaction mixtures were diluted 17-fold before adhesion to the electron microscopy grid. The horizontal width of the *bars* in the *left panels* denotes an unbound B-form duplex DNA that is 229 bp in length. The poly(dT) used in these experiments has an average length of 229 nt. For each of the *left panels*, magnification was identical to that in the panel immediately to its right.

branched "Y"-shaped molecules. The molecules counted were of variable lengths, although intermolecular association of filaments was more evident at this higher protein and DNA concentration.

When ATPγS was included in order to stabilize filaments (Fig. 6, *E* and *F*), the number of filaments observed with and without the DinI protein was approximately equal. However, the filaments observed on poly(dT) in the absence of DinI exhibited clear discontinuities (Fig. 6*E*), whereas those in the presence of DinI (Fig. 6*F*) did not. Yasuda (17) previously published electron microscopy experiments demonstrating that DinI does not promote measurable disassembly of RecA protein filaments.

### *The RecA C Terminus Affects the DinI-RecA Interaction*

*The RecAΔC17 Mutant Protein Is More Sensitive to DinI Protein Inhibition of DNA Strand Exchange Than the Wild-type RecA Protein*—Comparative DNA strand exchange reactions were carried out to determine how DinI protein affects the activity of the wild-type RecA and RecAΔC17 proteins. Each protein was tested initially under its optimal reaction conditions, which include $Mg^{2+}$ concentrations either identical to (the RecAΔC17 mutant) or in substantial excess of (wild-type RecA) the 3 mM ATP concentration (10). The amount of nicked circular product generated in reactions promoted by the 3 μM wild-type RecA protein in 60 min decreased as the DinI protein concentration was increased from 0 to 50 μM (Fig. 7*A*). A substantial decline was again evident only when an excess of DinI protein (relative to RecA) was added to the reaction. Nearly complete inhibition was seen in the presence of 50 μM DinI protein. In contrast, the same amount of RecAΔC17 mutant protein was unable to promote measurable DNA pairing or strand exchange in the presence of as little as 5 μM DinI protein. The C-terminal truncation mutant is thus much more sensitive to DinI protein than is the wild-type RecA under these conditions.

*A 3′ Overhang on the dsDNA Substrate Enhances RecA Protein-promoted DNA Strand Exchange in the Presence of DinI Protein*—The linear dsDNA substrates utilized in the experiment shown in Fig. 7*A* were prepared by digestion of circular dsDNA with the XhoI endonuclease, which generates 4-nt 5′ overhangs at the ends. An identical experiment was carried out using linear dsDNA substrates with 4-nt 3′ overhangs generated by the PstI endonuclease (Fig. 7*B*). DNA strand exchange promoted by the wild-type RecA protein was only modestly inhibited by DinI. The final yield of strand exchange products was reduced less than 50% by DinI concentrations up to 50 μM when the linear dsDNA substrate contained a 3′ overhang. Furthermore, the RecAΔC17 protein was much less inhibited by DinI than when 5′ overhangs were utilized (Fig. 7*A*).

This "overhang effect," where RecA protein-promoted DNA pairing under suboptimal conditions (*i.e.* low magnesium concentrations) is enhanced when duplex DNA substrates with short single-stranded 3′ overhangs are utilized, was observed previously and ascribed to an effect of the RecA C terminus (10). It is a hallmark of a partially activated RecA conformation now designated as form Ac (10, 41). Taken together, the results in Fig. 7 indicate that the RecA C terminus is protecting RecA from the effects of DinI.

*Magnesium Ion Enhances RecA-promoted DNA Strand Exchange in the Presence of DinI Protein*—The overhang effect described above prompted the investigation of the effect of magnesium ion on the DinI protein inhibition of RecA-promoted DNA strand exchange. The DNA strand exchange reaction promoted by the wild-type RecA or RecAΔC17 proteins in 60 min in the presence of 25 or 10 μM DinI protein, respectively, was assayed as a function of increasing magnesium acetate concentration from 10 to 35 mM (Fig. 8*A*, wild-type (*WT*)) or 3 to 40 mM (Fig. 8*B*, RecAΔC17). At 10 mM magnesium ion, the reaction promoted by the wild-type RecA protein in the presence of 25 μM DinI is quite weak, as seen in Fig. 7*A*. The yield of nicked circular DNA strand exchange products was less than 5%. Increasing the magnesium to 25 mM resulted in a more robust reaction, increasing the yield of nicked circular products in this same reaction series to almost 60% of the input DNA. A somewhat greater increase is observed for the RecAΔC17 protein when the $Mg^{2+}$ concentration is increased from 3 to 10 mM. We therefore repeated the DinI titration for the RecAΔC17 protein-promoted DNA strand exchange in the presence of 10 mM magnesium ion (Fig. 8*C*). At this higher magnesium ion

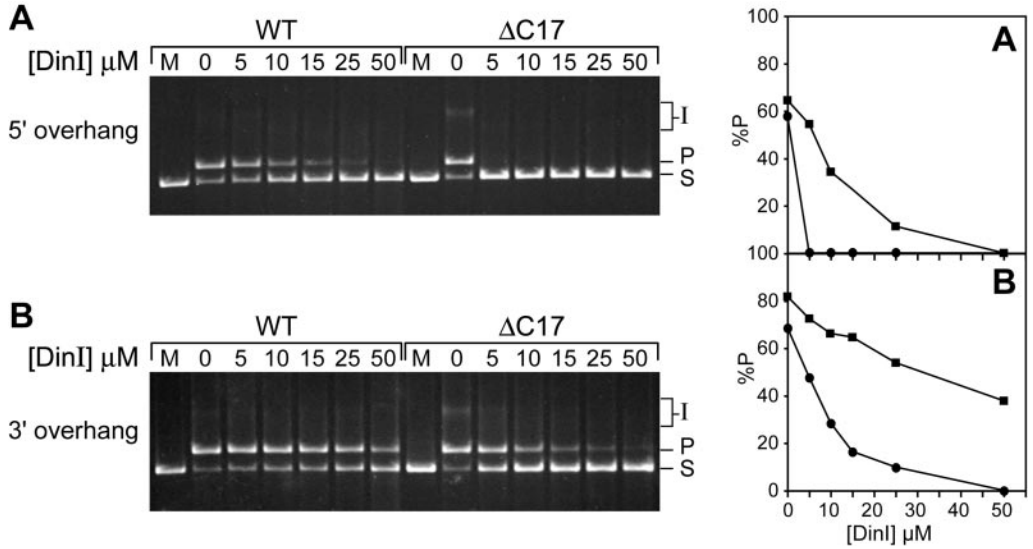



FIG. 7. **The effect of DinI protein on DNA strand exchange reactions promoted by the wild-type and ΔC17 RecA proteins.** The reactions contained 3 $\mu$M RecA protein, 10 $\mu$M circular ssDNA, 10 $\mu$M linear dsDNA, 3 mM ATP, and the amount of DinI protein indicated. The wild-type (*WT*) and RecAΔC17 mutant reactions were carried out with 10 and 3 mM $Mg(OAc)_2$, respectively. DinI protein was added after RecA, ssDNA, and SSB but before the linear duplex DNA. Reactions proceeded for 60 min at 37 °C. *A*, the linear dsDNA substrate (*S*) (shown in the *M* lane) was obtained by digestion with XhoI restriction endonuclease, generating 5′ overhangs. *B*, The linear dsDNA substrate (shown in the *M* lane) was obtained by digestion with PstI restriction endonuclease, generating 3′ overhangs. Joint molecule intermediates and final nicked circular dsDNA products are labeled *I* and *P*, respectively. Quantitation of the strand exchange data, presented as the fraction of total duplex DNA present as dsDNA products, is provided to the *right* of *both panels*. Data from the wild-type and RecAΔC17 mutant proteins are denoted by the symbols ■ and ●, respectively.

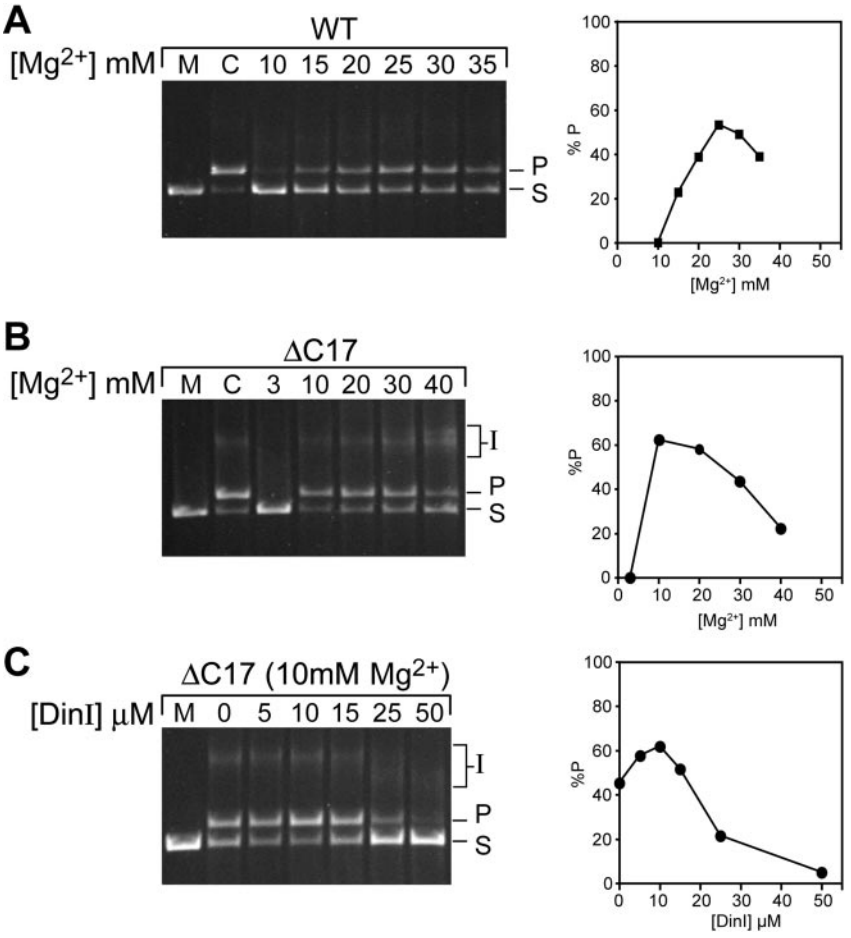
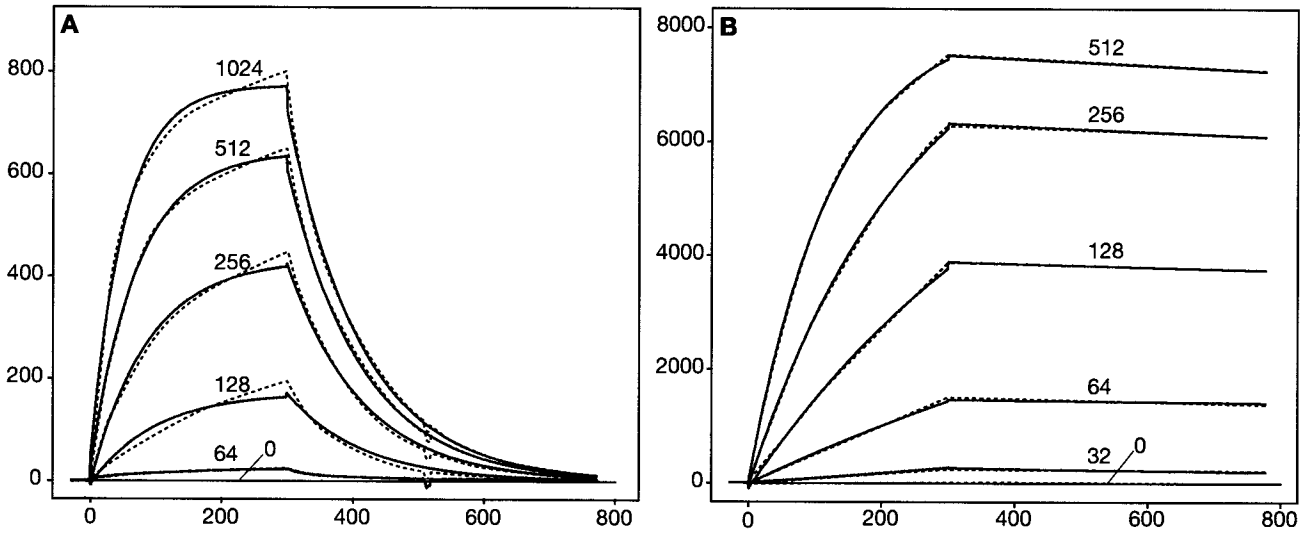



FIG. 8. **Effects of magnesium ion on the DNA strand exchange reactions catalyzed by the wild-type and ΔC17 RecA proteins in the presence of the DinI protein.** Gel labels are as described in the legend to Fig. 7, except as noted otherwise. Reactions contained 3 $\mu$M RecA protein, 3 mM ATP, 10 $\mu$M circular ssDNA, and 10 $\mu$M linear dsDNA *A*, wild-type RecA (*WT*) promoted reaction in the presence of 25 $\mu$M DinI protein and the indicated concentrations of $Mg(OAc)_2$. The lane labeled "*C*" shows an identical DNA strand exchange reaction carried out in 10 mM $Mg(OAc)_2$ in the absence of DinI protein. *B*, RecAΔC17 promoted reaction in the presence of 10 $\mu$M DinI protein and the indicated concentrations of $Mg(OAc)_2$. *Lane C* shows an identical DNA strand exchange reaction carried out in 3 mM $Mg(OAc)_2$ in the absence of DinI protein. *C*, RecAΔC17 promoted DNA strand exchange reaction containing 10 mM $Mg(OAc)_2$ and the concentration of DinI indicated. Quantitation of the data as the fraction of total duplex DNA present as *P* is presented to the *right* of *each panel*. Data from the wild-type and RecAΔC17 mutant proteins are denoted by the symbols ■ and ●, respectively.

concentration, more DinI protein is required to inhibit RecAΔC17 than is required to inhibit the reaction promoted by wild-type RecA protein (compare with Fig. 8*A*).

Under most conditions, the RecAΔC17 mutant protein is more sensitive to DinI protein than is the wild-type protein. The inhibitory effects of DinI are moderated both by added $Mg^{2+}$ ion and by the structure of the DNA ends on the duplex DNA used for the DNA strand exchange reaction.




C

| | $k_a$ ($M^{-1}s^{-1}$) | $k_d$ ($s^{-1}$) | $K_A$ ($M^{-1}$) | $K_D$ (M) |
|---|---|---|---|---|
| **WT RecA** | $1.03 \times 10^4$ | $8.82 \times 10^{-3}$ | $1.17 \times 10^6$ | $8.57 \times 10^{-7}$ |
| **ΔC17 RecA** | $1.56 \times 10^4$ | $7.73 \times 10^{-5}$ | $2.02 \times 10^8$ | $4.96 \times 10^{-9}$ |

FIG. 9. **Interaction between DinI and the wild-type and ΔC17 RecA proteins.** Real-time physical interactions were monitored with a biosensor Biacore 3000 instrument as described under "Experimental Procedures." DinI was immobilized on a CM5 chip to the level of 570 response units, and 0, 64, 128, 256, and 1024 nM wild-type protein (*A*) or 0, 32, 64, 128, 256, and 512 nM ΔC17 RecA protein (*B*) were used as the analyte. *Dotted line curves* represent experimental data. *Solid curves* were obtained by fitting the experimental data to the Langmuir model of interaction. *C*, apparent rate and equilibrium constants of the DinI-RecA interactions.

*Deletion of the 17 C-terminal Amino Acids Drastically Changes the Affinity of RecA to DinI*—We monitored the direct interaction between surface-immobilized DinI (ligand) and solution RecA (analyte) using biosensor technology (Fig. 9). A maximal binding capacity of the surface ($R_{max}$) depends on the molecular weights of the interacting molecules, the amount of immobilized ligand ($R_L$), and the stoichiometry of the interaction ($S_M$) and can be calculated from the following formula.

$$R_{max} = (\text{analyte } M_r/\text{ligand } M_r) \times R_L \times S_M \qquad \text{(Eq. 1)}$$

Assuming that one molecule of DinI can bind only one RecA molecule (17, 18), we calculated a theoretical $R_{max}$ value of 2,410 resonance units for the wild type and 2,272 for RecAΔC17. Fitting the data to the Langmuir binding model produces experimentally derived $R_{max}$ values of 1,660 and 8,320 for 512 nM wild type and 512 nM mutant proteins, respectively. Although the experimental $R_{max}$ for wild-type protein does not exclude 1:1 binding, it is obvious that the apparent stoichiometry of the RecAΔC17-DinI interaction is several times higher. There are two possible explanations for this difference; either RecAΔC17 forms stable oligomers in solution, or once bound to DinI, mutant protein undergoes a rapid polymerization. A comparison of the experimental and simulated curves in Fig. 9, *A* and *B*, shows a much stronger discrepancy between experiment and theory for the wild-type protein compared with the RecAΔC17 mutant. This is especially true for the association phase of the curves. This "misbehavior" of the wild-type protein can be explained by a requirement for complex conformational changes in order to accommodate DinI molecules.

The RecAΔC17-DinI complex is at least 100 times more stable than the wild-type RecA-DinI complex, as can be judged from estimated dissociation equilibrium constants $K_D$ (Fig. 9*C*). This difference in affinity is not determined by a faster formation of the complex by mutant protein (compare association rate constants ($k_a$) in Fig. 9*C*) but rather by a much slower dissociation of the RecAΔC17-DinI complex (Fig. 9*C*, $k_d$ column). Note that the models used to obtain the dissociation constants do not take into account the oligomerization of RecA protein and should be used as a reflection of relative affinity only.

## DISCUSSION

Our major conclusion is that the DinI protein stabilizes RecA filaments. This observation substantially alters our understanding of the potential effects of DinI expression on RecA function. The end-dependent disassembly of RecA filaments is suppressed by DinI and with it the exchange of RecA monomers into and out of established RecA filaments. DinI protein does not stimulate the binding of RecA protein to single-stranded DNA precoated with SSB (Fig. 4), so that suppression of RecA filament disassembly after the filaments are formed appears to be the primary effect of DinI. The stabilization occurs without affecting the intrinsic ATP hydrolytic activity of RecA protein and, indeed, does not affect LexA cleavage or many DNA strand exchange reactions. Combining our findings with other published data, we argue that Din I is not involved in the termination of the SOS response but instead has a more complex role in SOS.

The effects of DinI depend markedly on the concentration of DinI added to the reaction, revealing that the DinI-RecA interaction is highly complex. Stabilization is observed at DinI concentrations that are at or just above the concentrations of RecA protein used in these experiments. The stabilization is not a nonspecific effect of any added protein, as similar amounts of added bovine serum albumin have no detectable effect on RecA filament stability. As its concentration is further increased, DinI begins to destabilize RecA filaments. At very high DinI concentrations (typically 50–100-fold above the RecA concentration), the destabilization effect of DinI is catastrophic for RecA filaments, and virtually all RecA activities are strongly inhibited. These observations reconcile an apparent conflict in

the literature. Some of us previously have described the destabilization of RecA filaments by DinI (16). Ohmori and colleagues (17) reported no effects of DinI on the structure or ATP hydrolytic activities of assembled RecA filaments. The current experiments rationalize both sets of observations, with the apparent discrepancy explained by the use of higher concentrations of DinI in the former study (16) than in the latter (17).

The DinI protein has been presented as a RecA inhibitor that acts to help shut down the SOS response (16, 17, 20, 21). The present results might be interpreted as part of a general negative regulatory scheme. At relatively low DinI concentrations, RecA filaments would be stabilized. This could inhibit the redistribution of RecA protein in the cell to effect DNA repair at different locations. At higher DinI concentrations, RecA filaments would be destabilized, removing a key signal for maintaining the SOS response.

However, we must now entertain several alternatives to the view of DinI as a negative modulator that terminates the overall SOS response. The DinI protein is expressed early in the SOS response, not late (16). This is not the pattern that one would expect for a protein involved in SOS termination. Ohmori and colleagues (21) have reported that RecA-mediated LexA cleavage after UV irradiation is greatly inhibited in cells in which DinI protein is highly overexpressed from a multicopy plasmid. However, when the DinI protein is expressed normally from the single copy gene on the chromosome, there is no difference in the LexA cleavage patterns observed in *dinI*$^-$ *versus dinI*$^+$ cells (21). It is thus not clear that DinI concentrations ever reach the levels required to destabilize RecA filaments during the SOS response in wild-type cells or that DinI ever inhibits LexA cleavage in those cells. *In vitro*, when DinI is present at concentrations that we find stabilize RecA filaments, the autocatalytic cleavage of the UmuD protein is inhibited, but the cleavage of the LexA protein is not (21). We have confirmed the inability of DinI protein to inhibit LexA cleavage *in vitro* under the conditions in which DinI stabilizes RecA filaments.[2] It is notable that the only observation implicating DinI protein in a shutdown of the entire SOS response is the inhibition of SOS induction seen when DinI is highly overexpressed from a multicopy plasmid (20, 21).

At the lower levels of DinI that might be obtained when it is expressed from its chromosomal gene, DinI may act within the SOS system more as a highly selective governor than a brake. Expressed early in the SOS response, a primary role may be to inhibit the cleavage of UmuD protein and thereby delay the onset of mutagenic translesion bypass DNA replication by DNA polymerase V. At the same time, LexA cleavage would be left unaffected so that other aspects of the SOS response could proceed. The bound DinI protein inhibits the initiation of DNA strand exchange only when certain types of DNA ends are presented to the RecA filament (Fig. 7). Ongoing DNA strand exchange is not affected by DinI (16). DinI may thus modulate certain aspects of the recombinational DNA repair reactions promoted by RecA during the SOS response without eliminating them. With its filament-stabilizing capacity, DinI may actually enhance some RecA functions.

We do not know why DinI switches from a stabilizing to a destabilizing effect in a concentration-dependent manner, but a working hypothesis might go as follows. DinI-RecA interaction occurs in two modes. The higher affinity interaction, occurring at lower DinI concentrations, stabilizes RecA filaments. A second, lower affinity interaction at higher DinI concentrations destabilizes the filaments. Based on the limited work published to date, it is tempting to speculate further that a change in the oligomeric form of DinI could contribute to the change in DinI function we observe. Although the oligomeric form of DinI in solution has not been characterized in detail, it appears to exist primarily as a monomer (16, 18). A tendency for DinI to dimerize at quite high concentrations has been reported in two studies (16, 18). Alternatively, the destabilization of RecA filaments by high concentrations of DinI could reflect an ionic effect mediated by the negatively charged C terminus of DinI, as described below.

Recently, an NMR study was carried out on RecA-DNA complexes in the presence of the DinI protein (18). When 8- or 12-mer oligonucleotides were bound by the RecA protein, a broadening of the NMR signals was observed when DinI protein was present. The authors (18) suggest that RecA affinity for DNA and possibly the characteristic exchange of RecA monomers bound to DNA is altered in the presence of DinI. Our current data support this hypothesis.

An understanding of the transition from the stabilizing mode of DinI-RecA interaction to the destabilizing mode will require more detailed molecular analysis of these interactions. To begin such an analysis, we also demonstrate that the interaction between DinI and RecA filaments involves, at least in part, the C terminus of the RecA protein. The RecA C terminus has a protective effect on RecA protein, reducing the inhibition conferred by DinI. However, the comparison in Fig 7*A* has the complication that the wild-type and mutant proteins were both assayed at their optimal reaction conditions. The wild-type RecA protein requires 6–8 mM free $Mg^{2+}$ concentrations (above and beyond the $Mg^{2+}$ needed to chelate the available ATP) to promote strand exchange, whereas the RecAΔC17 protein does not (10). Addition of $Mg^{2+}$ to the RecAΔC17 reaction greatly moderates the effect of DinI on this mutant protein. However, the lower $Mg^{2+}$ concentration may be more relevant to the metabolic conditions *in vivo* (10), and the effect of DinI on the mutant protein under these conditions is dramatic. Further, the binding of DinI to RecA protein is clearly enhanced by the C-terminal deletion when the wild-type and mutant proteins are compared by surface plasmon resonance (Fig. 9). Previous work has shown that the functional state of RecA filaments is modulated by the RecA C terminus and by added $Mg^{2+}$ (3, 10, 41). Because the RecA C terminus and the $Mg^{2+}$ concentration also modulate the effects of DinI, it is likely that DinI has an altered affinity for different RecA functional states.

The selective binding of DinI to particular filament states could play an important role in the effects of DinI on RecA function *in vivo*. As already noted, the effects of DinI on the recombination functions of RecA are complex, and they may telegraph the functional state to which DinI binds most avidly. DinI protein primarily appears to inhibit the initiation of DNA strand exchange. When added to an ongoing DNA strand exchange reaction, DinI has little effect on the generation of products (16). DinI acts to inhibit the entry of duplex DNA into the RecA-ssDNA filament. However, duplexes with 3′ single strand extensions are blocked much less efficiently. A 3′ extension-mediated enhancement of the initiation of DNA strand exchange can also be seen with the wild-type RecA protein when suboptimal low $Mg^{2+}$ conditions are used (10). At low $Mg^{2+}$ concentrations, the C terminus of the wild-type protein restricts access to the filament by duplex but not ssDNA ends. This functional state of RecA protein has been designated Ac (activated/closed) (10, 41). As shown by the data in Fig. 7*B*, the binding of DinI protein to a RecA filament appears to have a similar effect. From this observation, we speculate that the DinI protein may be bound at a site normally occupied by the RecA C terminus under the low $Mg^{2+}$

[2] S. Lusetti and M. Cox, unpublished results.

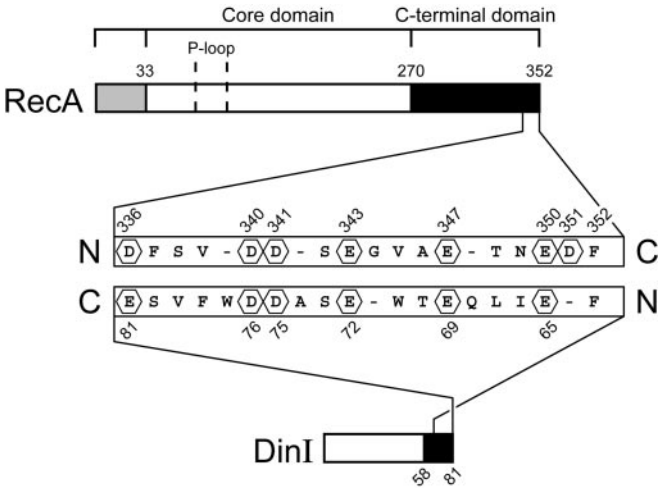



FIG. 10. **Similarity between the C termini of the RecA and DinI proteins.** At the *top* is a linear sequence representation of the *E. coli* RecA protein. The core domain contains the ATP binding motif (*P-loop*). The N- and C-terminal domains are the *shaded* and *black regions*, respectively. The primary sequence of the last 17 amino acids (residues 336–352) of RecA are expanded directly below. The *hexagons* highlight the acidic residues in this region. This RecA C terminus is aligned with the C-terminal 18 amino acids (residues 64–81) of the *E. coli* DinI protein. Note that the DinI sequence runs C- to N-terminally from *left* to *right*.

conditions. In the wild-type RecA protein, added $Mg^{2+}$ activates the RecA protein and eliminates the selective advantage of 3′ ssDNA extensions in the initiation of strand exchange (10). Similarly, added $Mg^{2+}$ appears to moderate the effects of the DinI protein (Fig. 8).

The C terminus of DinI, like the C terminus of RecA, is replete with amino acid residues that would contribute negative charges (Fig. 10). It has previously been suggested that the C terminus of DinI protein might bind within the RecA filament groove (16). The sequence of the C terminus of the DinI protein is also suggestive of a somewhat different model, one in which this segment of the protein might act as a competitor of the RecA C terminus in its effects on RecA function. The sequence alignment is especially intriguing if the DinI sequence is inverted in orientation relative to the RecA sequence (Fig. 10). The evident charge conservation in this comparison could provide a basis for DinI interactions with RecA. Of course, it is also possible that the RecA C terminus acts to repel DinI protein and to partially screen RecA filaments from its effects. The high concentration of negative charges in the DinI C terminus might also have a role in the destabilizing effect of DinI at high concentrations, producing a deleterious ionic effect.

More work will be required to determine whether the stabilization of RecA filaments by DinI is simply a consequence of the binding of DinI to RecA or a part of the overall mechanism by which DinI modulates RecA function. The combined results do indicate a robust interaction between the RecA and DinI proteins, with functional consequences that we have just begun to explore.

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