

Site-directed Mutagenesis of the RecA Protein of *Escherichia coli*

TYROSINE 264 IS REQUIRED FOR EFFICIENT ATP HYDROLYSIS AND STRAND EXCHANGE BUT NOT FOR LexA REPRESSOR INACTIVATION*

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The role of Tyr²⁶⁴ in nucleotide binding and hydrolysis catalyzed by the RecA protein of *Escherichia coli* was investigated by constructing Gly, Ser, and Phe substitution mutations using oligonucleotide-directed mutagenesis. The corresponding mutant *recA* genes neither restored resistance to killing by ultraviolet irradiation nor increased homologous recombination in a *recA* strain. The purified RecA(Gly²⁶⁴) protein was unable to bind nucleotide, hydrolyze ATP, or form stable ternary complexes with adenosine 5'-O-thiotriphosphate and DNA although the mutant protein bound DNA normally in the absence of nucleotide. The RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins hydrolyzed ATP poorly and the rates were reduced approximately 8- and 18-fold, respectively. Although capable of low levels of ATP hydrolysis, neither the RecA(Phe²⁶⁴) nor the RecA(Ser²⁶⁴) protein promoted DNA pairing or strand exchange reactions *in vitro*. Furthermore, these mutant RecA proteins were impaired in their ability to form salt-resistant ternary complexes with adenosine 5'-O-thiotriphosphate and DNA as judged by filter binding. Nevertheless, nucleoprotein complexes formed with either RecA(Phe²⁶⁴) or RecA(Ser²⁶⁴) protein directed efficient cleavage of LexA repressor *in vitro*. These results demonstrate that Tyr²⁶⁴ is required for efficient ATP hydrolysis and for homologous pairing of DNA but does not participate in activating RecA protein for LexA repressor autodigestion.

Despite its relatively modest size (approximately 38 kDa), the RecA protein of the enterobacterium *Escherichia coli* is a remarkably complex enzyme. *In vivo* the protein is required for mediating one or more early steps in the process of homologous recombination as well as for controlling expres-

sion of a coordinated cellular response to DNA damage, the SOS response (1, 2). *In vitro* the purified protein catalyzes several reactions which include (i) DNA-dependent nucleoside triphosphate hydrolysis; (ii) ATP-stimulated annealing of homologous single-stranded DNAs (3); (iii) an ATP-dependent three-strand exchange reaction (4, 5); and (iv) ATP-dependent stimulation of autoproteolysis by the LexA, λ CI, and umuD proteins (6–9). In all of these reactions ATP serves either as a substrate (i) or as an effector (ii–iv). For example, ATP hydrolysis greatly stimulates annealing of single-stranded DNA chains as well as extensive strand exchange between duplex and homologous single-stranded DNAs, whereas only ATP binding appears to be necessary for RecA protein to promote limited pairing of homologous segments in the formation of synaptic intermediates and to facilitate the autodigestion of repressors and UmuD protein.

Biochemical studies have provided information regarding the interaction of ATP with RecA protein. Certain nucleotides induce structural changes in RecA protein as judged by changes in the susceptibility of the protein to protease cleavage (10) and by electron microscopy (11). RecA protein binds tightly to DNA in the presence of ATP (12) and ADP stimulates dissociation of the RecA protein-DNA complexes (13). The nonhydrolyzable ATP analog, ATP γ S,¹ promotes formation of salt-resistant ternary complexes of RecA protein, nucleotide, and single-stranded DNA. In these stable nucleoprotein complexes, ATP γ S is noncovalently bound and dissociation of RecA protein is blocked (14, 15). There appears to be a single ATP-binding site/RecA protein monomer (16) and, based upon hydrolysis competition experiments, it is likely that this site participates in the hydrolysis of several nucleoside triphosphates (17, 18). Using a photoaffinity analog of ATP, N₃ATP, a single tyrosine residue was identified which has been proposed to constitute part of the ATP-binding domain (19). This same tyrosine, Tyr²⁶⁴, was covalently modified by another ATP analog, 5'-*para*-fluorosulfonylbenzoyl-adenosine, which irreversibly inhibits ATP hydrolysis by RecA protein (20). These labeling and modification results suggest that Tyr²⁶⁴ plays an important role in mediating protein-nucleotide interactions in RecA protein. This tyrosine residue is conserved among more than a dozen RecA proteins from different Gram-negative bacteria, although phenylalanine is substituted at this position in at least two other bacterial species (21, 22).

In order to elucidate the role of Tyr²⁶⁴ in the interaction of

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¹ The abbreviations used are: ATP γ S, adenosine 5'-O-(thiotriphosphate); DTT, dithiothreitol; SDS, sodium dodecyl sulfate; RFI, RFII, and RFIII, the circular double-stranded replicative form derivatives of M13 which are covalently closed, nicked, and linear, respectively; N₃ATP, 9-(3'-azido-3'-deoxy- β -D-xylofuranosyl)adenine 5'-triphosphate.

RecA protein with ATP, we have constructed, using site-directed mutagenesis techniques, *recA* genes containing substitutions of this tyrosine codon and have characterized the properties of the resultant RecA enzymes *in vivo* as well as *in vitro*. The mutant proteins containing phenylalanine, serine, or glycine replacements are partly or completely deficient in hydrolysis of ATP although binding of the nucleotide is not seriously affected for the RecA(Phe) and RecA(Ser) proteins. Significantly, the mutant RecA proteins are impaired in their ability to form stable ternary complexes with DNA and ATP- γ S and are unable to catalyze homologous exchange *in vivo* or to promote strand transfer or DNA annealing *in vitro*. Nevertheless, the RecA(Phe) and RecA(Ser) proteins induce efficient cleavage of LexA repressor in solution. These results indicate that Tyr²⁶⁴ is required for the formation of presynaptic RecA-DNA complexes but is dispensable for the formation of "co-protease" complexes.

EXPERIMENTAL PROCEDURES

Materials—RecA protein was purified to homogeneity from *E. coli* strain KM1842 as described (23) and aliquots were stored at -70°C in R buffer (20 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 1 mM dithiothreitol) containing 25% glycerol. RecA protein concentrations were calculated using a value of $\epsilon_{280}^{1\%} = 5.16$.

LexA protein was a generous gift of Dr. John Little (Department of Biochemistry and Molecular and Cellular Biology, University of Arizona, Tucson). Phage P22 DNA and ³H-labeled P22 DNA were generously provided by James Halbrook of this laboratory. Unlabeled ATP, ADP, and S₁ nuclease were purchased from Sigma; ³H-labeled ATP was obtained from Amersham Corp. and [α -³²P]N₃ATP was purchased from ICN. The ATP analog, adenosine 5'-O-(thiotriphosphate) (ATP- γ S), was purchased from Boehringer Mannheim. The oligonucleotide-directed *in vitro* mutagenesis kit, based on the phosphorothiolate substitution procedure of Eckstein (24), was purchased from Amersham Corp. Nitrocellulose filters (0.45 μm) were obtained from Schleicher and Schuell. Restriction enzymes and Polymin P were from Bethesda Research Laboratories. Phosphocellulose (P11) and GF/C filters were from Whatman. Bacteriophage M13 RFI and single-stranded DNAs were prepared as described (25).

Oligonucleotide-directed Mutagenesis of the *recA* Gene—The 3.0-kilobase *Bam*HI restriction fragment containing the wild type *recA* gene was excised from plasmid pBR*recA* and ligated into the unique *Bam*HI site of M13mp8 RFI DNA. Recombinant phage containing the fragment were isolated (mp8*recA*) and high titer phage stocks were prepared by polyethylene glycol precipitation of the supernatant and purified by equilibrium banding in a CsCl gradient. Viral DNA was isolated from purified phage by repeated phenol extraction in the presence of SDS (0.5%) and was precipitated by addition of 2 volumes of absolute ethanol (-20°C). The orientation of the insert in the recombinant phage was determined by restriction endonuclease cleavage of the RFI DNA and by hybridization with strand-specific oligonucleotides. The purified mp8*recA* DNAs were spotted onto nitrocellulose filters in high salt, baked at 80°C for 2 h, and hybridized with 5'-³²P-end-labeled oligonucleotides that were complementary to coding or noncoding strand of the *recA* gene.

The oligonucleotides used to prepare the mutationally altered *recA* genes were, Phe²⁶⁴: AGATCCTCTTCGGCGAAG; Ser²⁶⁴: AGATCCTCTCCGGCGAAG; and Gly²⁶⁴: TCCAGATCCTCGGCGCGAAGGTAT. All mutagenic oligonucleotides as well as the DNA sequencing primer, GACATCCGTCGTATCGGC, which annealed approximately 120 base pairs upstream from the site of mutagenesis were prepared using a Du Pont Generator DNA synthesizer. The mutagenic oligonucleotides were phosphorylated at the 5' end using T₄ polynucleotide kinase and annealed to the mp8*recA* viral template. The three mutant *recA* genes were prepared according to the protocols of the Amersham *In vitro* Mutagenesis System. Phage containing each of the *recA* mutations were identified by picking several individual plaques from each of the mutagenesis reactions, preparing single-stranded DNA, and sequencing the region of interest. Isolates containing the appropriate allele were identified from each mutagenesis and the *recA* genes were completely sequenced in order to confirm that no additional sequence changes other than at the targeted Tyr²⁶⁴ codon had been introduced during the mutagenesis procedure.

Subcloning Wild Type and Mutant *recA* Genes—For each of the

recA mutant-containing phages, the corresponding RFI DNA was prepared by buoyant density centrifugation in a CsCl gradient containing ethidium bromide, and digested with *Bam*HI to liberate the 3.0-kilobase *recA*-containing fragment. After electrophoresis in low melting temperature agarose, the fragments were excised, purified, and ligated into *Bam*HI-digested pBR322 DNA to yield plasmids pBR*recA*(Phe²⁶⁴), pBR*recA*(Ser²⁶⁴), and pBR*recA*(Gly²⁶⁴). Following purification in CsCl EtBr gradients, the inserts were confirmed by a final round of dideoxy sequencing using a double-stranded sequencing protocol (26). Plasmids were transformed into competent *E. coli* strains DM1187 Δ 21, and JC14604 containing deletions of the *recA* gene and ampicillin-resistant cells were selected on LB agar containing 50 $\mu\text{g}/\text{ml}$ ampicillin (LB-amp) (27).

Measurement of Cell Survival Following UV Irradiation—Cells were grown in LB-amp liquid medium to a density of $2-4 \times 10^8$ cells/ml, collected by centrifugation, and resuspended at a density of 10^8 cells/ml in M9 salts (28). Cell suspensions were irradiated for the indicated time using a low pressure mercury lamp (GE G8T5). Following UV treatment, cells were placed on ice in the dark, serially diluted, and spread onto LB-amp plates. Survival was determined after incubation for 24 h at 37°C . The fraction surviving was determined by dividing the number of survivors at a given UV dose by the number of survivors in the unirradiated sample and correcting for the dilution factor.

Semiquantitative Measure of Recombination Proficiency—Recombination was measured in *E. coli* strain JC14604 which contains two defective copies of the *lacZ* gene. The mutations are nonidentical and noncomplementing and therefore the cells are Lac⁻. Lac⁺ cells arise by homologous recombination between *lac* alleles when a functional *recA* gene is expressed. Cultures of strain JC14604 transformed with either the mutant *recA* plasmids, pBR*recA*⁺, or pBR322 were spread onto Lactose MacConkey plates containing ampicillin (50 $\mu\text{g}/\text{ml}$). Plates were incubated for 2 days at 37°C and Lac⁺ papillae were counted.

Purification of RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) Proteins—Strain DM1187 Δ 21 was transformed with each of the pBR*recA* mutant plasmids and recloned isolates were used to inoculate 5 liters of LB-amp medium. Cultures were grown to saturation and cells were collected by centrifugation and washed in 1 volume of TE (0.01 M Tris-HCl, pH 7.5, 1 mM EDTA). Cell pellets (16 g wet weight) were resuspended in a solution of 50 mM Tris-HCl (pH 7.5) and 25% sucrose (24 ml). Mutant RecA proteins were purified to greater than 95% purity following the procedure developed for wild type RecA protein (23) with the following modifications: NaCl gradients (0–250 mM in R buffer) were used to elute the mutant proteins from single-stranded DNA cellulose. The purity of the final material was assessed by silver staining of polyacrylamide gels (11%) containing 4 μg of each RecA protein.

N₃ATP Photoaffinity Labeling of RecA Proteins—Covalent attachment of N₃ATP to the purified wild type and mutant RecA proteins was performed as previously described (29) in reactions containing 74 μM [α -³²P]N₃ATP and 21 μM RecA protein.

ATP Hydrolysis Assay—Hydrolysis of [³H]ATP was measured as previously described (30). Rate measurements for wild type RecA protein were performed using 2 μM RecA protein, 103 μM heat-denatured calf thymus DNA, and the ATP concentrations indicated in the figure. For initial velocity measurements, the reaction mixture was incubated at 37°C and hydrolysis was initiated by addition of RecA protein. Aliquots (5 μl) were removed after 0, 20, 40, 60, and 150 s of incubation and were mixed with an equal volume of ice-cold EDTA (25 mM) to stop hydrolysis. Samples (1 μl) were spotted onto polyethyleneimine-cellulose thin layer plates and developed by ascending chromatography. Reactions using the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins contained 2 μM enzyme, 103 μM heat-denatured calf thymus DNA, and the following ATP concentrations; for RecA(Phe²⁶⁴): 25, 50, 75, 100, 200, and 500 μM ; and for RecA(Ser²⁶⁴): 75, 100, 200, 300, and 400 μM . Reaction mixtures were incubated at 37°C and samples (1 μl) were taken at 0, 2.5, 5, 10, 20, and 30 min, spotted onto polyethyleneimine-cellulose plates and developed as described above.

S₁ Nuclease Assay for DNA Reannealing—The ability of the RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) proteins to catalyze reannealing of denatured DNA was measured essentially as described by Weinstock *et al.* (31) using S₁ nuclease. Wild type or mutant RecA protein (2 μM) was incubated with 40 μM heat-denatured ³H-labeled phage P22 DNA in renaturation buffer (20 mM Tris-HCl, pH 7.5, 10 mM KCl, 10 mM MgCl₂, 1 mM dithiothreitol, 0.1 mM EDTA, and 3% (v/v) glycerol) in the presence of 1 mM ATP at 37°C . The final

volume was 110 μ l. Aliquots (20 μ l) were removed at the times indicated and added to 10% SDS (2.5 μ l) followed by addition of S₁ digestion buffer (270 μ l, 75 mM Na acetate, pH 4.6, 150 mM NaCl, 1 mM zinc acetate) and 45 units of S₁ nuclease. Digestions were performed at 37 °C for 30 min and stopped by addition of 20 μ g of heat-denatured calf thymus DNA and cold trichloroacetic acid (10%, 1 ml). Nuclease-resistant ³H-labeled DNA was precipitated, collected on Whatman GF/C filters, and radioactivity was determined by liquid scintillation counting. The values presented in the figures for each of the RecA proteins were corrected for renaturation in the absence of added enzyme.

DNA Strand Exchange Reaction—DNA strand exchange reactions were performed in Tris acetate buffer (pH 7.5) and contained 16.8 μ M linear duplex M13 DNA, 9.9 μ M circular single-stranded M13 DNA, 1 mM ATP, 0.9 μ M single-strand binding protein, and 6 μ M RecA protein (either the wild type or mutant). The reaction mixtures (40 μ l) also contained 0.08 units of pyruvate kinase and 10 mM phosphoenolpyruvate for ATP regeneration. Preincubation of the reaction mixture (minus RecA protein and single-strand binding protein) was performed at 37 °C for 2 min and the reaction was started by addition of RecA protein and single-strand binding protein. After 90 min the reactions were stopped by addition of 4.6 μ l of 10% SDS, and the reaction products were separated by electrophoresis in 1% agarose gels and visualized by ethidium bromide staining.

Following staining and photography, the DNA in the gel was transferred to nitrocellulose, hybridized with nick-translated M13 RFIII DNA, washed, and placed under film (Cronex) for autoradiography as previously described (32).

³H-Labeled DNA-binding Assay—The ability of RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) to bind ³H-labeled M13 circular single-stranded DNA in the absence and presence of ATP γ S (1 mM) was measured as previously described (33). Reaction mixtures (100 μ l) contained 9 μ M wild type or mutant RecA protein and 210 μ M ³H-labeled M13 single-stranded DNA (2×10^4 cpm/nmol) in binding buffer (20 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 1 mM dithiothreitol, 0.5 mM EDTA, and 3% (v/v) glycerol). Reactions were incubated at 37 °C for 30 min and applied to alkaline-treated nitrocellulose filters under gentle suction. Filters were washed with low salt buffer (R buffer containing 50 mM NaCl, 3 ml) or high salt buffer (R buffer containing 500 mM NaCl, 3 ml) and subsequently washed with low salt buffer (2 ml). The filters were dried and the amount of radioactivity was determined by liquid scintillation counting.

³⁵S-Labeled ATP γ S-binding Assay—Binding of ³⁵S-labeled ATP γ S to the RecA proteins was measured in the presence and absence of M13 single-stranded DNA (101 μ M) using conditions modified from those used to measure single-stranded DNA binding. Reaction mixtures (50 μ l) contained 2.6 μ M wild type or mutant RecA protein and 3.7 μ M [³⁵S]ATP γ S (0.33 μ Ci/nmol) in the buffers described above. After incubating at 37 °C for 30 min, samples were applied to alkaline-treated nitrocellulose filters under gentle suction and washed with a low salt buffer solution (R buffer containing 30 mM NaCl, 4 ml) or high salt buffer (R buffer containing 1 M NaCl, 2 ml) followed by washing with low salt buffer (2 ml). Filters were dried and radioactivity was determined by liquid scintillation counting.

RecA Protein-dependent Cleavage of LexA Repressor—LexA repressor cleavage was measured in the presence or absence of ATP γ S (0.5 mM) in buffer containing: 20 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM dithiothreitol and 30 mM NaCl. Reaction mixtures (100 μ l) contained 5 μ g of wild type or mutant RecA protein, 1.2 μ g of M13 single-stranded DNA, and digestion was started by the addition of LexA repressor (20 μ g). Incubations were at 37 °C for the indicated times and samples (20 μ l) were removed, added to 10 μ l of gel loading buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 5% β -mercaptoethanol, 0.001% bromophenol blue, and 10% (v/v) glycerol) and heated at 100 °C for 3 min. Samples were analyzed by electrophoresis in polyacrylamide gels containing SDS and the proteins were visualized by silver staining.

RESULTS

Construction of Substitution Mutations at Codon 264 in the *recA* gene—The 3.0-kilobase *Bam*HI fragment containing the entire coding and regulatory regions of the *E. coli recA* gene was cloned into M13mp8 and the viral DNA was used for oligonucleotide-directed mutagenesis of codon 264 using the method of Eckstein (24). As shown in Fig. 1, the TAC tyrosine codon was changed to a TTC triplet encoding phenylalanine,

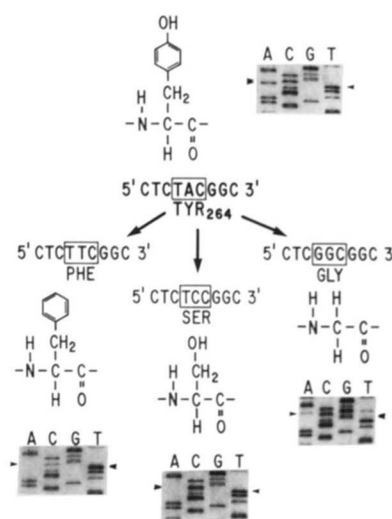


FIG. 1. Site-directed mutagenesis of codon 264 of the *recA* gene. The method of Nakamaye and Eckstein (24) was used to introduce single- and double-base substitution mutations at the TAC codon specifying Tyr²⁶⁴. The mutagenic oligonucleotides used for the construction of these mutations are described under "Experimental Procedures." The DNA sequencing ladders corresponding to the regions containing the mutations are shown.

to a TCC triplet encoding serine, and to a GGC triplet encoding glycine. One candidate phage from each mutagenesis was picked, its DNA purified and analyzed by dideoxy sequencing. All three desired mutations were identified in this initial screening. The mutant *recA* genes, designated *recA*(Phe²⁶⁴), *recA*(Ser²⁶⁴), and *recA*(Gly²⁶⁴) were completely sequenced to verify that only the desired nucleotide substitutions had been introduced into the gene during the mutagenesis procedure. The *Bam*HI fragments containing the mutant *recA* alleles were subsequently subcloned into plasmid vector pBR322 to produce plasmids pBR*recA*(Phe²⁶⁴), pBR*recA*(Ser²⁶⁴), and pBR*recA*(Gly²⁶⁴). A pBR322 plasmid containing the wild type *recA* gene on a *Bam* fragment was also prepared and was designated pBR*recA*(Tyr²⁶⁴).

In Vivo Characterization of *recA* Mutants—Strain JC14604 containing a deletion of the *recA* gene was transformed with each of the pBR*recA* plasmids. All transformants showed similar rates of growth. Crude protein fractions were prepared from each of the strains, separated by electrophoresis in a polyacrylamide gel containing SDS, transferred to nitrocellulose, and hybridized with a polyclonal antibody prepared against wild type RecA protein for Western analysis (data not shown). These results demonstrated that each of the transformants produced significant levels of the plasmid-encoded RecA protein.

The pBR*recA* plasmids were tested for their ability to complement the repair deficiency of strain JC14604 by measuring survival of cells following UV irradiation. The level of survival of strain JC14604 is extremely low after exposure to UV (Fig. 2). A derivative containing the pBR*recA*(Tyr²⁶⁴) plasmid was dramatically more resistant to killing as expected. At the highest UV dose examined (540 s) the *recA*⁺ plasmid-containing strain was approximately 4×10^4 -fold more resistant to killing than the strain containing vector pBR322. The pBR*recA*(Phe²⁶⁴), pBR*recA*(Ser²⁶⁴), and pBR*recA*(Gly²⁶⁴) plasmids were unable to confer UV resistance to the *recA*⁺ parent strain and the transformants were as sensitive to killing as the JC14604 (pBR322).

Recombination proficiency was also determined in the JC14604 strain background by measuring the relative frequency of Lac⁺ papillae formed as a result of recombination

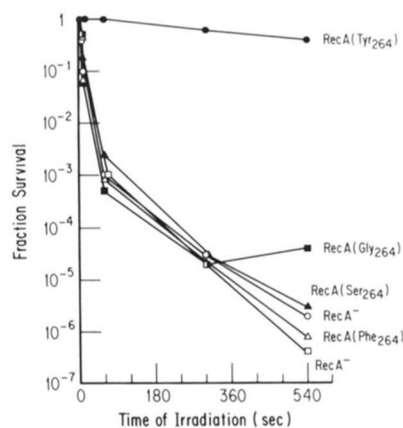


FIG. 2. The pBRrecA(Phe²⁶⁴), pBRrecA(Ser²⁶⁴), and pBRrecA(Gly²⁶⁴) plasmids do not restore UV resistance to a *recA*⁻ strain. Strain JC14604 containing plasmids pBRrecA(Tyr²⁶⁴) (wild type RecA), pBRrecA(Phe²⁶⁴), pBRrecA(Ser²⁶⁴), pBRrecA(Gly²⁶⁴), or pBR322 were grown at 37 °C in LB-amp medium, suspended in M9 salts, and irradiated for the indicated times with a low pressure Hg lamp. Survivors were measured by plating serial dilutions of cells onto LB-amp plates and incubating for 2 days at 37 °C. The open circle and open square correspond to two different transformants of strain JC14604 containing pBR322 plasmid.

TABLE I
Effects of tyrosine substitution mutations
on lac recombination in strain JC14604

Plasmid	Lac ⁺ papillae
pBR322	<10 ⁻⁸
pBRrecA ⁺	≥10 ⁻² –10 ⁻¹
pBRrecA(Phe ²⁶⁴)	<10 ⁻⁸
pBRrecA(Ser ²⁶⁴)	<10 ⁻⁸
pBRrecA(Gly ²⁶⁴)	<10 ⁻⁸

between the duplicated *lacZ* regions in this strain. As shown in Table I, cells transformed with the wild type *recA* gene on pBR322 became recombination proficient and produced approximately 10³ Lac⁺ papillae/10⁵ cells after 2 days at 37 °C. No Lac⁺ papillae were seen on MacConkey plates spread with strain JC14604 containing plasmid pBR322. The pBRrecA(Phe²⁶⁴), pBRrecA(Ser²⁶⁴), and pBRrecA(Gly²⁶⁴) plasmids did not restore recombination proficiency to this strain based on the observation that no Lac⁺ cells were detected when up to 10⁸ cells were spread onto indicator plates. We conclude that the *recA* mutations at position 264 are unable to restore repair or recombination proficiency in a *recA* strain.

Purification of RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) Proteins—Plasmids containing the *recA* mutations were transformed into strain DM1187Δ21 which contains a deletion of the *recA* gene and inactive LexA repressor (*lexA51*). The RecA proteins were purified from 5 liters of cells as described under "Experimental Procedures." The final yields of enzyme were approximately 28 mg of RecA(Phe²⁶⁴), 20 mg of RecA(Ser²⁶⁴), and 1.5 mg of RecA(Gly²⁶⁴). Four micrograms of the purified material was fractionated by electrophoresis in polyacrylamide gels containing SDS and stained with silver to assess purity of the material. All three proteins were greater than 95% homogeneous and no individual contaminant represented more than 1% of the protein (data not shown).

Photoaffinity Labeling of RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) with [³²P]N₃ATP—Photoaffinity labeling of mutant RecA proteins was performed in order to investigate whether these proteins could bind ATP. The conditions for labeling with [³²P]N₃ATP were those developed in this laboratory (29). Labeling of the protein was determined by

autoradiography and more quantitative measurements were made by excising the RecA protein bands from polyacrylamide gels and determining the associated radioactivity by scintillation counting (right panel of Fig. 3). As shown in Fig. 3, both the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) were labeled by this analog whereas labeling of the RecA(Gly²⁶⁴) protein was extremely low. Interestingly, the RecA(Phe²⁶⁴) protein was labeled to a greater extent (0.09 mol of N₃ATP/mol of RecA) than was obtained for the wild type RecA protein (0.06 mol of N₃ATP/mol of RecA) after 120 s of irradiation. The RecA(Ser²⁶⁴) protein reached a maximal incorporation of 0.028 mol of N₃ATP/mol of protein, whereas the RecA(Gly²⁶⁴) protein showed essentially background levels of incorporation. In these experiments we did not detect any labeling of the minor protein contaminants in any of the RecA protein preparations and all of the incorporated label co-migrated with the RecA protein in polyacrylamide gels (data not shown). Moreover, covalent attachment of N₃ATP to the RecA proteins required UV treatment (data not shown). The results of these experiments suggest that the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins bound ATP whereas the RecA(Gly²⁶⁴) was unable to bind nucleotide efficiently.

ATP Hydrolysis Catalyzed by the RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) Proteins—The ability of the mutant RecA proteins to catalyze ATP hydrolysis was measured in the presence and absence of single-stranded M13 DNA. The results (Fig. 4) indicate that both the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins catalyzed low but significant levels of ATP hydrolysis that was dependent upon the presence of a DNA cofactor. The level of ATP hydrolysis catalyzed by the RecA(Gly²⁶⁴) protein was the same in the absence or presence of DNA and this level was comparable to that measured for the wild type RecA protein in the absence of DNA. The rates of ATP hydrolysis catalyzed by the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins were 0.75 and 0.50 mol of ADP/mol of enzyme/min, respectively, which were 17- and 26-fold lower than RecA(Tyr²⁶⁴) protein measured under the same conditions.

The kinetics of DNA-dependent ATP hydrolysis were more carefully examined for RecA(Tyr²⁶⁴), RecA(Phe²⁶⁴), and RecA(Ser²⁶⁴) proteins and the values of *K_m*, *V_{max}*, and *k_{cat}*

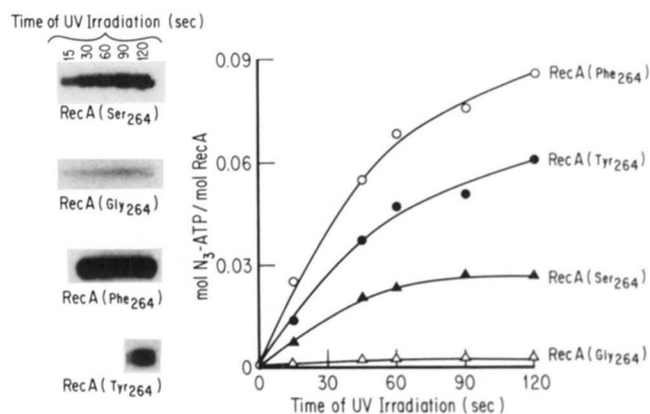


FIG. 3. Photoaffinity labeling of RecA proteins with N₃ATP. Reaction mixtures (110 μl) contained 74 μM γ-³²P-labeled N₃ATP and 21 μM RecA protein (wild type or mutant). Other reaction components, and conditions for UV cross-linking are described under "Experimental Procedures." Aliquots (6.3 μl) were withdrawn at the indicated times and analyzed for the extent of labeling. The results of two separate experiments are shown. The left panel contains autoradiographs of RecA proteins after different periods of cross-linking. The data on the right were obtained by excising the labeled RecA protein band from polyacrylamide gels and determining radioactivity by liquid scintillation counting.

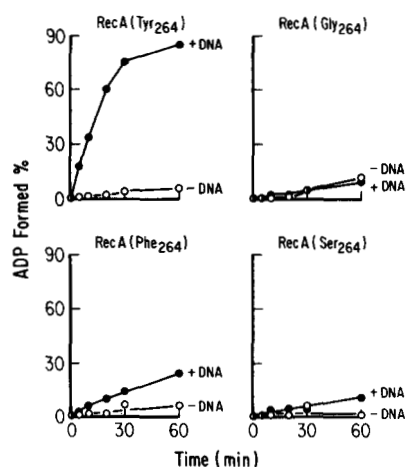


FIG. 4. Hydrolysis of ATP catalyzed by RecA(Tyr²⁶⁴), RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) proteins. Reaction conditions and assay procedures were as described under "Experimental Procedures." Reaction mixtures (60 μ l) contained 2 μ M wild type or mutant RecA protein and 500 μ M [³H]ATP. M13 single-stranded DNA was present at 21 μ M (closed circles). Open symbols indicate hydrolysis in the absence of DNA.

were determined from Eadie-Hofstee plots of the kinetic data (Fig. 5 and Table II). The K_m values for the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins were 2- and 5-fold higher than that for wild type RecA. The higher K_m value for the RecA(Phe²⁶⁴) protein was interesting in view of the N₃ATP photoaffinity labeling results indicating that this enzyme bound nucleotide more efficiently. It should be noted, however, that the photo-labeling was performed in the absence of DNA which has been shown to modulate the binding affinity of ATP (12).

The substitution of phenylalanine or serine for tyrosine at position 264 had a much greater effect upon the rate of ATP cleavage as demonstrated by the nearly 10-fold reduction in k_{cat} for RecA(Phe²⁶⁴) and almost 20-fold reduction for RecA(Ser²⁶⁴) compared to wild type protein. Thus, these results demonstrate a significant role for Tyr²⁶⁴ in ATP hydrolysis and to a lesser extent for ATP binding.

DNA Reassociation and Strand Exchange Activities of RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) Proteins—As shown in Fig. 1 and Table I, none of the mutant *recA* genes was able to complement the repair or recombination defects of a *recA*⁻ strain. We examined the ability of the purified RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) proteins to promote two "recombinational" reactions *in vitro*, the reassociation of complementary single-strands of DNA, and the exchange of strands between duplex and single-stranded homologs. In the first experiments, the mutant RecA proteins were incubated with heat-denatured ³H-labeled P22 DNA in the presence of ATP and the amount of duplex formed was measured following S₁ nuclease digestion. The results shown in Fig. 6 demonstrate that none of the three substitution mutant proteins was capable of catalyzing DNA reassociation under conditions where the wild type RecA protein efficiently promoted duplex formation. Moreover, no ATP-independent reassociation activity was detected with any of the RecA proteins (data not shown). Thus the mutant RecA proteins are totally deficient in single-stranded DNA pairing activity.

The three-stranded DNA exchange reaction likely represents a more complete experimental model for the action of RecA protein during recombination *in vivo* (1). We therefore examined the ability of RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) proteins to promote strand exchange between circular single-stranded M13 viral DNA and the homologous

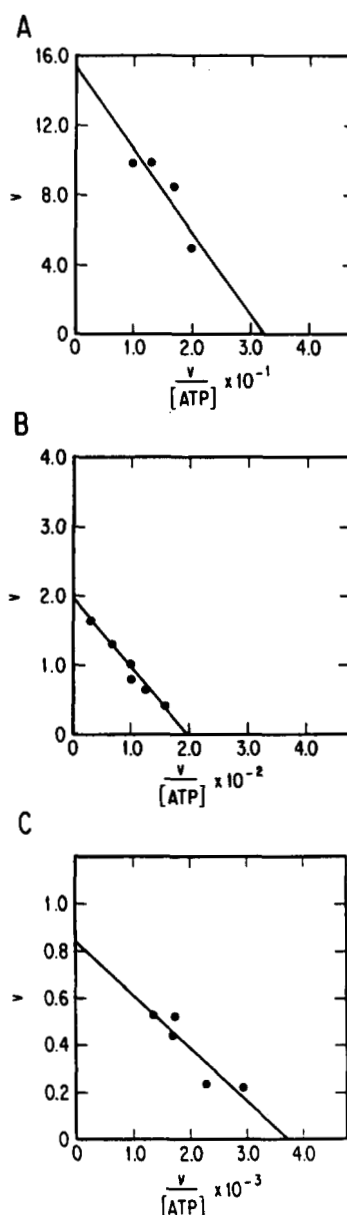


FIG. 5. Eadie-Hofstee plots of ATP hydrolysis by wild type and mutant RecA proteins. A, RecA(Tyr²⁶⁴) protein; B, RecA(Phe²⁶⁴) protein; and C, RecA(Ser²⁶⁴) protein. Reactions contained 2 μ M protein and 103 μ M heat-denatured calf thymus DNA. The ATP concentrations and other reaction conditions are described under "Experimental Procedures." v , rate of hydrolysis in mole of ADP formed per mole of enzyme/min.

TABLE II
Kinetic characterization of DNA-dependent ATP hydrolysis by wild type and mutant RecA proteins

Protein	K_m μ M	V_{max} μ M ADP/min	K_{cat} mol ADP/mol enzyme/min
RecA(Tyr ²⁶⁴) (wild type)	45	30	15
RecA(Phe ²⁶⁴)	100	4	2.0
RecA(Ser ²⁶⁴)	210	1.8	0.9

linearized RF DNA. The products of this three-strand exchange reaction are a linear single-stranded DNA and a nicked circular duplex which were efficiently formed in the presence of wild type RecA protein in a reaction containing single-strand binding protein and an ATP-regenerating system (Fig. 7A, lane 4). However, when the RecA(Tyr²⁶⁴) protein

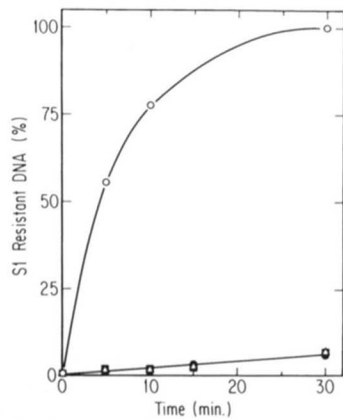


FIG. 6. Pairing of complementary single-stranded DNA by mutant RecA proteins. Reaction mixture contained 40 μ M heat-denatured 3 H-labeled P22 DNA, 1 mM ATP, and 2 μ M RecA(Tyr²⁶⁴) (wild type) protein, \circ ; 2 μ M RecA(Phe²⁶⁴) protein, \bullet ; 2 μ M RecA(Ser²⁶⁴) protein, \blacksquare ; or 2 μ M RecA(Gly²⁶⁴) protein, \triangle . The conditions for annealing and for measuring double-stranded DNA using S₁ nuclease are described under "Experimental Procedures." Approximately 5% of the labeled DNA became S₁ resistant in the absence of protein during a 30-min incubation. This same level was seen with either the wild type or mutant proteins after 30 min in the absence of ATP.

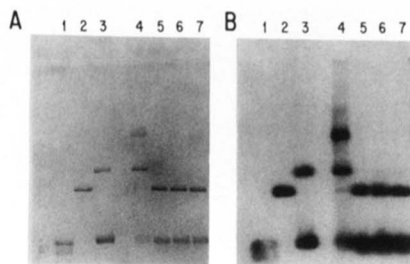


FIG. 7. Strand exchange promoted by RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) proteins. The DNA strand exchange reaction was performed as described under "Experimental Procedures" using 6 μ M RecA protein (wild type or mutant). Panel A, ethidium bromide-stained 1% agarose gel of reaction products. Panel B, autoradiogram of gel in panel A probed with 32 P-labeled M13 DNA. Lane 1, circular M13 single-stranded DNA; lane 2, M13 linear duplex DNA; lane 3, M13 circular duplex DNAs, forms I (lower) and II (upper). Lanes 4–7, products from strand exchange reaction using: lane 4, wild type RecA protein; lane 5, RecA(Gly²⁶⁴) protein; lane 6, RecA(Phe²⁶⁴) protein; lane 7, RecA(Ser²⁶⁴) protein. The slow mobility DNA in lane 4 likely represents incomplete or partial exchange products.

was replaced by the RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), or RecA(Gly²⁶⁴) enzymes, no strand exchange products were detected by ethidium bromide staining of the DNA. In order to determine whether joint molecule intermediates might have been formed but were produced at levels too low to be detected by staining, the DNA in the agarose gel was transferred to nitrocellulose by the method of Southern and the filter was hybridized with a 32 P-labeled M13 DNA probe. The results of this experiment are shown in Fig. 7B. Although we estimate that conversion of 0.1% of the input duplex DNA to product would be readily detected by hybridization, neither full-length product nor discreet intermediates were detected when the mutant RecA proteins were used in the reactions (lanes 5–7). Thus, these 3 tyrosine substitution RecA proteins were completely deficient in DNA pairing ability *in vitro*, a result which was consistent with their Rec⁻ phenotypes *in vivo*.

DNA Binding by RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) Proteins—The failure of the tyrosine-substituted RecA proteins to promote DNA pairing or strand exchange

reactions *in vitro* prompted us to examine their DNA-binding properties. Based upon their binding to single-stranded DNA cellulose during purification (see "Experimental Procedures") and the demonstrable but low levels of DNA-dependent ATP hydrolysis catalyzed by RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins, we anticipated that these proteins would bind single-stranded DNA. Binding to single-stranded M13 DNA was measured using a filter-binding assay (33) in the presence and absence of the ATP analog, ATP γ S. This nonhydrolyzable analog binds tightly to RecA protein and forms a stable ternary complex in the presence of DNA (14, 15).

The ability of the different RecA proteins to bind single-stranded DNA is shown in Fig. 8A. Approximately 30% of the input DNA was trapped on filters by RecA(Tyr²⁶⁴) protein in the absence of ATP γ S. These complexes were sensitive to treatment with 0.5 M NaCl which resulted in less than 2% binding. In the presence of the nonhydrolyzable nucleotide, between 95 and 100% of the M13 DNA was retained on filters and all of this binding was stable in the presence of high salt.

Although the RecA(Gly²⁶⁴) protein showed no ATP hydrolysis activity, it bound single-stranded DNA as well as wild type in the absence of ATP γ S and more than half of the label was removed by washing the complexes in high salt. The addition of ATP γ S to the reaction did not increase binding to DNA (in fact binding decreased) and there was no increase in the salt-resistant complexes compared to the level seen in the absence of ATP γ S. Thus, although the RecA(Gly²⁶⁴) protein bound DNA, this interaction was not stabilized by ATP γ S, a result which was consistent with the inability of

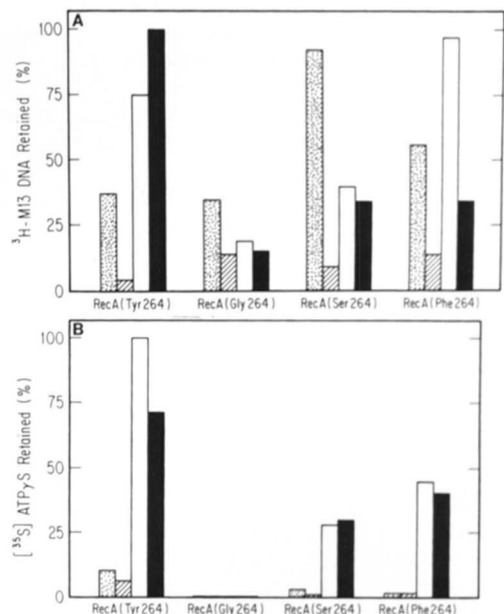


FIG. 8. DNA and ATP γ S binding by RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) proteins. Single-stranded, 3 H-labeled M13 DNA binding (A) and 35 S-labeled ATP γ S binding (B) were measured using alkaline-treated nitrocellulose filters as described under "Experimental Procedures." In A, open and solid bars indicate DNA bound in the presence of ATP γ S (1 mM). The stippled and cross-hatched bars correspond to DNA bound in the absence of nucleotide. Filters were washed with buffer containing low salt (50 mM NaCl): stippled and open bars; or with buffer containing high salt (500 mM NaCl): cross-hatched and solid bars. In B, the stippled and cross-hatched bars indicate the amount of ATP γ S bound in the absence of single-stranded DNA, whereas the open and solid bars correspond to the amount of ATP γ S bound in the presence of single-stranded DNA (100 μ M M13 viral DNA). Filters were washed with buffer containing low salt (30 mM NaCl): stippled and open bars; or filters were washed with buffer containing high salt (1 M NaCl): cross-hatched and solid bars.

this mutant RecA protein to bind nucleotide.

The RecA(Phe²⁶⁴) protein bound significant levels of DNA in the absence of ATP γ S and this value increased in the presence of ATP γ S to nearly 100% as seen with the wild type protein (Fig. 8A). However, unlike the RecA(Tyr²⁶⁴) protein, the ternary complexes formed with ATP γ S and the RecA(Phe²⁶⁴) protein were significantly more sensitive to salt such that only 35% of the DNA was retained in ternary complexes after high salt treatment.

The RecA(Ser²⁶⁴) protein retained nearly 100% of the labeled DNA in the absence of ATP γ S following a low salt wash. In the presence of ATP γ S, the amount of labeled DNA retained on filters actually decreased. However, as observed with the RecA(Phe²⁶⁴) protein, only approximately 35% of the labeled DNA was retained in salt-resistant complexes on filters indicating that most of the DNA is retained in a salt-sensitive complex with the mutant RecA proteins.

In order to determine whether this behavior was due to meaningful protein-DNA binding we examined binding of ³⁵S-labeled ATP γ S in complexes in a parallel series of experiments. As shown in Fig. 8B, retention of significant amounts of ATP γ S on the nitrocellulose filters was observed only in the presence of DNA with the RecA(Tyr²⁶⁴), RecA(Ser²⁶⁴), and RecA(Phe²⁶⁴) proteins. Moreover, most of the ATP γ S bound in the presence of DNA remained associated with the complexes after a high salt wash, indicating that the RecA(Ser²⁶⁴) and RecA(Phe²⁶⁴) formed stable ternary complexes with ATP γ S and DNA albeit at significantly reduced levels compared to wild type RecA protein. We have also used a gel retardation assay to assess the DNA-binding properties of these mutationally altered RecA proteins. This independent assay was consistent with the results of the filter-binding studies (data not shown).

LexA Cleavage Promoted by RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴) Proteins—The filter-binding experiments demonstrated that the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins were capable of binding single-stranded DNA and forming stable ternary complexes in the presence of the nonhydrolyzable nucleotide, ATP γ S. Ternary complexes of RecA protein, single-stranded DNA, and ATP (or ATP γ S) are also capable of promoting cleavage of LexA repressor *in vitro*. We investigated whether the nucleoprotein complexes formed using the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins promoted cleavage of LexA repressor. As shown in Fig. 9, both of these mutant proteins were “activated” for stimulating LexA repressor cleavage in the presence of single-stranded DNA and ATP γ S. No cleavage was detected when ATP γ S was omitted

from the reactions (data not shown). Moreover, the kinetics of cleavage were not significantly different from those seen from the wild type protein. The RecA(Gly²⁶⁴) protein did not promote LexA cleavage at any appreciable level, a result which was consistent with the inability of this protein to form stable ternary complexes with DNA and nucleotide. We conclude that the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins are capable of forming nucleoprotein complexes which bind LexA protein and promote its cleavage. Nonetheless, these same nucleoprotein complexes are incompetent for strand exchange.

DISCUSSION

The results presented in this paper provide evidence that Tyr²⁶⁴ in the RecA protein is required for efficient ATP hydrolysis and strand transfer. Substitution of this residue with Phe, Ser, or Gly either greatly reduced or completely abolished DNA-dependent ATP hydrolysis catalyzed by this protein. We have characterized the three mutationally altered RecA proteins: RecA(Phe²⁶⁴), RecA(Ser²⁶⁴), and RecA(Gly²⁶⁴), with respect to both ATP and DNA interactions. Both RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) bind nucleotide as judged by photoaffinity labeling with [α -³²P]N₃ATP. Based upon the levels of ³²P incorporation, the RecA(Phe²⁶⁴) protein was more efficiently labeled than the wild type protein. This enhanced cross-linking might be due to a more favorable binding interaction between the phenylalanine residue and the adenine base of N₃ATP within the binding site. By stabilizing the azido-adenine moiety within the active site, the Phe residue could promote more efficient insertion of the nitrene-free radical into this or nearby residues. The lower efficiency of photolabeling of RecA(Ser²⁶⁴) protein by N₃ATP further supports the idea that the adenine moiety interacts with aromatic residues in the binding site.

The RecA(Gly²⁶⁴) protein was not photolabeled at an appreciable efficiency with N₃ATP. The substitution of glycine at this position could lead to major structural perturbations in and around the ATP-binding site. However, since the protein retained its ability to bind DNA, it is likely that the structural integrity of this protein was not completely compromised by this replacement. Moreover, the extremely low level of photoaffinity labeling of the RecA(Gly²⁶⁴) protein demonstrates that little nonspecific cross-linking of RecA protein occurred under conditions of photolabeling.

Kinetic measurement of the mutant RecA proteins for ATP hydrolysis demonstrated that substitutions of Tyr²⁶⁴ by Phe or Ser affected both the binding of ATP as well as the rate of bond cleavage. Although the RecA(Phe²⁶⁴) protein showed enhanced photolabeling compared to wild type enzyme, paradoxically, the *K_m* for ATP hydrolysis increased approximately 2-fold. This apparent inconsistency is likely to be related to the observation that single-stranded DNA which is present in the hydrolysis experiments but not during photoaffinity labeling of RecA protein enhances binding of N₃ATP as well as ATP at the nucleotide-binding site (12, 34). It is possible that the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins are partially or completely defective in this response and are therefore characterized by relatively high Michaelis constants.

Filter-binding studies revealed that the RecA(Ser²⁶⁴) and RecA(Phe²⁶⁴) proteins were altered in their ability to form salt-resistant ternary complexes containing DNA and the nonhydrolyzable ATP analog, ATP γ S. Incubation of the wild type RecA protein with single-stranded DNA and ATP γ S leads to the formation of protein-DNA complexes that are stable to high salt treatment such that under conditions of protein excess, all of the DNA is retained in complexes on alkaline-treated nitrocellulose filters. In contrast, the levels

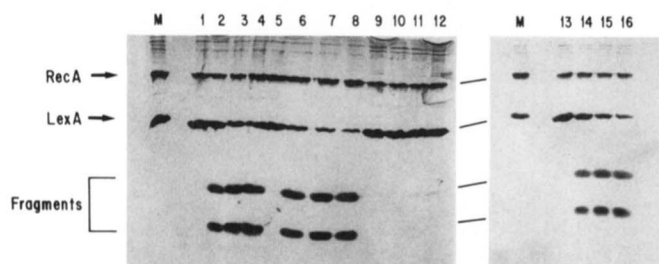


FIG. 9. RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins promote LexA repressor cleavage *in vitro*. The conditions of cleavage of LexA repressor are described under “Experimental Procedures.” At the times indicated below, samples were removed from each reaction and analyzed by electrophoresis in polyacrylamide gels. Lanes 1–4, RecA(Tyr²⁶⁴) protein (wild type); lanes 5–8, RecA(Phe²⁶⁴) protein; lanes 9–12, RecA(Gly²⁶⁴) protein; lanes 13–16, RecA(Ser²⁶⁴) protein. Samples were taken at 0 min, lanes 1, 5, 9, and 13; 30 min, lanes 2, 6, 10, and 14; 60 min, lanes 3, 7, 11, and 15; and 90 min, lanes 4, 8, 12, and 16. M, marker lanes containing RecA and LexA proteins.

of salt-resistant complexes formed with the RecA(Ser) or RecA(Phe) proteins were reduced by 3-fold. Both RecA(Ser²⁶⁴) and RecA(Phe²⁶⁴) proteins retained single-stranded DNA in the absence of nucleotide as judged by filter binding and most of this binding was sensitive to high salt treatment. Using a gel retardation assay rather than a filter-binding assay, we have observed that both RecA(Ser²⁶⁴) and RecA(Phe²⁶⁴) proteins were altered in their ability to form stable complexes with single-stranded DNA and ATP γ S (data not shown). Moreover, using [³⁵S]ATP γ S in filter-binding studies, we also observed that the RecA(Ser²⁶⁴) and RecA(Phe²⁶⁴) proteins retained less of this nucleotide in salt-resistant complexes with DNA. These results suggest that both RecA(Ser²⁶⁴) and RecA(Phe²⁶⁴) proteins are defective in their ability to undergo a transition from a "low affinity" DNA-binding conformation to the "high affinity" DNA-binding state that characterizes presynaptic filaments.

The complexes formed with the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins with single-stranded DNA and ATP γ S were capable of interacting with LexA protein and stimulating its proteolysis. We detected no differences in the kinetics of LexA cleavage promoted by these mutant RecA proteins, a result suggesting that these complexes were almost as efficient as those formed with wild type RecA protein in binding and stimulating LexA protein autodigestion. The RecA(Gly²⁶⁴) protein was incapable of directing LexA repressor inactivation under identical conditions. This latter observation was consistent with the demonstrated inability of the RecA(Gly²⁶⁴) protein to bind nucleotide.

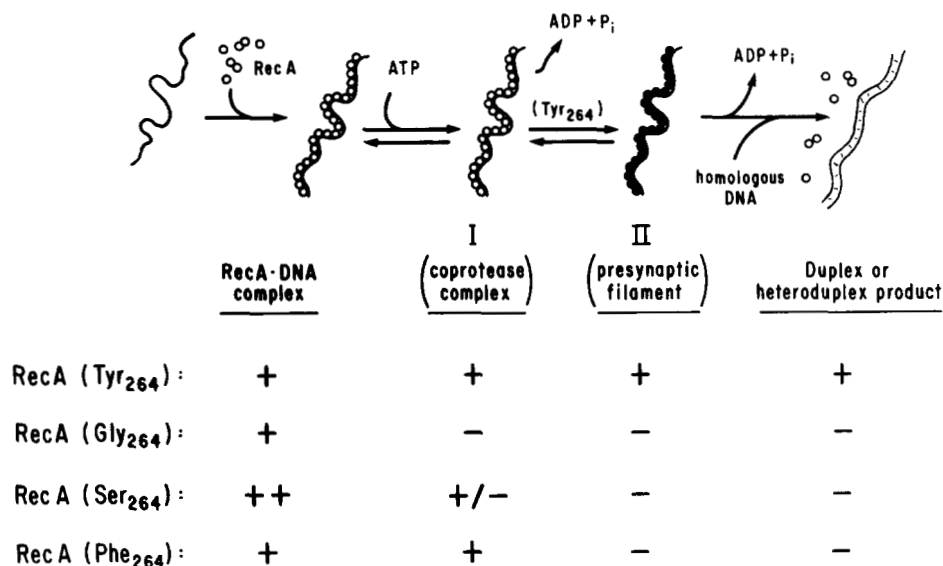
Although the RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins could form complexes with DNA and ATP γ S, they catalyzed no detectable DNA pairing or strand exchange. It is possible that the low level of ATP hydrolysis catalyzed by RecA(Phe²⁶⁴) and RecA(Ser²⁶⁴) proteins was not sufficient for RecA protein-catalyzed single-stranded DNA pairing. If this interpretation is correct, then there would be a minimum level of RecA protein-catalyzed ATP hydrolysis which would be required for DNA pairing activity. Recently, however, Menetski *et al.* (35) have demonstrated that RecA protein promotes considerable DNA strand exchange in the presence of nucleotide (ATP γ S) but in the absence of ATP hydrolysis. Therefore, it appears unlikely that there is a threshold level of hydrolysis needed for strand exchange.

Our results provide genetic and biochemical evidence that

RecA protein forms two functionally distinct complexes with single-stranded DNA and that Tyr²⁶⁴ is needed only in the formation of complexes that are necessary for efficient ATP hydrolysis and homologous DNA pairing. By contrast, so-called "protease" complexes of RecA protein, DNA, and ATP γ S were formed efficiently with either RecA(Ser²⁶⁴) or RecA(Phe²⁶⁴) proteins. Although these results do not indicate how these complexes might be structurally and functionally related, the results of Tessman and Peterson (36) suggest that the protease complex might be a precursor to the recombination complex. These investigators identified novel *recA* mutations which showed altered protease activity *in vivo*. Although they isolated mutations which inactivated both protease and recombination functions of RecA protein, they were unable to isolate mutations that abolished the protease function without eliminating the recombination activity of the protein. These results argue that the protease and recombination complexes of RecA protein and DNA are not independent but that the protease complex is required for the formation of the recombination and hydrolysis competent complex. One possible scheme is depicted in Fig. 10 in which the protease complex (complex I) is the immediate precursor of the recombination or presynaptic complex (complex II). However, the protease complex need not be the immediate precursor and several other intermediate forms are possible in this pathway. It is worth noting that one *recA* mutation, *recA430* appears to be deficient in LexA cleavage *in vivo* but is capable of promoting DNA pairing *in vivo* as well as *in vitro* (37, 38). Although these latter results appear to be inconsistent with the scheme presented in Fig. 10, we have recently suggested that the likely defect in the RecA430 protein is its reduced binding affinity for LexA repressor (39). Thus this mutation likely allows normal formation of complexes I and II shown in Fig. 10 but LexA binds poorly to the protease complex (I). Additional genetic and biochemical experiments will be necessary in order to determine the structural relationship between complexes I and II and to identify other residues in RecA protein which are required for coupling ATP binding and hydrolysis to repressor cleavage and strand exchange.

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FIG. 10. A possible functional relationship between co-protease complexes (I) and presynaptic complexes (II) formed by RecA protein. In this model RecA protein binds to DNA in the absence of ATP. Upon ATP addition, complex I is formed which is capable of binding and stimulating LexA repressor cleavage. Complex I is converted to complex II in RecA protein and requires the participation of Tyr²⁶⁴. Homologous pairing occurs on complex II to produce a duplex (or heteroduplex) product. ATP hydrolysis occurs on both complex I as well as complex II. Note that the three mutant RecA proteins are capable of binding single-stranded DNA in the absence of nucleotide, however, only RecA(Ser²⁶⁴) and RecA(Phe²⁶⁴) proteins can form complex I.



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