Proc. Natl. Acad. Sci. USA
Vol. 78, No. 10, pp. 6061–6065, October 1981
Biochemistry

tif-1 mutation alters polynucleotide recognition by the recA protein of *Escherichia coli*

(ATPase/oligonucleotide/λ repressor cleavage/cold-sensitive revertant/SOS induction)

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Communicated by I. Robert Lehman, June 26, 1981

ABSTRACT **The requirements for polynucleotide-dependent hydrolysis of ATP and for proteolytic cleavage of phage λ repressor have been examined for both the wild-type (recA$^+$ protein) and the tif-1 mutant form [tif(recA) protein] of the *recA* gene product. The recA$^+$ and tif(recA) proteins catalyze both reactions in the presence of long single-stranded DNAs or certain deoxyhomopolymers. However, short oligonucleotides [$(dT)_{12}$, $(dA)_{14}$] stimulate neither the protease nor the ATPase activities of the recA$^+$ protein. In contrast, these short oligonucleotides activate tif(recA) protein to cleave λ repressor without stimulating its ATPase activity. Moreover, both the ATPase and protease activities of the tif(recA) protein are stimulated by poly(rU) and poly(rC) whereas the recA$^+$ protein does not respond to these ribopolymers. We have purified the recA protein from a strain in which the tif mutation is intragenically suppressed. This mutant protein (recA629) is inactive in the presence of $(dT)_{12}$, $(dA)_{14}$, poly(rU), and poly(rC) for λ repressor cleavage and ATP hydrolysis. These results argue that the the *tif*-1 mutation (or mutations) alters the DNA binding site of the recA protein. We suggest that *in vivo* the tif(recA) protein is activated for cleaving repressors of SOS genes by complex formation with short single-stranded regions or gaps that normally occur near the growing fork of replicating chromosomes and are too short for activating the recA$^+$ enzyme. This mechanism can account for the expression of SOS functions in the absence of DNA damage in *tif* mutant strains.**

The molecular mechanism of λ induction involves proteolytic cleavage of the phage-encoded repressor protein (cI protein). Both *in vivo* and *in vitro*, this proteolytic inactivation requires recA protein, a multifunctional enzyme that catalyzes DNA-dependent ATP hydrolysis, ATP-dependent reassociation of complementary single strands of DNA, and pairing of single strands with homologous duplex DNA segments (strand assimilation and exchange) (1–6). *In vivo*, recA protein is also required for coordinately regulating expression of several cellular functions in response to DNA damage that includes mutagenesis, enhanced error-free DNA repair, inhibition of septation during cell division, colicin induction, increased expression of its own gene, and at least five additional genetic elements (7–9). Genetic and biochemical evidence support the idea that expression of these functions (SOS functions) ensues from the proteolytic inactivation of one or more bacterial repressors controlling their expression in a manner analogous to the destruction of λ repressor (7, 8). Little *et al.* (10) have shown that, *in vivo* and *in vitro*, recA protein is needed to cleave lexA protein, the repressor of the *recA* gene, and possibly several other SOS functions.

Craig and Roberts (11) have shown that λ repressor is cleaved *in vitro* into two fragments in a reaction requiring recA protein, ATP [or the phosphothiolate analog, adenosine-5′-*O*-(3-thiotriphosphate) (ATP-γ-S), and polynucleotide. Their results demonstrate that recA protein must be activated to cleave λ repressor by binding single-stranded polynucleotide. The active form of the enzyme for proteolysis *in vitro* and *in vivo* was inferred to be a recA protein–DNA complex. *In vivo*, the single-stranded DNA effectors are likely to be found in the form of gaps that result from excision repair or from blocking chromosomal replication at sites of DNA damage (12). Thus, the wide variety of DNA-damaging agents and DNA synthesis inhibitors that stimulate the "SOS response" in *Escherichia coli* presumably do so by creating or stabilizing single-stranded regions in the chromosome.

The studies of Roberts *et al.* (2) and Craig and Roberts (11) used a mutant form of the recA protein, tif(recA). The *tif*-1 missense mutation partially uncouples regulation of SOS activities from DNA damage. at 30°C, prophage induction and expression of other SOS functions in *tif*-1 strains depend on DNA damage or arrest of DNA synthesis, as in *recA*$^+$ strains. However, at 42°C, *tif*-1 mutant cells express these activities constitutively (7). Moreover, strains carrying an *spr* mutation in the *lexA* gene and a *tif*-1 allele are constitutive for SOS functions at all temperatures. Thus, *in vivo*, the tif(recA) protein can be activated without DNA damage although, *in vitro*, purified tif(recA) protein absolutely requires a polynucleotide cofactor for endopeptidase activity (11). Taken together, these results suggest that other factors or DNA structures in the cell might preferentially activate tif(recA) protein but would be unable to activate recA$^+$ protein.

This hypothesis has been tested *in vitro* using extensively purified recA$^+$ and tif(recA) enzymes and different polynucleotide cofactors. The results indicate that tif(recA) protein is uniquely able to recognize short oligonucleotides and certain other polynucleotides that are inactive with recA$^+$ protein and suggest a model for the differential activation of tif(recA) protein for cleaving λ repressor and other SOS repressors *in vivo*.

MATERIALS AND METHODS

The wild-type (recA$^+$) and tif(recA) proteins were purified from strains KM1842 (4) and DM1187 (13), respectively, by ATP elution from DNA cellulose as described (14). The enzymes are >98% pure as judged by Coomassie blue staining after electrophoresis in $NaDodSO_4$/polyacrylamide gels. The recA629 protein was purified as described (4). λ repressor, purified by the method of Reichardt (15), was a gift of A. D. Kaiser. ATP hy-

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Abbreviations: ATP-γ-S, GTP-γ-S, and UTP-γ-S, adenosine-, guanosine-, and uridine-5′-*O*-(3-thiotriphosphate), respectively; φXRFI, covalently-closed duplex replicative form of φX174 DNA.

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drolysis was measured as described (4). The conditions for repressor cleavage and analysis of the reaction products by polyacrylamide gel electrophoresis will be described in detail elsewhere. For the experiments reported here, the reaction mixtures (30 μl) were 20 mM Tris·HCl, pH 7.5/1 mM dithiothreitol/30 mM NaCl/3 mM $MgCl_2$/330 μM ATP-γ-S or 3 mM $MnCl_2$/500 μM ATP/ ≈3 μM λ repressor/35 μM polynucleotide/15 μM recA or tif(recA) protein. Incubations were performed at 37°C for 2 hr. Oligonucleotides and polynucleotides were from P-L Biochemicals and GIBCO. ATP-γ-S and guanosine-5′-*O*-(3-thiotriphosphate) (GTP-γ-S) were from Boehringer Mannheim; uridine-5′-*O*-(3-thiotriphosphate) (UTP-γ-S) was generously provided by Fritz Eckstein, Max Planck Institute for Experimental Medicine.

RESULTS

Polynucleotide Dependence of ATP Hydrolysis Catalyzed by recA$^+$ and tif(recA) Proteins. Both the recA$^+$ and the tif(recA) proteins hydrolyze ATP to ADP and P_i in the presence of single-stranded DNA or deoxyhomopolymers (refs. 3, 4, 11; Fig. 1). In the case of the recA$^+$ protein, the initial rate of ATP hydrolysis is similar with φX174 viral DNA, poly(dT), poly(dU), poly(dC), and poly(dA). Under the conditions of these experiments, the turnover number [(mol of ADP formed/mol of recA protein)/min] is 18 at 37°C, a value somewhat higher than previously reported (unpublished). With either poly(dT) or poly(dU) as cofactor, the extent of ATP hydrolysis by the recA$^+$ protein was only 50% whereas more than 80% of the ATP was hydrolyzed by the recA$^+$ protein in the presence of φX174 DNA or poly(dA). No further hydrolysis occurred in the presence of poly(dT) after 2 hr of incubation at 37°C even though the protein was stable under these conditions (data not shown). We detect little or no hydrolysis with poly(dG), presumably because this polynucleotide does not bind the recA$^+$ protein (unpublished). Unlike their deoxy counterparts, ribo homopolymers fail to stimulate ATP hydrolysis by recA$^+$ protein although poly(rC) stimulates it slightly (Fig. 1*C*). However, both the rate and extent of poly(rC)-stimulated ATP hydrolysis are significantly lower than with any deoxyhomopolymer. As previously reported, short defined-length oligonucleotides [$(dT)_{16}$, $(dA)_{14}$] fail to stimulate ATP hydrolysis by recA$^+$ protein (ref. 11, unpublished). Duplex circular DNA (φXRFI) stimulates ATP hydrolysis by recA$^+$ protein after a lag of 20 min.

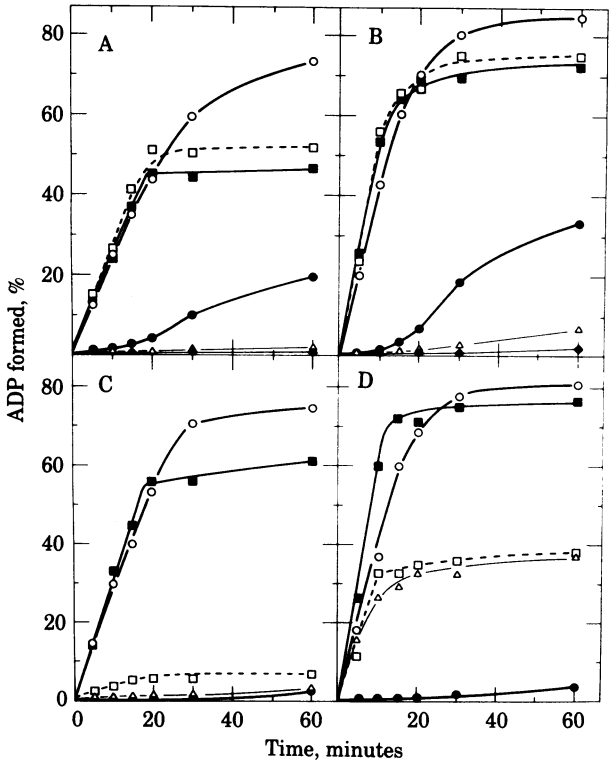

FIG. 1. Kinetics of ATP hydrolysis catalyzed by recA$^+$ and tif(recA) proteins. Reaction mixtures (60 μl) were 20 mM KCl/20 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$/0.5 mM EDTA/1 mM dithiothreitol/500 μM [^{3}H]ATP (67 μCi/ml; 1 Ci = 3.7 × 10^{10} becquerels)/21 μM polynucleotide or oligonucleotide/0.75 μM recA$^+$ protein (*A* and *C*) or 0.83 μM tif(recA) protein (*B* and *D*) in 1.5-ml plastic microcentrifuge tubes (Eppendorf). The reactions were started by addition of enzyme, and 1-μl samples were taken at the indicated times, applied to polyethyleneimine cellulose strips (Polygram MN300), and developed by ascending chromatography as described. In the absence of any polynucleotide cofactor, <1.2% of the ATP was hydrolyzed by either recA$^+$ or tif(recA) protein. (*A* and *B*) ○, φX174 single-stranded DNA; ●, φX174 RFI DNA; △, poly(rA); ▲, $(dT)_{16}$; ■, poly(dU); □, poly(dT). (*C* and *D*) ○, poly(dA); ●, $(dA)_{14}$; △, poly(rU); □, poly(rC); ■, poly(dC).

The tif(recA) enzyme responds differently to several of these polynucleotide cofactors. The ATPase activity of the mutant enzyme is stimulated by φX174 viral DNA, poly(dT), poly(dC), and poly(dU) as with the recA$^+$ enzyme, although both the rate and extent of ATP hydrolysis are nearly identical with these polynucleotides. The lower extent of ATP hydrolysis catalyzed by recA$^+$ protein compared with that catalyzed by tif(recA) protein in the presence of poly(dT) or poly(dU) may be due to differential sensitivity of the enzymes to ADP inhibition, as suggested by other experiments (see below). Although unable to stimulate the ATPase of recA$^+$ enzyme, both poly(rC) and poly(rU) stimulate ATP hydrolysis by the tif(recA) protein. Approximately 30% of the ATP is hydrolyzed within 10 min with an initial rate that is comparable with those of the deoxyribohomopolymer-stimulated reactions [turnover no. = 23 (mol of ADP/mol of recA protein)/min]. Nevertheless, the extent of hydrolysis does not exceed 40% of the initial ATP in the reaction, although the enzyme remains active for the duration of the incubation (data not shown). The ATPase activity of the tif(recA) protein is not stimulated by poly(rA) or poly(rG), a result that is similar to that for the recA$^+$ enzyme. Furthermore, tRNA does not stimulate ATP hydrolysis by tif(recA) protein (data not shown).

Short oligonucleotides such as $(dT)_{16}$ and $(dA)_{14}$ are poor effectors of tif(recA) protein ATPase activity, although a slight stimulation can be detected. Duplex DNA (φXRFI) stimulates ATP hydrolysis catalyzed by the tif(recA) enzyme, with a lag in the kinetics as observed with the recA$^+$ enzyme (see Fig. 1*A* and *B*).

Competition binding studies indicate that both tif(recA) and recA$^+$ proteins bind both ribo- and deoxyribo-homopolymers (unpublished results). Thus, although the recA$^+$ protein binds poly(rU) and poly(rC), the complex formed is incompetent for ATP hydrolysis. In contrast, the tif(recA) mutant enzyme forms a complex with these ribohomopolymers that results in significant ATP hydrolysis. In the case of poly(rA), which binds to both proteins (data not shown), no stimulation of ATP hydrolysis is observed. Therefore, polynucleotide binding by the recA$^+$ or the tif(recA) protein is necessary but not sufficient for ATP hydrolysis.

Polynucleotide Size Requirement for λ Repressor Cleavage. Craig and Roberts (11) have shown that short oligonucleotides [$(dT)_9$, $(dA)_{16}$] serve as cofactors for the cleavage of λ repressor by tif(recA) protein. As shown in Fig. 2*A*, the tif(recA) protein uses the oligonucleotides $(dT)_{12}$, $(dT)_{16}$, $(dA)_{10}$, $(dA)_{12}$, and $(dA)_{14}$ as cofactors for λ-repressor cleavage. In the absence of any oligonucleotide, no cleavage of λ repressor can be de-

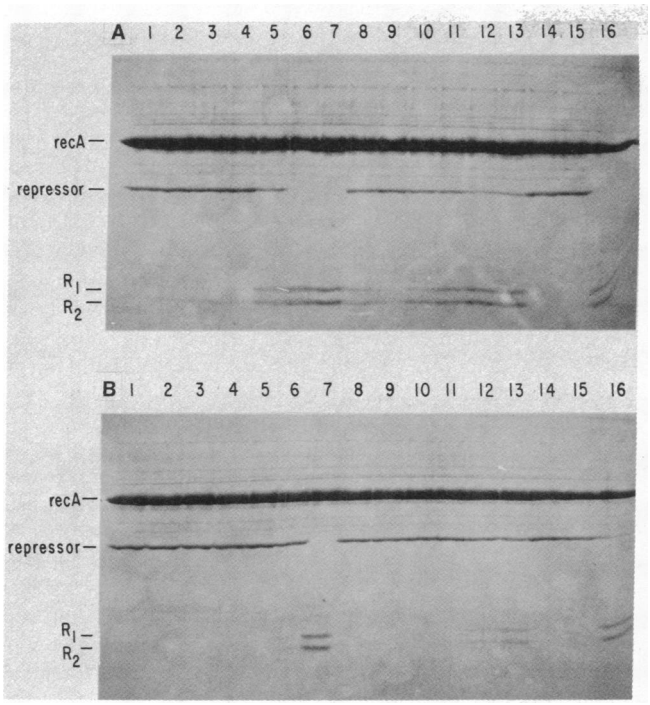

FIG. 2. Effect of polynucleotide chain length on λ-repressor cleavage by tif(recA) or recA$^+$ protein. The faint protein bands are contaminants in the λ-repressor preparation, which is ≈70% pure. (*A*) tif(recA) protein. (*B*) recA$^+$ protein. Lanes: 1, no polynucleotide; 2, $(dT)_4$; 3, $(dT)_8$; 4, $(dT)_{10}$; 5, $(dT)_{12}$; 6, $(dT)_{16}$; 7, poly(dT) (≈2000 residues per chain); 8, $(dA)_7$; 9, $(dA)_8$; 10, $(dA)_{10}$; 11, $(dA)_{12}$; 12, $(dA)_{14}$; 13, poly(dA); 14, $(rA)_2$; 15, $(rA)_6$; 16, poly(rA) (40–50 residues per chain). R_1 and R_2, cleavage fragments.

tected (<5%). Under the reaction conditions used in these experiments, which differ from those of Craig and Roberts (11), neither the tetranucleotide, $(dT)_4$, nor the octanucleotide, $(dT)_8$, stimulates repressor cleavage by the tif(recA) protein, whereas long-chain polynucleotides such as poly(dT), poly(dA), and poly(rA) effectively stimulate repressor cleavage (Fig. 3).

In striking contrast, the recA$^+$ protein is unable to cleave λ repressor in the presence of the oligonucleotides, $(dT)_{12}$ and $(dT)_{16}$, that stimulate the tif(recA) protein-dependent cleavage

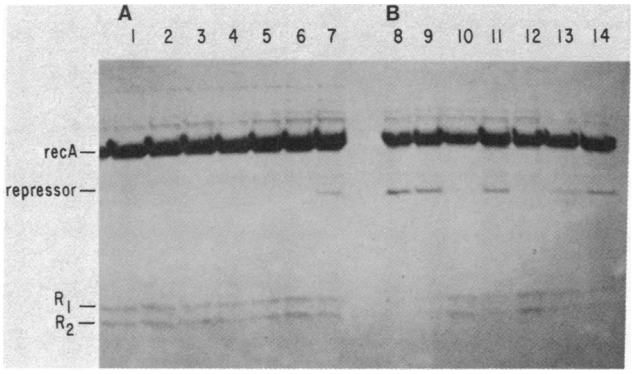

FIG. 3. Effects of various polynucleotides on repressor cleavage by tif(recA) (*A*) and recA$^+$ (*B*) proteins. Cleavage reactions were performed as described in *Materials and Methods*, except that 300 μM UTP-γ-S or GTP-γ-S was substituted for ATP-γ-S where indicated. Lanes: 1 and 8, poly(rC); 2 and 9, poly(rU); 3 and 10, poly(dU); 4 and 11, poly(rA); 5 and 12, φX single-stranded DNA; 6 and 13, φX DNA and UTP-γ-S; 7 and 14, φX single-stranded DNA and GTP-γ-S. R_1 and R_2, cleavage fragments.

reaction. Incubating λ repressor up to 5 hr with recA$^+$ protein and $(dT)_{16}$ results in cleavage of <10% of the repressor (data not shown), even though the recA$^+$ protein is in excess (≈5–7 recA protein monomers per repressor monomer). Moreover, $(dA)_{14}$ only weakly stimulates repressor cleavage by the recA$^+$ protein. Comparison of the results shown in Fig. 2 *A* and *B* indicates that the tif(recA) protein is considerably more active than the recA$^+$ protein for cleaving λ repressor when short oligonucleotides are used as cofactors. However, in the presence of the longer polynucleotides, both proteins show comparable levels (within a factor of two) of cleaving activity for λ repressor. Polynucleotides such as poly(dT), poly(dA), and poly(rA) stimulate repressor cleavage by the recA$^+$ protein under conditions in which the oligonucleotides are inert (Fig. 2*B*). As in the case of tif(recA) protein, recA$^+$ protein-directed cleavage of λ repressor is absolutely dependent on polynucleotide. Although the data of Fig. 2 *A* and *B* do not allow accurate comparison of the specific activities of the mutant and wild-type recA proteins with the longer polynucleotides, kinetic data suggest that, in the presence of poly(dT), the tif(recA) protein is approximately twice as active as the recA$^+$ protein for cleaving λ repressor.

The differential effect of short oligonucleotides on the recA$^+$ and tif(recA) proteins has also been observed using the reaction conditions described by Craig and Roberts (11) (data not shown). We believe the differences in repressor-cleavage activity between these proteins in the presence of short oligonucleotide cofactors reflect differences in their abilities to recognize short single-stranded DNA segments. Despite this obvious difference in repressor-cleavage activity neither recA$^+$ nor tif(recA) protein efficiently recognizes these oligonucleotides as cofactors for catalyzing ATP hydrolysis (refs. 11, 16; Fig. 1*A* and *B*).

Effects of Ribohomopolymers on Repressor Cleavage by recA$^+$ and tif(recA) Proteins. The ability of various polynucleotides to stimulate repressor cleavage by the recA$^+$ and tif(recA) enzymes was examined (Fig. 3). The recA$^+$ protein cleaves λ repressor in the presence of φX174 single-stranded DNA, poly(dU), poly(dC), poly(dT), and poly(rA). No repressor cleavage was detected (<5%) when poly(rC) or poly(rU) was incubated with the recA$^+$ protein under the same conditions. The set of polynucleotides that stimulate repressor cleavage by the recA$^+$ protein also stimulates repressor cleavage by the tif(recA) enzyme. Moreover, both poly(rU) and poly(rC) stimulate repressor cleavage by the tif-(recA) protein (Fig. 3). These results are consistent with the idea that the tif(recA) mutation alters the ability of the recA gene product to interact with certain oligonucleotides and polynucleotides. Thus, the tif(recA) protein forms a complex with poly(rU) and poly(rC) that is productive for both ATP hydrolysis and λ-repressor cleavage. Although the recA$^+$ protein binds both poly(rU) and poly(rC) and forms stable complexes with these polynucleotides in the presence of ATP-γ-S (data not shown), this association does not lead to λ-repressor cleavage. Mixing experiments with recA$^+$ protein, tif(recA) protein, and poly(rU) indicate that the recA$^+$ protein does not inhibit repressor cleavage directed by the tif(recA) enzyme in the presence of this polynucleotide (data not shown), demonstrating that the inability of poly(rU) to stimulate repressor cleavage by the recA$^+$ protein is not due to the presence of an inhibitor in the recA$^+$ protein preparation.

Polynucleotide Requirements for recA629 Protein-Directed Cleavage of λ Repressor. Is the activation *in vitro* of tif(recA) protease by certain ribohomopolymers and oligonucleotides related to the mechanism of λ induction *in vivo*? To answer this question, we examined the properties of the recA protein isolated from a strain carrying an intragenic suppressor mutation (recA629) of *tif*-1 that prevents λ-prophage induction at 42°C. In addition, the *rec*A629 mutation renders cells cold sensitive

for homologous recombination and repair of UV damage and produces a recA protein that is cold labile for DNA strand-pairing reactions *in vitro* (ref. 4; unpublished). The *rec*A629 mutant protein was purified to >85% homogeneity and examined for its ability to cleave λ repressor under conditions in which the "parental" tif(recA) protein is active. As shown in Fig. 4, the recA629 protein fails to cleave λ repressor in the presence of short oligonucleotides [e.g., $(dT)_{12}$, $(dT)_{16}$, $(dA)_{12}$] or ribohomopolymers [e.g., poly(rC) and poly(rU)]. However, in the presence of poly(dT) or φX174 DNA, it is active. The *rec*A629 protein does not catalyze ATP hydrolysis in the presence of either poly(rU) or poly(rC) (data not shown), although both φX174 single-stranded DNA and poly(dT) stimulate the ATPase of this mutant enzyme. Thus, "reversion" of the Tif phenotype *in vivo* is correlated with alterations in polynucleotide and oligonucleotide recognition by the *rec*A gene product.

Effects of Nucleoside Diphosphates and Analogs on λ-Repressor Cleavage by recA$^+$ and tif(recA) Proteins. Several recA protein-dependent reactions (ATPase, strand assimilation, and strand reassociation) are sensitive to nucleoside diphosphates and dTTP (5). The ATP hydrolysis data (Fig. 1) suggested that the tif(recA) enzyme might be less sensitive to inhibition by nucleoside diphosphates (ADP and UDP) and dTTP. As shown in Fig. 5, this hypothesis was confirmed for the repressor-cleavage activity. The tif(recA) protein is relatively insensitive to inhibition and retains most of its activity in the presence of 660 μM ADP, UDP, or dTTP, whereas the recA$^+$ protein is substantially inhibited under the same conditions. These results are consistent with the notion that ADP, UDP, and dTTP bind more tightly to the recA$^+$ protein than to the tif(recA) enzyme in the presence of polynucleotide.

We have also observed that UTP-γ-S substitutes for ATP-γ-S in the repressor-cleavage reaction (Fig. 3, lanes 6 and 13). UTP-γ-S also promotes formation of stable recA protein–DNA complexes (unpublished) and competitively inhibits the ATPase activity of recA$^+$ protein (data not shown). GTP-γ-S substitutes partially for ATP-γ-S in the cleavage reaction, although the rate of cleavage is reduced (Fig. 3, lanes 7 and 14; data not shown). GTP-γ-S partially blocks binding of ATP-γ-S to the recA protein (unpublished), indicating that it binds to the enzyme at or near the same site as the ATP analog.

DISCUSSION

Several differences between the enzymatic properties of the recA$^+$ protein and the mutationally altered tif(recA) protein are documented in this paper. Although the ATPase activities of both proteins are stimulated by relatively long single-stranded DNA or deoxyhomopolymers, the recA$^+$ protein fails to hydrolyze ATP in the presence of ribohomopolymers such as poly(rU) and poly(rC) whereas the tif(recA) protein hydrolyzes ATP in the presence of these polynucleotides. The initial rate of ATP hydrolysis catalyzed by tif(recA) protein in the presence of these ribohomopolymers is near that seen in the presence of φX174 single-stranded DNA. Nevertheless, the extent of ATP hydrolysis is only 50%, while >80% of the ATP is hydrolyzed when deoxyhomopolymers are used as cofactors. Under these reaction conditions, tif(recA) protein is stable for several hours, suggesting that premature cessation of hydrolysis is not due to enzyme inactivation.

Neither recA$^+$ nor tif(recA) protein catalyzes significant hydrolysis of ATP in the presence of oligonucleotides such as $(dT)_{16}$ or $(dA)_{14}$. However, the tif(recA) enzyme is uniquely able to use short oligonucleotides as cofactors for cleaving λ repressor. Craig and Roberts (11) have reported that the rate of repressor cleavage by tif(recA) protein in the presence of oligonucleotides such as $(dA)_{16}$ is ≈50% of the rate of cleavage in the presence of long polynucleotide cofactors. The findings reported here are not inconsistent with their results although only the extents of cleavage were determined in this work. Using shorter incubation periods and less recA protein, we estimate that the tif(recA) protein is at least 20–50 times more active than the recA$^+$ protein for cleaving λ repressor in the presence of oligonucleotides. These proteins display comparable protease activities when long deoxypolynucleotides are used as cofactors.

Both poly(rU) and poly(rC) stimulate λ-repressor cleavage by the tif(recA) enzyme. In contrast, these polynucleotides are inactive as cofactors for the recA$^+$ protein-directed cleavage of repressor. This difference cannot be due to differential binding of these polynucleotides to tif(recA) protein compared with recA$^+$ protein as we have shown that poly(rU) and poly(rC) compete with single-stranded DNA for recA protein binding. Mixing experiments indicate that recA$^+$ protein does not inhibit activation of tif-1(recA) protein by these polynucleotides. We conclude that the recA$^+$ protein does not contain an inhibitor but may in fact form active multimers with the tif(recA) subunits.

We also note that the tif(recA) enzyme is less sensitive to inhibition by nucleoside diphosphates (UDP, ADP) and the nucleoside triphosphate, dTTP. This observation is not incompatible with the notion that the *tif*-1 mutation alters the DNA

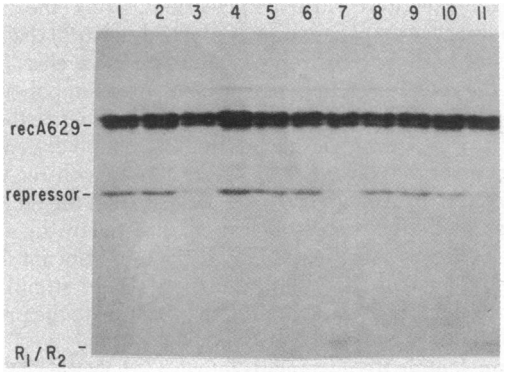

FIG. 4. Polynucleotide-dependent repressor cleavage by recA629 protein. Incubations were performed as described in *Materials and Methods* except that ≈15 μM recA629 protein and 33 μM polynucleotide were used. Lanes: 1, $(dT)_{12}$; 2, $(dT)_{16}$; 3, poly(dT); 4, $(dA)_{12}$; 5, poly(dA); 6, poly(rA); 7, poly(dU); 8, poly(rU); 9, poly(rC); 10, poly(dC); 11, φX single-stranded DNA. The cleavage fragments R_1 and R_2 are not well stained and appear at the bottom of the polyacrylamide gel.

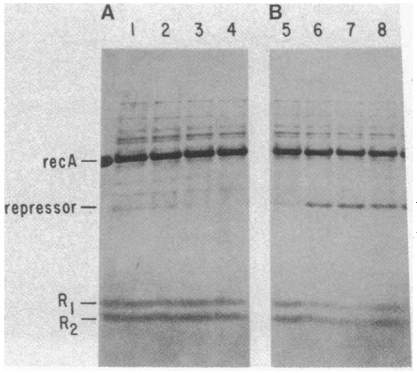

FIG. 5. Inhibition of repressor cleavage by ADP, UDP, and dTTP. Repressor cleavage experiments were performed as described in *Materials and Methods* except that 5 μM tif(recA) (*A*) protein or recA$^+$ (*B*) protein and 13.5 μM φX single-stranded DNA were used. Lanes: 1 and 5, no additions; 2 and 6, 660 μM ADP; 3 and 7, 660 μM UDP; 4 and 8, 660 μM dTTP. R_1 and R_2, cleavage fragments.

binding site of the recA protein because polynucleotides alter the affinity of the recA protein for binding the ATP analog ATP-γ-S and the UTP analog UTP-γ-S (unpublished observations). Thus, changes in the polynucleotide binding site could indirectly effect nucleoside triphosphate and diphosphate binding.

A model has been proposed for induction of λ prophage and SOS functions in *tif*-1 mutant strains in the absence of DNA damage (8). According to this model, activation of the tif form of the recA protein does not require an effector but results from a temperature-induced conformational change in the tif(recA) protein. The *in vitro* results of Craig and Roberts (11) and those described here indicate that tif (recA) protein-directed cleavage of λ repressor, and presumably other repressors of SOS functions (e.g., lexA protein), requires a polynucleotide cofactor for activity. Evidence has been presented that, for both $recA^+$ and tif(recA) proteins, the active proteolytic form is an enzyme–DNA complex (11). Our results indicate that the tif(recA) protein can be activated *in vitro* by polynucleotides [poly(rU) and poly(rC)] and oligonucleotides that do not activate $recA^+$ protein under identical conditions. Moreover, we have shown that intragenic suppression of the *tif* mutation *in vivo* produces an altered recA protein that no longer recognizes these polymers as effectors for λ-repressor cleavage or ATP hydrolysis. We conclude that the Tif phenotype results from qualitative and perhaps quantitative alterations in DNA binding by the *rec*A gene product.

Which DNA cofactor is likely to be important *in vivo* for constitutive expression of SOS function and λ-prophage induction? We suggest that short single-stranded regions in replicating chromosomes can bind and activate the tif(recA) protein but cannot activate the $recA^+$ enzyme in a manner analogous to the role of short oligonucleotides *in vitro*. These single-stranded regions are likely to be in and around the replication fork and, based on *in vitro* results, are probably 10–20 nucleotides long. This model predicts that the tif(recA) enzyme can be activated *in vivo* only in cells actively replicating (or repairing) their DNA. Consistent with this idea is the observation that the Tif phenotype can be suppressed in *dna*A mutant cells at 42°C when chromosomes are fully replicated and no new replication forks can initiate (16). According to this model, suppression would be achieved by eliminating the cellular effector of the enzyme. The enhanced expression of tif(recA) at 42°C may indicate that the enzyme binds more tightly to these single-stranded regions at the high temperature or that these single-stranded regions are more accessible at 42°C.

Our results indicate that the tif(recA) enzyme is not activated for λ-repressor cleavage by oligonucleotides shorter than 10 residues. Although we have not examined a wide variety of reaction conditions or oligonucleotide effectors, we have found no evidence indicating that that tif(recA) protein cleaves λ repressor in response to very short oligonucleotides such as dimers or trimers. Irbe *et al.* (17) have shown that the dinucleotides d(A-G) and d(G-G) stimulate φ80 prophage induction in a permeable system. Their results, however, do not show that these dinucleotides are direct effectors of the recA protein for induction.

The *rec*A629 mutation, an intragenic suppressor of *tif*-1, renders the recA protein cold labile for DNA-pairing reactions (ref. 4; unpublished). Moreover, the mutant protein is unable to recognize short oligonucleotides or ribohomopolymers as cofactors for repressor cleavage. Although the *rec*A629 mutant is conditionally deficient in recombination and DNA-damage repair functions, it shows detectable levels of spontaneous prophage induction at 30°C, where it displays a Rec^- phenotype (unpublished results). Therefore, a second-site suppressor mutant selected for loss of *tif*-1-mediated induction of λ prophage produces a recA protein that no longer responds to oligonucleotides as cofactors for cleaving λ repressor. Moreover, the recA629 protein does not promote λ-repressor cleavage or hydrolyze ATP in the presence of poly(rU) or poly(rC). The change in polynucleotide-recognition properties of the recA protein resulting from the *tif*-1 mutation and the reversion of these properties in the *rec*A629 strain argues that these mutations are localized in the DNA binding domain of the recA polypeptide chain. This hypothesis can be directly tested by further reversion studies of the *tif*-1 and *rec*A629 mutations, as well as by direct DNA and protein sequence analyses.

We gratefully acknowledge the support and interest of I. R. Lehman. K.M. wishes to thank Ms. Judy Kojima for her assistance. K.M. was a Fellow of the American Cancer Society (California Chapter) and G.M.W. was supported by a Bank of America–A. P. Giannini Fellowship. This work was supported by grants from the National Institutes of Health (GM 06196) and the National Science Foundation (PCM 74-00865).

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