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Construction of a Recombinase-deficient Mutant recA Protein That Retains Single-stranded DNA-dependent ATPase Activity*

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The recA1 mutation is a single point mutation that replaces glycine 160 of the recA polypeptide with an aspartic acid residue. The mutant recA1 protein has a greatly reduced single-stranded DNA-dependent ATPase activity at pH 7.5 compared to the wild-type protein. Interestingly, the recA1 protein does exhibit a vigorous ATPase activity at pH 6.2. To explore the molecular basis of this pH effect, we used site-directed mutagenesis to replace aspartic acid 160 of the recA1 polypeptide with an isosteric, but nonionizing, asparagine residue. The new [Asn[160]]recA protein catalyzes ATP hydrolysis at pH 7.5 with the same turnover number as the wild-type protein. This result suggests that the activation of the recA1 protein ATPase activity that occurs at pH 6.2 may be due, in part, to neutralization of the negatively charged aspartic acid 160 side chain. Although it is an active single-stranded DNA-dependent ATPase, the [Asn[160]]recA protein is unable to complement a recA deletion *in vivo* and is unable to carry out the three-strand exchange reaction *in vitro*. Further examination of ATP hydrolysis (under strand exchange conditions) revealed that the ATPase activity of the [Asn[160]]recA protein is strongly suppressed in the presence of *Escherichia coli* single-stranded DNA-binding protein (a component of the strand exchange assay), whereas the ATPase activity of the wild-type recA protein is stimulated by the *E. coli* protein. To account for these results, we speculate that ATP may induce specific conformational changes in the wild-type recA protein that are essential to the DNA pairing process and that these conformational changes may not occur with the [Asn[160]]recA protein.

The recA protein of *Escherichia coli* is essential for homologous genetic recombination and for the postreplicative repair of damaged DNA (Radding, 1982). The sequence of the recA gene shows that the recA protein is composed of 352 amino acids and has a molecular weight of 37,852 (Horii *et al.*, 1980; Sancar *et al.*, 1980). The purified recA protein binds cooperatively to ssDNA,[1] forming a polymeric, filament-like structure. This recA-ssDNA complex catalyzes the hydrolysis of ATP to ADP and inorganic phosphate. In addition, the recA protein will promote a variety of ATP-dependent DNA pairing activities that presumably reflect *in vivo* functions. These reactions include the assimilation of linear single strands into duplex DNA (D-loop formation) and the exchange of strands between linear duplex and homologous single-stranded DNAs (three-strand exchange) (Radding, 1982). We are presently studying the mutant recA1 protein in an effort to determine the mechanistic role of ATP binding and hydrolysis in the recA protein-promoted DNA pairing process.

recA1 mutants of *E. coli* are defective in all known *in vivo* functions of the recA gene (Clark and Margulis, 1965). The recA1 mutation has been identified as a single point missense mutation which replaces glycine 160 of the recA polypeptide with an aspartic acid residue (Kawashima *et al.*, 1984). We have previously shown that the recA1 protein, like the wild-type protein, will bind to ssDNA and also will promote the ATP-independent renaturation of complementary ssDNA molecules (Bryant and Lehman, 1986). The recA1 protein, however, has a greatly reduced ssDNA-dependent ATPase activity at pH 7.5 compared to the wild-type protein and is unable to carry out ATP-dependent strand exchange reactions (Bryant and Lehman, 1986; Rusche *et al.*, 1985; West *et al.*, 1980). In this paper, we examine the ssDNA-dependent ATPase activity of the recA1 protein in detail and characterize an activation of the ATPase activity that occurs at pH 6.2 (Kawashima *et al.*, 1984). We further investigate the mechanism of this pH activation by using site-directed mutagenesis to replace aspartic acid 160 of the recA1 polypeptide with an isosteric, but nonionizing, asparagine residue. The enzymatic properties of the new [Asn[160]]recA protein provide insight into the role of ATP hydrolysis in recA protein-promoted DNA strand exchange.

EXPERIMENTAL PROCEDURES

Materials

ATP, carbenicillin, and nalidixic acid were from Sigma. [^{3}H]ATP was from ICN. DNA polymerase I (large fragment), T4 DNA ligase, and deoxy- and dideoxynucleoside triphosphates were from Amersham Corp. *Bam*HI restriction endonuclease and T4 polynucleotide kinase were from New England Biolabs. SeaPlaque agarose was from FMC Corp. *E. coli* DNA polymerase III holoenzyme was from Dr. Mike O'Donnell (Stanford University) or Dr. Roger McMacken (Johns Hopkins University). *E. coli* SSB protein was from Dr. Mike O'Donnell or from Pharmacia LKB Biotechnology Inc. Plasmids pUC19 (Yanisch-Perron *et al.*, 1985) and pRecA (Hickson *et al.*, 1981) were from Dr. Steve Elledge (Stanford University). Phage M13mp18 (Yanisch-Perron *et al.*, 1985) was from Dr. Per Elias (Stanford University). Authentic recA1 protein (West *et al.*, 1980) was from Dr. Steve West (Yale University).

E. coli strains MC1061 (Casabadan and Cohen, 1980) and TG1 (Gibson, 1984) were from Dr. Per Elias. MC1061 was used as a cloning host for M13 derivatives, and TG1 was used for obtaining

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[1] The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; ϕX, bacteriophage ϕX174; SSB, *E. coli* single-stranded DNA-binding protein; SDS, sodium dodecyl sulfate; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-propane-1,3-diol; LB, Luria-Bertani.

M13 phage plaques and for growing M13 phage. *E. coli* strains JM107, LE392, and BNN124 were from Dr. Steve Elledge. JM107 was used as a cloning host for pUC19 derivatives (Yanisch-Perron *et al.*, 1985). LE392 and BNN124 were used for the expression of the wild-type and mutant recA genes (Elledge and Davis, 1987). BNN124 was constructed by transducing the Tn10-linked Δ(srlR-recA)306 deletion of strain JC10289 (Willis *et al.*, 1981) into LE392 by P1 transduction of the Tet^r marker (Elledge and Davis, 1987). BNN124 has been shown to lack recA protein by immunoblotting (Elledge and Davis, 1987).

Circular φX ssDNA ((+)-strand) and linear φX dsDNA were prepared as previously described (Cox and Lehman, 1981). Single- and double-stranded DNA concentrations were determined by absorbance at 260 nm using the conversion factors 36 and 50 μg/ml/A_{260}, respectively. All DNA concentrations are expressed as total nucleotides.

Methods

Oligonucleotide-directed Mutagenesis of the recA Gene—The plasmid pRecA is pBR322 with a 3-kilobase *E. coli* fragment carrying the recA gene and promoter cloned into the *Bam*HI site (Hickson *et al.*, 1981). The *Bam*HI fragment was excised from pRecA by *Bam*HI digestion, purified by agarose gel electrophoresis, and cloned into the single *Bam*HI site of M13mp18 using standard techniques (Maniatis *et al.*, 1982). Recombinant M13mp18 phage carrying the *Bam*HI fragment (M13recA) were selected, and single-stranded phage DNA was purified as described by Messing (1983).

The mutagenic primers used to produce the Asp^{160} mutation (GGCGAAATCGACGACTCTCAC) and the Asn^{160} mutation (GGCGAAATCAACGACTCTCAC), as well as the sequencing primer (see below), were synthesized using a 380A DNA synthesizer (Applied Biosystems, Inc., Foster City, CA). The mutagenic primers were phosphorylated at the 5′ terminus using T4 polynucleotide kinase as described by Zoller and Smith (1983).

The [Asp^{160}]recA gene was prepared as follows. The appropriate phosphorylated primer (6.25 pmol) was mixed with M13recA ssDNA (0.25 pmol) in 10 μl of 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl and heated at 55 °C for 5 min. The annealed primer-template was converted to closed-circular duplex DNA with DNA polymerase III holoenzyme as described by O'Donnell and Kornberg (1985). The polymerase reaction solution (40 μl) contained 0.25 pmol of primed M13recA ssDNA, 20 mM Tris-HCl (pH 7.5), 8 mM $MgCl_2$, 500 μM ATP, 4% glycerol, 3.4 μg of SSB, 3 μg of holoenzyme, and 60 μM each of dATP, dGTP, dTTP, and dCTP. Primer elongation was initiated by the addition of the holoenzyme and was carried out at 25 °C for 20 min. The mixture was quenched by heating at 70 °C for 10 min. T4 DNA ligase (10 units) and ATP (1 mM) were added, and the mixture was kept at 4 °C overnight. The mixture was quenched at 70 °C and then subjected to agarose gel electrophoresis (0.8% SeaPlaque agarose). The band of closed-circular duplex DNA was cut from the gel, mixed with 2 volumes of TE buffer (20 mM Tris-HCl (pH 7.5), 1 mM EDTA), and heated to 65 °C for 10 min. Portions of the liquified mixture were used to transform competent MC1061 cells (Messing, 1983). The transformants were plated on a lawn of TG1 cells, individual plaques were selected, and phage ssDNA was prepared as described (Messing, 1983). Phage ssDNA carrying the Asp^{160} mutation were identified by dideoxy sequencing (Sanger *et al.*, 1977) using a synthetic 20-mer (CGCAGTAGACGTTATCGTCG) that primed 50 nucleotides upstream from the mutation site. Approximately 15% of the plaques that were screened contained the desired mutation. Samples of phage containing the mutation were plaque-purified, and homogeneous ssDNA was prepared as described (Zoller and Smith, 1983).

Next, the [Asn^{160}]recA gene was prepared by the above procedure using the appropriate primer and purified M13recA(Asp^{160}) ssDNA as the template. Both the [Asp^{160}]recA and the [Asn^{160}]recA genes were completely sequenced in order to confirm that only the desired changes had been introduced during the mutagenesis procedure.

Expression of Wild-type and Mutant recA Genes—*Bam*HI fragments carrying the mutant recA genes were excised from the duplex DNA of the mutant phage by *Bam*HI digestion and then cloned into the single *Bam*HI site of pUC19 to yield the plasmids pUCrecA(wt), pUCrecA(Asp^{160}), and pUCrecA(Asn^{160}). Competent LE392 or BNN124 cells were transformed with the modified pUC plasmids, and the transformants were selected for growth on LB/carbenicillin plates (Messing, 1983).

Purification of Wild-type and Mutant recA Proteins—Individual colonies of appropriately transformed BNN124 cells were used to inoculate 100 ml of L broth containing 50 μg/ml carbenicillin. The cultures were grown to A_{595} = 1 at 37 °C and then treated with nalidixic acid (40 μg/ml) for 90 min. The cells (0.55 g) were collected by centrifugation and resuspended in 0.8 ml of Tris-HCl (pH 8.1), 25% sucrose. The wild-type and mutant recA proteins were purified to greater than 95% homogeneity using the procedure of Cotterill *et al.* (1982) scaled down proportionally. The three recA protein variants behaved similarly at all stages of purification. The concentrations of the purified proteins were determined by the Bradford (1976) method using a previously purified wild-type recA protein stock as a concentration standard (Cox *et al.*, 1981). The concentration of the recA protein stock was determined by absorbance at 280 nm using an extinction coefficient of 0.59 A_{280} mg^{-1} ml (Cox *et al.*, 1981). SDS-polyacrylamide gel electrophoresis of the recA protein variants at various stages of purification was performed as described (Laemmli and Favre, 1973).

ATPase Assay—ATP hydrolysis was measured using a thin-layer chromatography method as previously described (Weinstock *et al.*, 1979). The standard ATPase assay mixtures contained 25 mM Tris-HCl (pH 7.5) or 25 mM BisTris-HCl (pH 6.2), 10 mM $MgCl_2$, 5% glycerol, 1 mM dithiothreitol, 30 μM φX ssDNA, 1.0 μM recA protein, and the indicated concentrations of ATP. All reactions were initiated by the addition of recA protein after preincubation of all other components for 10 min at 37 °C. All reactions were carried out at 37 °C unless otherwise indicated.

Three-strand Exchange Reactions—Three-strand exchange reactions were carried out as described by Cox and Lehman (1981). Reaction mixtures contained 25 mM Tris-HCl (pH 7.5) or 25 mM BisTris-HCl (pH 6.2), 10 mM $MgCl_2$, 5% glycerol, 1 mM dithiothreitol, 3.3 μM circular φX ssDNA, 5.6 μM linear φX dsDNA, 2.0 μM recA protein, 0.3 μM SSB, and 1.0 mM ATP. Reactions were initiated by the simultaneous addition of SSB and ATP after preincubation of all other components for 10 min at 37 °C. All reactions were carried out at 37 °C. Aliquots (40 μl) of the reaction solution were quenched at various times by the addition of 5 μl of SDS (10%). The samples were loaded onto a 0.8% agarose gel and electrophoresed in TAE buffer (40 mM Tris acetate, 1 mM EDTA). The substrates and products were visualized by ethidium bromide staining.

The hydrolysis of ATP during the strand exchange reactions was measured using the thin-layer chromatography assay cited above. Based on a ssDNA concentration of 3.3 μM and a binding stoichiometry of one recA monomer/four nucleotides of ssDNA (Bryant *et al.*, 1985), the maximum concentration of recA protein that will be active as a ssDNA-dependent ATPase under strand exchange conditions is 0.82 μM. This recA protein concentration was used to calculate rates of ATP hydrolysis under strand exchange conditions.

RESULTS

Construction of recA Mutants—A *Bam*HI fragment carrying the entire recA gene and promoter (Horii *et al.*, 1980) was cloned into M13mp18, and the resulting vector was used as a template for site-directed mutagenesis of the recA gene. Two mutations were made. One mutation changed the amino acid at position 160 of the recA polypeptide from glycine (GGC) to aspartic acid (GAC). This mutation corresponds to a reported sequence for the recA1 mutation (Kawashima *et al.*, 1984). The second mutation changed this aspartic acid (GAC) to an asparagine (AAC). The mutant recA genes were completely sequenced to confirm that only the desired changes had been introduced during the mutagenesis procedure. The *Bam*HI fragments carrying the wild-type and mutant recA genes were cloned into pUC19, generating three plasmids designated pUCrecA(wt), pUCrecA(Asp^{160}), and pUCrecA-(Asn^{160}).

In Vivo Properties of Mutant recA Proteins—*E. coli* strains LE392 and BNN124 were employed for the expression of the wild-type and mutant recA genes. LE392 carries the wild-type recA gene in the chromosome and has normal recA function. BNN124 is a derivative of LE392 with a Tn10-linked Δ(srlR-recA)306 deletion that removes the entire recA structural gene (Elledge and Davis, 1987; Willis *et al.*, 1981).

BNN124 cells were transformed with each of the plasmids

carrying variant recA genes, and the resulting transformants were analyzed for recA function using a UV sensitivity assay (Table I). Untransformed BNN124 cells were sensitive to UV radiation, consistent with the recA$^-$ phenotype (Clark and Margulis, 1965). BNN124/pUCrecA(wt) transformants, on the other hand, were as resistant to UV radiation as was LE392, indicating that pUCrecA(wt) is able to direct the production of active wild-type recA protein that can complement the recA deletion. In contrast, BNN124/pUCrecA(Asp^{160}) transformants were sensitive to UV radiation, demonstrating that the conversion of glycine 160 to aspartic acid is sufficient to inactivate recA function. This finding is consistent with the recA1 phenotype and supports the reported sequence assignment for the recA1 mutation (Kawashima *et al.*, 1984). BNN124/pUCrecA(Asn^{160}) transformants also were sensitive to UV radiation, indicating that conversion of aspartic acid 160 of the recA1 protein to an asparagine residue is not sufficient to restore recA function *in vivo*. Gel electrophoresis of cell lysates showed that all three transformed strains produced abundant amounts of the plasmid-encoded recA polypeptide (see below). Therefore, the inability of the [Asn^{160}]recA gene to complement the recA deletion of BNN124 was not due to lack of expression of the mutant protein.

LE392 cells were also transformed with each of the three plasmids carrying variant recA genes and analyzed for UV sensitivity (Table I). Untransformed LE392 cells were resistant to UV radiation, as were the LE392/pUCrecA(wt) transformants. LE392/pUCrecA(Asp^{160}) transformants, on the other hand, were sensitive to UV radiation. This negative complementation is consistent with the known properties of the recA1 gene and is probably the result of coaggregation of chromosomally encoded wild-type recA protein with plasmid-encoded, inactive [Asp^{160}]recA protein to produce an inactive mixed complex (Yancey and Porter, 1984). LE392/pUCrecA(Asn^{160}) transformants also were sensitive to UV radiation, indicating that pUCrecA(Asn^{160}) also directs the production of a defective recA polypeptide that can interfere with the functioning of the wild-type recA protein.

Purification of Wild-type and Mutant recA Proteins—Small cultures (100 ml) of BNN124/pUCrecA(wt), BNN124/pUCrecA(Asp^{160}), and BNN124/pUCrecA(Asn^{160}) cells were grown, and cell extracts were prepared. Gel electrophoresis of the extracts showed that the wild-type recA, [Asp^{160}]recA, and [Asn^{160}]recA proteins were expressed at levels corresponding to about 50, 5, and 25% of the total soluble protein, respectively. Each recA protein variant was purified to greater than 95% homogeneity using the magnesium precipitation method of Cotterill *et al.* (1982). The yield of pure protein from 100-ml cultures (0.55 g of cells) was 5.1 mg of wild-type recA protein, 0.5 mg of [Asp^{160}]recA protein, and 2.5 mg of [Asn^{160}]recA protein (Fig. 1).

ATP Hydrolysis by Mutant recA Proteins at 25 °C—The ssDNA-dependent ATPase activities of the wild-type and mutant recA proteins were initially measured under conditions compatible with our previous studies of the recA protein-promoted renaturation of complementary ssDNA molecules (Bryant and Lehman, 1986). The assays contained 30 μM ϕX ssDNA, 1.0 μM recA protein, and 250 μM ATP and were carried out at 25 °C (Fig. 2).

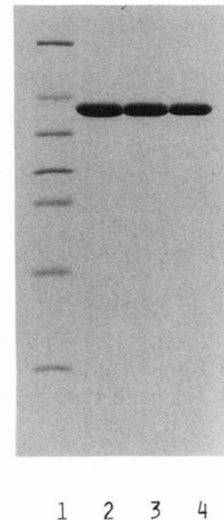

FIG. 1. **SDS-polyacrylamide gel electrophoresis of purified wild-type and mutant recA proteins.** *Lane 1,* molecular weight standards (Sigma) comprised of bovine albumin (66,000), ovalbumin (45,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), trypsinogen (24,000), trypsin inhibitor (20,100), and α-lactalbumin (14,200); *lane 2,* wild-type recA protein; *lane 3,* [Asp^{160}]recA protein; *lane 4,* [Asn^{160}]recA protein. Electrophoresis was performed as described (Laemmli and Favre, 1973). The acrylamide concentration was 6% in the stacking gel and 15% in the separating gel. The gel was stained with a solution containing 25% (v/v) 2-propanol, 10% (v/v) acetic acid, and 250 μg/ml Coomassie Brilliant Blue R-250 and destained with a solution of 10% (v/v) 2-propanol and 10% acetic acid.

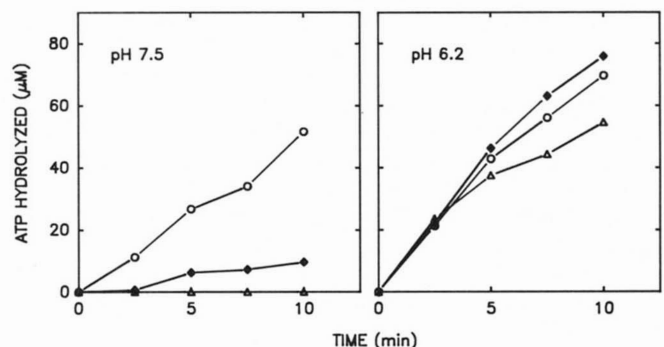

FIG. 2. **ATP hydrolysis by mutant recA proteins at 25 °C.** Reactions were carried out as described under "Experimental Procedures." Reaction solutions contained either 25 mM Tris-HCl (pH 7.5) (*left*) or 25 mM BisTris-HCl (pH 6.2) (*right*), 10 mM $MgCl_2$, 30 μM ϕX ssDNA, 250 μM [^{3}H]ATP, and a 1.0 μM concentration of wild-type recA protein (○), [Asp^{160}]recA protein (△), or [Asn^{160}]recA protein (◆). Reactions were carried out at 25 °C. The points represent the amount of ATP hydrolyzed as a function of time.

TABLE I

UV resistance of the mutant recA strains

A single colony of each strain was suspended in a drop of sterile water and then streaked in a single line on an LB plate (plates for the pUC transformants also contained carbenicillin (75 μg/ml) to prevent loss of the plasmid). Portions (25%) of each streak were exposed to UV light (2 J/m^2/s) for 0, 5, 10, or 15 s and then incubated overnight at 37 °C. Strains designated with the plus sign showed no apparent reduction in growth (compared to the portion of the streak which received no irradiation) after the maximum 15 s of irradiation. Strains designated with the minus sign showed no growth after the minimum 5 s of irradiation.

Strain	UV resistance
BNN124	−
BNN124/pUCrecA(wt)	+
BNN124/pUCrecA(Asp^{160})	−
BNN124/pUCrecA(Asn^{160})	−
LE392	+
LE392/pUCrecA(wt)	+
LE392/pUCrecA(Asp^{160})	−
LE392/pUCrecA(Asn^{160})	−

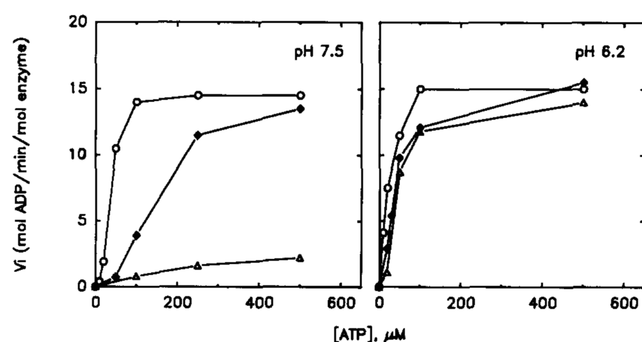

FIG. 3. **Dependence of ATP hydrolysis on ATP concentration.** Reactions were carried out as described under "Experimental Procedures." Reaction solutions contained either 25 mM Tris-HCl (pH 7.5) (*left*) or 25 mM BisTris-HCl (pH 6.2) (*right*), 10 mM $MgCl_2$, 30 μM φX ssDNA, the indicated concentrations of [^{3}H]ATP, and a 1.0 μM concentration of wild-type recA protein (○), 1.0 μM [Asn[160]] recA protein (♦), or 1.0 μM [Asp[160]]recA protein (△). The points represent initial rates of ATP hydrolysis. The reactions were carried out at 37 °C.

At pH 7.5 (Fig. 2), the wild-type recA protein catalyzed ATP hydrolysis at a rate of 6 μmol/min, whereas neither [Asp[160]]recA protein nor authentic recA1 protein catalyzed any detectable ATP hydrolysis. These results are consistent with our previous study (Bryant and Lehman, 1986). Under these conditions, the [Asn[160]]recA protein catalyzed ATP hydrolysis at a rate of 1 μmol/min.

At pH 6.2 (Fig. 2), the wild-type recA protein catalyzed ATP hydrolysis at a rate of 8 μmol/min, slightly higher than that measured at pH 7.5. More significantly, the [Asp[160]]recA protein also catalyzed ATP hydrolysis at a rate of 8 μmol/min at pH 6.2. Identical results were obtained with authentic recA1 protein. Thus, the [Asp[160]]recA protein becomes activated as an ATPase at the lower pH. Under these conditions, the [Asn[160]]recA protein also catalyzed ATP hydrolysis at a rate of 8 μmol/min.

The ssDNA-dependent ATP hydrolysis activity of the wild-type recA protein was not linearly dependent on recA protein concentration (from 1.0 to 2.5 μM protein) at 25 °C, possibly due to inefficient binding of recA protein to regions of secondary structure in the ssDNA (Menge and Bryant, 1988). Therefore, further characterization of the ATPase activities of the recA protein variants was carried out at 37 °C.

Dependence of ATPase Activity on ATP Concentration at pH 7.5—The dependence of the rate of ATP hydrolysis on ATP concentration was determined for each of the recA protein variants at pH 7.5 and 37 °C. The ATPase assay conditions for the kinetic measurements described below consisted of 30 μM φX ssDNA and 1.0 μM recA protein. At 37 °C and 30 μM ssDNA, the rate of ATP hydrolysis by the wild-type recA protein is linearly dependent on recA concentration (from 0.25 to 2.0 μM recA). Therefore, these assay conditions ensure that there is sufficient ssDNA to bind all of the recA protein (Menge and Bryant, 1988). The ATP hydrolysis data are presented in Fig. 3, and the kinetic parameters are summarized in Table II (double-reciprocal plots and Hill plots not shown).

The turnover number for ATP hydrolysis by the wild-type recA protein at pH 7.5 was 15 min^{-1}, and the $S_{0.5}$ for ATP was 38 μM.[2] A double-reciprocal plot of the data is concave upward, indicating positive cooperativity for the ATPase activity with respect to ATP concentration; and a Hill coefficient of 2.6 was determined at ATP concentrations near $S_{0.5}$ (10–100 μM ATP). These results are consistent with previous reports (Weinstock *et al.*, 1981).

TABLE II

Kinetic parameters for ssDNA-dependent ATP hydrolysis by the mutant recA proteins

The kinetic parameters were derived from double-reciprocal plots and Hill plots of the data presented in Fig. 3 (plots not shown).

Kinetic parameter	recA protein		
	Wild-type	[Asp[160]]	[Asn[160]]
pH 7.5			
$S_{0.5}$ (μM)	38	300	150
$V_{max}/[E_t]$ (min^{-1})	15	3	15
n_H	2.6		2.4
pH 6.2			
$S_{0.5}$ (μM)	25	45	45
$V_{max}/[E_t]$ (min^{-1})	17	15	17
n_H	1.0	2.4	2.6

The [Asp[160]]recA protein exhibited a low, but significant, ATPase activity at pH 7.5 and 37 °C. The turnover number for ATP hydrolysis was estimated as 3 min^{-1}, and the $S_{0.5}$ for ATP as 300 μM. However, because of the low rate of ATP turnover, neither V_{max} nor the cooperativity of the reaction could be precisely determined. Similar results were obtained with authentic recA1 protein.

The turnover number for ATP hydrolysis by the [Asn[160]] recA protein at pH 7.5 was 15 min^{-1}, identical to that for the wild-type protein. Furthermore, a double-reciprocal plot of the data is concave upward, and a Hill coefficient of 2.4 was determined at ATP concentrations near $S_{0.5}$ (50–500 μM ATP), also similar to that for the wild-type protein. The $S_{0.5}$ for ATP of 150 μM, however, was approximately 4-fold higher than that determined for the wild-type protein. Note that these parameters are quite different from those determined for the [Asp[160]]recA protein at this pH.

Dependence of ATPase Activity on ATP Concentration at pH 6.2—The dependence of the ATPase activity of each recA protein variant on ATP concentration was also determined at pH 6.2 and 37 °C. The data are presented in Fig. 3, and the kinetic parameters are summarized in Table II (double-reciprocal plots and Hill plots not shown).

The turnover number for ATP hydrolysis by the wild-type recA protein at pH 6.2 was 17 min^{-1}, and the $S_{0.5}$ for ATP was 25 μM. These parameters are similar to those determined at pH 7.5. The double-reciprocal plot is linear, however; and a Hill coefficient of 1.0 was determined at ATP concentrations near $S_{0.5}$ (10–100 μM ATP). Thus, at pH 6.2, the ATPase activity of the wild-type recA protein does not exhibit the cooperative dependence on ATP concentration that is found at pH 7.5.

The kinetic parameters for the [Asp[160]]recA protein ATPase activity at pH 6.2 were markedly different from those determined at pH 7.5. The turnover number for ATP hydrolysis was 15 min^{-1}, the $S_{0.5}$ for ATP was 45 μM, and a Hill coefficient of 2.4 was determined at ATP concentrations near $S_{0.5}$ (20–100 μM ATP). Identical results were obtained with authentic recA1 protein. Thus, the [Asp[160]]recA protein is activated as an ATPase at the lower pH, as indicated both by a lower $S_{0.5}$ for ATP and a higher turnover number for ATP hydrolysis.

The turnover number for ATP hydrolysis by the [Asn[160]] recA protein at pH 6.2 was 17 min^{-1}, similar to that determined at pH 7.5. The $S_{0.5}$ for ATP of 45 μM, however, was significantly lower than at pH 7.5. The Hill coefficient at ATP concentrations near $S_{0.5}$ (10–50 μM ATP) was 2.6. Note

[2] $S_{0.5}$ is the substrate concentration required for half-maximal velocity. In the absence of cooperativity, the half-saturation point is equivalent to the Michaelis constant (K_m) but is more properly termed $S_{0.5}$ in cooperative systems (Neet, 1980).

that these parameters are very similar to those for the [Asp^{160}] recA protein at this pH.

Mutant recA Proteins in Three-strand Exchange Reaction—The three-strand exchange reaction was employed to determine if either the [Asp^{160}]recA or [Asn^{160}]recA protein could function in an ATP-dependent DNA pairing process. In the three-strand exchange reaction, a circular ϕX ssDNA molecule and a homologous linear ϕX dsDNA molecule are recombined by the action of the recA protein to form a nicked-circular dsDNA molecule and a linear ssDNA molecule. The reaction is strongly stimulated by *E. coli* SSB, which is included in the assay mixture. The substrates and products of this reaction are readily monitored by agarose gel electrophoresis (Cox and Lehman, 1981).

At pH 7.5 and 1 mM ATP (Fig. 4, *upper*), the wild-type recA protein-promoted strand exchange reaction proceeded efficiently when SSB was included in the assay mixture. When SSB was omitted, only a low amount of strand exchange by the wild-type protein was detected. In contrast, the [Asp^{160}] recA protein did not promote any detectable strand exchange in 90 min, with or without SSB present. More interestingly, the [Asn^{160}]recA protein also failed to carry out detectable strand exchange under these conditions. Thus, even though the [Asn^{160}]recA protein can catalyze ATP hydrolysis at the same rate as the wild-type protein at pH 7.5 (Fig. 3), the mutant protein is unable to promote DNA strand exchange.

Strand exchange reactions were also attempted at pH 6.2 and 1 mM ATP (Fig. 4, *lower*) since both the [Asp^{160}]recA and [Asn^{160}]recA proteins were found to catalyze ATP hydrolysis at rates comparable to the wild-type recA protein at this pH (Fig. 3). As was found at pH 7.5, however, neither the [Asp^{160}] recA nor [Asn^{160}]recA protein promoted any detectable strand exchange in 90 min, with or without SSB. The wild-type recA protein-promoted strand exchange reaction, on the other hand, proceeded as efficiently at pH 6.2 as at pH 7.5 when SSB was included in the assay mixture. No strand exchange by the wild-type protein was detected at pH 6.2 in the absence of SSB.

To investigate further the inactivity of the mutant recA proteins in strand exchange, ATP hydrolysis by the wild-type and mutant recA proteins was measured under strand exchange conditions. Strand exchange conditions differ from the standard ATPase assay conditions that were used for the kinetic measurements described above in that during strand exchange, the recA protein is in excess relative to the amount of ssDNA present (3.3 μM ssDNA, 2.0 μM recA protein). Therefore, under strand exchange conditions, the rate of ATP hydrolysis is limited by the amount of ssDNA present, and maximal ATP hydrolysis rates will be observed only if the ssDNA is completely coated by recA protein.

At pH 7.5 (strand exchange conditions; Fig. 5), the rate of ATP hydrolysis (1 mM ATP) by the wild-type recA protein was 11 mol/min/mol of enzyme in the absence of SSB and increased to 19 mol/min/mol of enzyme in its presence. In contrast, the rate of ATP hydrolysis by the [Asn^{160}]recA protein was 7 mol/min/mol of enzyme in the absence of SSB and decreased to 0.5 mol/min/mol of enzyme in its presence. Thus, at pH 7.5, SSB stimulates ATP hydrolysis by the wild-type recA protein, but suppresses ATP hydrolysis by the [Asn^{160}]recA protein.

The [Asp^{160}]recA protein catalyzed little ATP hydrolysis (less than 1 mol/min/mol of enzyme) under strand exchange conditions at pH 7.5, with or without SSB present. Therefore, ATP hydrolysis by the wild-type and [Asp^{160}]recA proteins was examined at pH 6.2 (Fig. 6). At pH 6.2, the rate of ATP hydrolysis (1 mM ATP) by the wild-type recA protein was 25 mol/min/mol of enzyme, both in the presence and absence of SSB. In contrast, the rate of ATP hydrolysis by the [Asp^{160}] recA protein was 20 mol/min/mol of enzyme in the absence of SSB and decreased to 1 mol/min/mol of enzyme in its presence. Thus, at pH 6.2, SSB strongly suppresses the ATPase activity of the [Asp^{160}]recA protein, but not that of the wild-type recA protein ATPase.

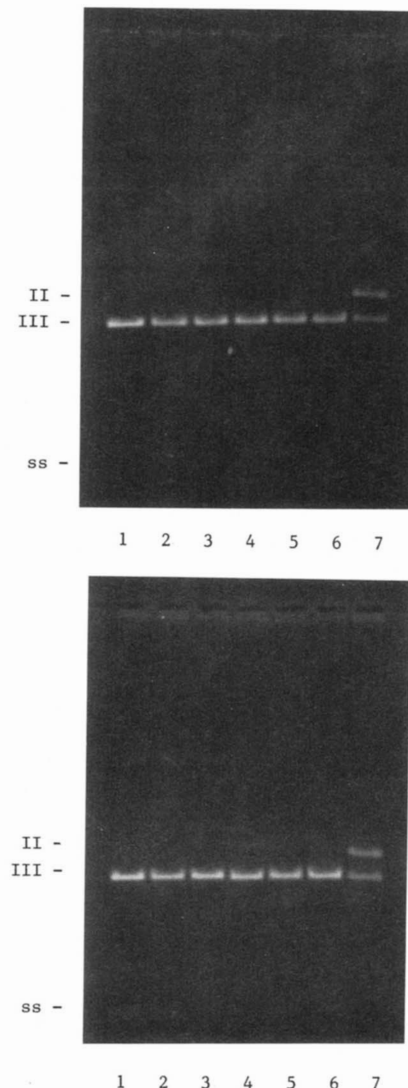

FIG. 4. **Mutant recA proteins in three-strand exchange reaction.** Reactions were carried out at pH 7.5 (*upper*) or pH 6.2 (*lower*) as described under "Experimental Procedures." Reaction solutions contained 3.3 μM ϕX ssDNA, 5.6 μM ϕX dsDNA, 1.0 mM ATP, and the following: *lane 1*, no protein; *lane 2*, 2.0 μM [Asp^{160}] recA protein; *lane 3*, 2.0 μM [Asp^{160}]recA protein plus 0.3 μM SSB; *lane 4*, 2.0 μM [Asn^{160}]recA protein; *lane 5*, 2.0 μM [Asn^{160}]recA protein plus 0.3 μM SSB; *lane 6*, 2.0 μM wild-type recA protein; and *lane 7*, 2.0 μM wild-type recA protein plus 0.3 μM SSB. The reactions were carried out for 90 min at 37 °C and analyzed by electrophoresis through a 0.8% agarose gel. *II*, circular duplex DNA containing a nick (replicative form II); *III*, linear duplex DNA (replicative form III); *ss*, single-stranded DNA.

DISCUSSION

The ssDNA-dependent ATPase activity of the mutant recA1 protein exhibits a pronounced dependence on pH. At pH 7.5 (37 °C), the turnover number for ATP hydrolysis by the recA1 protein is about 5-fold lower, and the $S_{0.5}$ for ATP is about 8-fold higher than the corresponding values for the

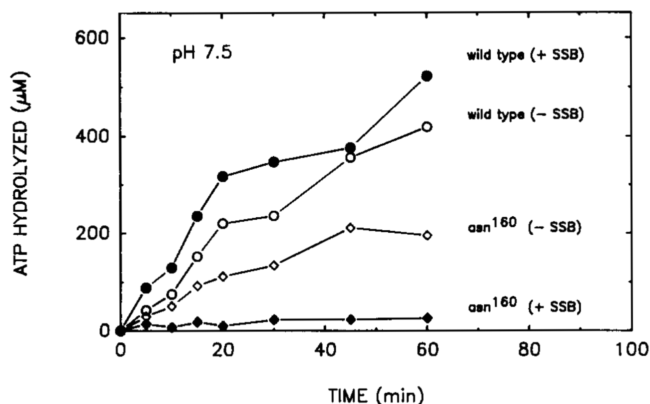

FIG. 5. **ATP hydrolysis by wild-type and [Asn[160]]recA proteins under strand exchange conditions (pH 7.5).** Reactions were carried out as described under "Experimental Procedures." Reaction solutions contained 3.3 μM ϕX ssDNA, 5.6 μM ϕX dsDNA, 1.0 mM [^{3}H]ATP, 2.0 μM wild-type recA protein (●, ○) or 2.0 μM [Asn[160]]recA protein (♦, ◇), and 0.3 μM SSB where indicated (●, ♦). The reactions were carried out at pH 7.5 and 37 °C. The points represent the amount of ATP hydrolyzed as a function of time.

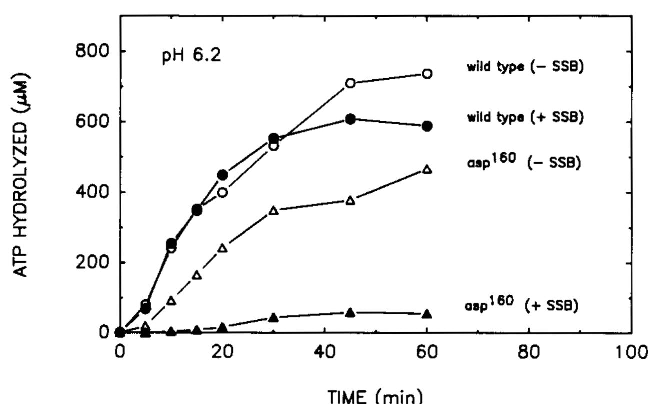

FIG. 6. **ATP hydrolysis by wild-type and [Asp[160]]recA proteins under strand exchange conditions (pH 6.2).** Reactions were carried out as described under "Experimental Procedures." Reaction solutions contained 3.3 μM ϕX ssDNA, 5.6 μM ϕX dsDNA, 1.0 mM [^{3}H]ATP, 2.0 μM wild-type recA protein (●, ○) or 2.0 μM [Asp[160]]recA protein (▲, △), and 0.3 μM SSB where indicated (●, ▲). The reactions were carried out at pH 6.2 and 37 °C. The points represent the amount of ATP hydrolyzed as a function of time.

wild-type recA protein. At pH 6.2, on the other hand, the turnover numbers and the $S_{0.5}$ values for the wild-type and recA1 protein are nearly the same. Since the pK_a of aspartic acid side chains in proteins is normally about 4 (Fersht, 1985), the aspartic acid 160 side chain of the recA1 protein is probably ionized at pH 7.5, and the resulting negative charge may be responsible for the deleterious effects of the recA1 mutation on recA protein function. If this is the case, then a possible explanation for the activation of the ATPase activity of the recA1 protein that occurs at pH 6.2 might involve protonation of the ionized aspartic acid 160 side chain, which would eliminate the negative charge. This mechanism would require that the aspartic acid 160 side chain have a pK_a of about 7. Alternatively, a nearby histidine residue (p$K_a \sim 7.0$; Fersht, 1985) may become protonated at pH 6.2, and the resulting positively charged imidazolium ring could conceivably neutralize the negatively charged aspartic acid 160 side chain and restore the ATPase activity of the recA1 protein. The recA1 protein does have 2 histidine residues, one at position 163, just 3 residues away from the recA1 mutation site (Horii *et al.*, 1980; Sancar *et al.*, 1980).

In order to determine: 1) whether a negatively charged aspartic acid 160 side chain is responsible for the inactivation of the ATPase activity of the recA1 protein at pH 7.5, and 2) whether neutralization of the negative charge is a plausible explanation for the activation of the recA1 ATPase that occurs at pH 6.2, we used site-directed mutagenesis to replace aspartic acid 160 of the recA1 polypeptide with an asparagine residue. The asparagine side chain is nearly identical, sterically and geometrically, to a protonated aspartic acid side chain and has similar hydrogen bonding properties (Knowles, 1987). Unlike the aspartic acid side chain, however, the asparagine side chain does not ionize significantly in aqueous solution.

The turnover number for ssDNA-dependent ATP hydrolysis by the [Asn[160]]recA protein is equivalent to that of the wild-type protein at both pH 7.5 and 6.2. This result is consistent with the idea that neutralization of the negative charge of the aspartic acid 160 side chain may be responsible for the increase in the turnover number for ATP hydrolysis by the recA1 protein that occurs between pH 7.5 and 6.2. The $S_{0.5}$ value for the [Asn[160]]recA protein, on the other hand, exhibits a dependence on pH that is similar to that found for the recA1 protein, decreasing from a value 4-fold higher than that for the wild-type protein at pH 7.5 to a value comparable to the wild-type protein at pH 6.2. This indicates that the decrease in the $S_{0.5}$ value for the recA1 protein that occurs between pH 7.5 and 6.2 cannot be attributed to neutralization of the aspartic acid 160 side chain. Another possible explanation for the pH dependence of the recA1 ATPase activity may be that the recA1 protein assumes a conformation at pH 6.2 in which a negatively charged aspartic acid residue at position 160 no longer exerts a deleterious effect on the ATPase activity. The change in the cooperativity of the ssDNA-dependent ATPase activity of the wild-type recA protein that occurs between pH 7.5 and 6.2 may also be the result of pH-dependent conformational changes in the protein. Whatever the mechanism, it is clear that the conversion of aspartic acid 160 of the recA1 protein to a neutral asparagine residue increases the ATPase activity of the protein at pH 7.5, as indicated by the turnover number for ATP hydrolysis.

Neither the [Asp[160]]recA nor [Asn[160]]recA gene is able to complement an recA1 deletion *in vivo*, as indicated by UV sensitivity assays (Table I). Although the precise role of the wild-type recA protein in the repair of UV damage to DNA *in vivo* is not completely understood, it is well-established that the recA protein can act indirectly as a DNA damage-activated protease to induce a network of DNA repair genes as part of the SOS response (Radding, 1982). In addition, it is believed that the recA protein can act directly to repair UV damage in a process known as recombination-repair, which presumably involves the strand exchange activity of the recA protein. Recently, the UV sensitivity of a number of randomly generated mutant recA strains has been shown to correspond to the *in vivo* recombination proficiency of the strains, indicating that recombination activity is the major factor that accounts for UV sensitivity in *E. coli* (Wang and Tessman, 1986). Consistent with their lack of complementation *in vivo*, the [Asp[160]]recA and [Asn[160]]recA proteins are both unable to carry out the three-strand exchange reaction *in vitro*.

The inactivity of the [Asn[160]]recA protein in the three-strand exchange reaction is particularly interesting because at pH 7.5 and saturating concentrations of ATP, the [Asn[160]] recA and wild-type recA proteins catalyze ATP hydrolysis at the same rate and with the same cooperativity (Table II). When ATP hydrolysis was measured under strand exchange conditions, however, it was found that the ATPase activity of

the [Asn[160]]recA protein is strongly suppressed by the SSB that is included in the strand exchange assay mixture. The ATPase activity of the wild-type recA protein, on the other hand, is stimulated by SSB.

The molecular basis for the stimulatory effects of SSB on wild-type recA protein-catalyzed ATP hydrolysis and strand exchange is currently the subject of some controversy. Morrical *et al.* (1986) have proposed that recA protein and SSB bind simultaneously to ssDNA, in stoichiometric amounts, to form a unique complex that is the reactive species in strand exchange. Kowalczykowski *et al.* (1987) and Kowalczykowski and Krupp (1987), on the other hand, have suggested that SSB serves only to melt out secondary structure in ssDNA before being displaced by recA protein and that the reactive complex in strand exchange consists of only ssDNA and stoichiometric amounts of recA protein. Regardless of the exact mechanism of SSB stimulation, there is general agreement that SSB will displace the wild-type recA protein from ssDNA unless ATP is present (Tsang *et al.*, 1985; Morrical *et al.*, 1986; Kowalczykowski *et al.*, 1987). Presumably, ATP induces a conformation of the wild-type recA protein that is resistant to displacement by SSB. Our results with the [Asn[160]]recA and the [Asp[160]]recA proteins suggest that these mutant recA proteins may be unable to assume an SSB-resistant conformation in the presence of ATP. As a result, the mutant recA proteins would be displaced from ssDNA by SSB, and ATP hydrolysis by the mutant proteins would be prevented. Alternatively, the mutant proteins may bind to ssDNA in the presence of SSB, but not recognize it as an effector for the ATPase activity. Experiments aimed at resolving these possibilities are in progress.

To investigate further the relationship between the SSB suppression of ATP hydrolysis and the lack of strand exchange activity by the mutant recA proteins, we have recently carried out strand exchange experiments using a magnesium shift protocol that by-passes the requirement for SSB (Muniyappa *et al.*, 1984). In this procedure, recA-ssDNA complexes are initially formed at 1 mM Mg^{2+}. The low Mg^{2+} concentration disfavors the formation of secondary structure in ssDNA and presumably allows recA protein to bind more efficiently to the ssDNA. After the recA-ssDNA complexes are formed, the linear dsDNA is added, and the Mg^{2+} concentration is increased to 10 mM to allow optimal strand exchange to occur. Using this protocol, we find that the wild-type recA protein is able to carry out substantial strand exchange in the absence of SSB (75% completion in 30 min). The [Asn[160]] recA protein, however, still fails to carry out any detectable strand exchange.[3] These results indicate that whereas the SSB suppression of ATP hydrolysis undoubtedly reflects a fundamental defect in the [Asn[160]]recA protein, it is not, in itself, the reason that this protein is unable to carry out DNA strand exchange.

Although the three-dimensional structure of the recA protein has not yet been determined, a recent prediction of the secondary structure of the recA protein places glycine 160 in a 20-amino acid loop region that connects an α-helix to a β-strand (Blanar *et al.*, 1984). Glycine residues are generally well-conserved in proteins and often serve to increase main chain flexibility (Schulz and Schirmer, 1979). Thus, the replacement of glycine 160 of the wild-type recA protein with an aspartic acid residue (in the recA1 protein) may disrupt recA function, not only because of the introduction of a negative charge, but also because of a decrease in the flexibility of this postulated loop region. Our results demonstrate that the replacement of aspartic acid 160 with an asparagine residue, which removes the negative charge, is sufficient to restore the turnover number for ATP hydrolysis of the protein to a value identical to that of the wild-type protein at pH 7.5. The SSB suppression of ssDNA-dependent ATPase activity and the lack of strand exchange activity by the [Asn[160]]recA protein, however, clearly indicate that a neutral asparagine residue at position 160 is sufficient to disrupt the recombinase activity of the recA protein.

In recent related studies, we have examined the hydrolysis of GTP by the wild-type recA protein (Menge and Bryant, 1988). At pH 7.5, the $S_{0.5}$ for GTP is 750 μM compared to 40 μM for ATP. Nevertheless, at saturating nucleotide concentrations, ATP and GTP are hydrolyzed by recA protein at nearly the same rate. GTP, however, will not substitute for ATP as a high energy cofactor in the recA protein-catalyzed strand exchange reaction. When GTP hydrolysis was measured under strand exchange conditions, we found that GTP hydrolysis was strongly suppressed by SSB. Since the wild-type protein presumably should be able to recognize ssDNA as an effector for nucleoside triphosphate hydrolysis, even in the presence of SSB, these results suggest that GTP may be unable to induce the conformation of the recA protein that is resistant to displacement by SSB. As a result, the recA protein would be displaced from ssDNA by SSB, and ssDNA-dependent GTP hydrolysis would be prevented.

Our studies on GTP hydrolysis by the wild-type recA protein parallel our studies of ATP hydrolysis by the [Asn[160]] recA protein in the following sense. One case involves the interaction of the wild-type recA protein with a "mutant" substrate (GTP), whereas the other case involves the interaction of a mutant recA protein with the "wild-type" substrate (ATP). In both cases, the catalytic competence of the system remains intact, as indicated by the normal turnover numbers for nucleoside triphosphate hydrolysis. However, the interactions between substrate and enzyme have clearly been perturbed in both cases, as indicated by the suppression of nucleoside triphosphate hydrolysis by SSB and the lack of strand exchange activity. Further study of these modified systems will hopefully lead to a molecular description of the role of ATP hydrolysis in recA protein-promoted DNA strand pairing processes.

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[3] F. R. Bryant, unpublished experiments.

REFERENCES

Blanar, M. A., Kneller, D., Clark, A. J., Karu, A. E., Cohen, F. E., Langridge, R., and Kuntz, I. D. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **49**, 507–510

Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254

Bryant, F. R. (1987) *J. Cell. Biochem.* **11C**, 240

Bryant, F. R., and Lehman, I. R. (1986) *J. Biol. Chem.* **261**, 12988–12993

Bryant, F. R., Taylor, A. R., and Lehman, I. R. (1985) *J. Biol. Chem.* **260**, 1196–1202

Casadaban, M. J., and Cohen, S. N. (1980) *J. Mol. Biol.* **138**, 179–207

Clark, A. J., and Margulis, A. D. (1965) *Proc. Natl. Acad. Sci. U. S. A.* **53**, 451–459

Cotterill, S. M., Satterthwait, A. C., and Fersht, A. R. (1982) *Biochemistry* **21**, 4332–4337

Cox, M. M., and Lehman, I. R. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78**, 3433–3437

Cox, M. M., McEntee, K., and Lehman, I. R. (1981) *J. Biol. Chem.* **256**, 4676–4678

Elledge, S. J., and Davis, R. W. (1987) *Mol. Cell. Biol.* **7**, 2783–2793

Fersht, A. (1985) *Enzyme Structure and Mechanism*, W. H. Freeman, New York
Gibson, T. (1984) Ph.D. dissertation, Cambridge University
Hickson, I. D., Gordon, R. C., Tomkins, A. E., and Emmerson, P. T. (1981) *Mol. Gen. Genet.* **184,** 68–72
Horii, T., Ogawa, T., and Ogawa, H. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 313–317
Kawashima, H., Horii, T., Ogawa, T., and Ogawa, H. (1984) *Mol. Gen. Genet.* **193,** 288–292
Kowalczykowski, S. C., and Krupp, R. A. (1987) *J. Mol. Biol.* **193,** 97–113
Kowalczykowski, S. C., Clow, J., Somani, R., and Varghese, A. (1987) *J. Mol. Biol.* **193,** 81–95
Knowles, J. R. (1987) *Science* **236,** 1252–1258
Laemmli, U. K., and Favre, M. (1973) *J. Mol. Biol.* **80,** 575–599
Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Menge, K. L., and Bryant, F. R. (1988) *Biochemistry* **27,** 2635–2640
Messing, J. (1983) *Methods Enzymol.* **101,** 20–79
Morrical, S. W., Lee, J., and Cox, M. M. (1986) Biochemistry *25*, 1482–1494.
Muniyappa, K., Shaner, S. L., Tsang, S. S., and Radding, C. M. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 2757–2761
Neet, K. E. (1980) *Methods Enzymol.* **64,** 139–192
O'Donnell, M. E., and Kornberg, A. (1985) *J. Biol. Chem.* **260,** 12884–12889
Radding, C. M. (1982) *Annu. Rev. Genet.* **16,** 405–437
Rusche, J. R., Konigsberg, W., and Howard-Flanders, P. (1985) *J. Biol. Chem.* **260,** 949–955
Sancar, A., Stachelek, C., Konigsberg, W., and Rupp, W. D. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 2611–2615
Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 5463–5467
Schulz, G. E., and Schirmer, R. H. (1979) *Principles of Protein Structure*, Springer-Verlag, New York
Tsang, S. S., Muniyappa, K., Azhderian, E., Gonda, D. K., Radding, C. M., Flory, J., and Chase, J. W. (1985) *J. Mol. Biol.* **185,** 295–309
Wang, W.-B., and Tessman, E. T. (1986) *J. Bacteriol.* **168,** 901–910
Weinstock, G. M., McEntee, K., and Lehmann, I. R. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 126–130
Weinstock, G. M., McEntee, K., and Lehman, I. R. (1981) *J. Biol. Chem.* **256,** 8845–8849
West, S. C., Cassuto, E., Muraslim, J., and Howard-Flanders, P. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 2569–2573
Willis, D. K., Uhlin, B. E., Amini, K. S., and Clark, A. J. (1981) *Mol. Gen. Genet.* **183,** 497–504
Yancey, S. D., and Porter, R. D. (1984) *Mol. Gen. Genet.* **193,** 53–57
Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) *Gene (Amst.)* **33,** 103–119
Zoller, M. J., and Smith, M. (1983) *Methods Enzymol.* **100,** 468–500