Article No. jmbi.1998.2457 available online at http://www.idealibrary.com on IDEAL J. Mol. Biol. (1999) 286, 437–445

JMB

On the *in Vivo* Function of the RecA ATPase

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The *Escherichia coli* RecA protein is the prototype of the RecA/RAD51/DMC1 family of strand transferases acting in genetic recombination. The *E96D* mutant was previously isolated in a screen for toxic *recA* mutants and was found to constitutively derepress the SOS genes and inhibit chromosome segregation in *E. coli*. Here, we have found that the *E96D* mutation lowers the RecA k_{cat} value for ATP hydrolysis 100-fold. Use of this mutant reveals that the ATPase and branch migration activities of RecA are not necessarily required for catalyzing *in vivo* recombinational pairing and LexA cleavage. In addition to its effect on ATP hydrolysis, the mutation causes ATP to more strongly promote the transition to the biologically active, extended conformation of the RecA enzyme. The enhanced ATP binding is apparently the cause for a broader nucleic acid ligand specificity. The use of RNA and double-stranded DNA as cofactors for LexA cleavage could give rise to the inappropriate, constitutive derepression of the SOS genes. This underscores the need for the ATP affinity to be optimized so that RecA becomes selectively activated only during DNA repair and recombination through binding single-stranded DNA.

Keywords: ATP hydrolysis; allosteric effector; recombination; branch migration; RecA

Introduction

The RecA enzyme is a DNA-dependent ATPase which plays key roles in genetic recombination and DNA repair. The biochemically active conformation of RecA is a helical polymer which requires ATP and single-stranded DNA (ssDNA) binding for assembly (Kowalczykowski, 1991). The role of this complex in genetic recombination is to catalyze homologous DNA sequence searches between the bound DNA strand and its complement in intact chromosomes (Cox & Lehman, 1987; Radding, 1982). The RecA-ATP-ssDNA complex also stimulates the autocleavage of the LexA transcriptional repressor (Little *et al*., 1980), giving rise to the derepression of SOS DNA repair genes (Little & Mount, 1982).

RecA binds DNA in two different conformations, depending on the absence or presence of ATP (DiCapua *et al*., 1992). In the absence of ATP, RecA binds DNA in a form known as the compact conformation that does not catalyze DNA sequence alignment or LexA cleavage (Kowalczykowski, 1991; DiCapua *et al*., 1992). The ATP-bound conformation has a larger helical pitch (DiCapua *et al*., 1992) and is known as the extended conformation.

The interconversion between the two conformations of RecA is a result of nucleotide binding and hydrolysis. ATP binding stabilizes the extended conformation and destabilizes the compact conformation (Kowalczykowski, 1991). ATP therefore pulls the conformational equilibrium toward the extended form. ADP has an antagonistic effect by destabilizing the extended conformation, pushing the equilibrium toward the compact conformation (Lee & Cox, 1990). Therefore, hydrolysis of ATP converts a nucleotide which stabilizes the extended form to one which destabilizes it. Because interconversion between the two conformations requires RecA to transiently dissociate from DNA, ATP hydrolysis is accompanied by enzyme turnover (Lee & Cox, 1990).

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Abbreviations used: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; εssDNA, etheno-ssDNA (modified ssDNA containing 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine residies); RFI, relative fluorescence increase; ATPγS, adenosine 5′-γ-(thiotriphosphate); AMP-PNP, adenylyl β,γ-imidophosphate.

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0022-2836/99/070437–09 $30.00/0

In addition to destabilizing the extended conformation of the enzyme, ADP promotes a third conformation which is intermediary in pitch between the compact and extended conformations of RecA (Ellouze *et al.*, 1995). The ADP stabilized form and the nucleotide-free form of RecA both appear to be unable to catalyze LexA cleavage and DNA strand exchange.

In addition to its role in RecA-DNA dissociation, ATP hydrolysis plays a role in branch migration of D-loops and Holliday intermediates (MacFarland *et al.*, 1997). Reconstitution of RecA-dependent synapsis *in vitro* has shown that ATP binding, but not ATP hydrolysis, is required for finding and pairing the complementary DNA strands in intact chromosomes (Menetski *et al.*, 1990). RecA subsequently catalyzes extensive heteroduplex formation. This latter step is greatly enhanced by ATP hydrolysis (MacFarland *et al.*, 1997). Despite extensive work on the role of the RecA ATPase *in vitro*, its importance for *in vivo* function is unclear. As described in the accompanying paper, the E96D allele was isolated in a screen for toxic *recA* mutants (Campbell & Davis, 1999). Here, we show that this mutation greatly reduces the ATPase of RecA without affecting the ATP-dependent conformational transition to the biologically active, extended form of the enzyme. We use this mutant to test whether the ATPase and branch migration activities of RecA are needed for *in vivo* function. We also test the mutation's effect on the allosteric properties of ATP binding, and its consequences on the LexA cleavage reaction.

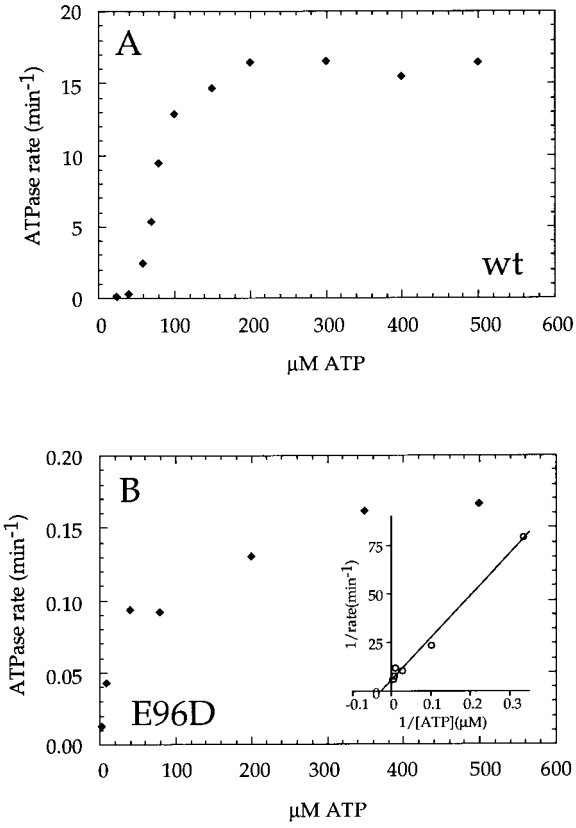

Figure 1. [ATP] dependence of ATPase activity. The rate of wild-type ATPase (a) and the rate of E96D ATPase (b) are plotted as a function of ATP. The inset of (b) contains a double-reciprocal plot of the E96D data which was used to determine the $K_{M,ATPase}$.

Results

ATP binding and hydrolysis

The accompanying paper describes the isolation of a group of mutations in the *Escherichia coli recA* gene which cause the protein to become toxic to the cell, but which constitutively stimulate LexA cleavage and which catalyze significant levels of recombination (Campbell & Davis, 1999). This group includes the E96D mutation, which occurs on the distal side of the water molecule proposed to act as the nucleophile during ATP hydrolysis (Story & Steitz, 1992). The E96D mutation shortens the amino acid side-chain and should increase the distance between the water molecule and the activating carboxylate. We predicted that the mutation would significantly reduce the ATPase rate.

To test this prediction, the ATP hydrolysis rate was determined. Two ATPase assays were used, an assay coupled to NADH oxidation and a radioactive P_i production assay. The measurements between assays were consistent within 15% of one another. Figure 1 shows that ATPase activity of RecA$^+$ becomes activated at 70 µM ATP. E96D is activated at half the concentration of ATP (36 µM; Figure 1(b) inset; $R^2 = 0.99$) but the cooperativity is lost in this mutant. The [ATP]-dependence of ATP hydrolysis is sigmoidal for RecA$^+$, but is hyperbolic for E96D (see Discussion). Moreover, the E96D mutation lowers the k_{cat} 100-fold from 16 per minute to 0.17 per minute. The majority of the E96D and RecA$^+$ ATPase activities are DNA-dependent, with an ATPase rate without DNA of 0.017 per minute for E96D and 0.018 per minute for RecA$^+$. No effort was made to determine whether the DNA-independent ATPase activities were due to trace contaminants.

Potency of ATP as an allosteric effector of E96D

As shown in the accompanying paper, the E96D mutation enhances RecA's affinity for DNA. We considered two possibilities to explain this phenomenon. A lower ATPase rate will decrease the amount of ADP, which antagonizes the tight-binding state of RecA (Lee & Cox, 1990). Alternatively, a higher ATP affinity will enhance the allosteric effect that ATP has on DNA binding (Kowalczykowski, 1991). To distinguish between these possibilities, we determined the potency of the allosteric effect of the non-hydrolyzable analog AMP-PNP (adenyl β,γ-imidophosphate) by measuring the concentration needed to promote

the conversion of the compact conformation of RecA into the extended conformation ($S_{0.5,AMP\text{-}PNP}$). This equilibrium shift was measured through the enhancement of fluorescence as the etheno-ssDNA (εssDNA)-RecA complex is converted to the εssDNA-RecA-ATP complex (Silver & Fersht, 1982; Cazenave *et al.*, 1983). Figure 2(a) shows that AMP-PNP acts as a much more potent allosteric effector for the E96D enzyme than it does for the wild-type enzyme. The wild-type enzyme has an $S_{0.5,AMP\text{-}PNP} = 900$ μM for the transition, whereas the E96D enzyme has an $S_{0.5,AMP\text{-}PNP} = 18$ μM.

In order to understand how well the allosteric effect of AMP-PNP reflects that of ATP, the experiment was repeated using ATP. Figure 2(b) shows that the allosteric effect of ATP parallels that of AMP-PNP with the $S_{0.5,ATP}$ for wild-type at 70 μM and that the $S_{0.5,ATP}$ for E96D at 1.6 μM. The relative allosteric enhancement of the E96D mutation was the same with AMP-PNP as it was for ATP (40 to 50-fold). The E96D mutant displayed a cooperative transition by ATP in this assay, in contrast to the ATPase assay.

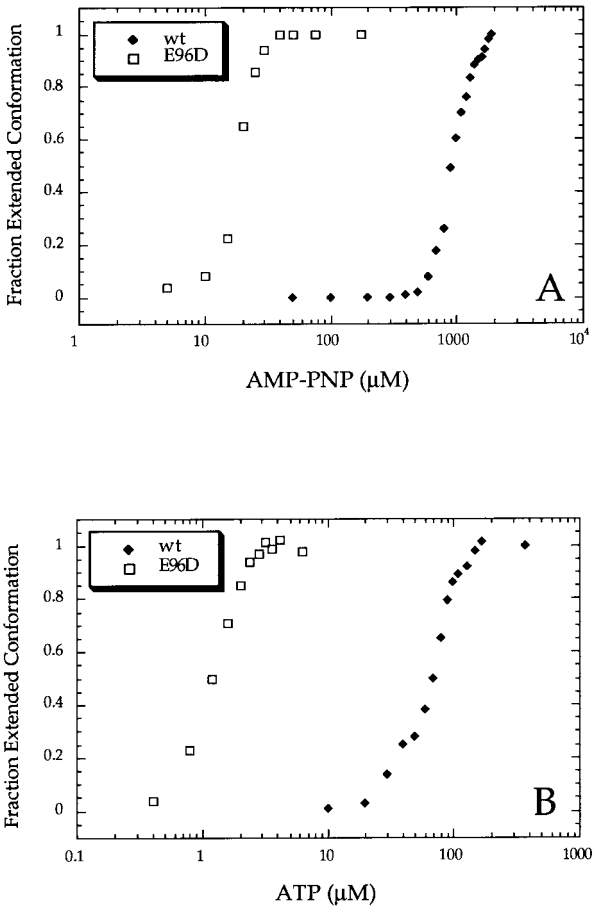

Figure 2. The potency of ATP and AMP-PNP as allosteric effectors for the transition from the compact to the extended conformation. RecA was bound to etheno-ssDNA and the fluorescence was measured as (a) AMP-PNP or (b) ATP was titrated into the reaction. The species giving the greater fluorescence is the extended conformation.

Correlation between LexA cleavage and conformational transition

The extended conformation of the E96D mutant was more easily stabilized by ATP than was RecA$^+$. In order to assess the biochemical consequences of the enhancement, the ATP titration was repeated for the LexA cleavage assay in Figure 3. We found that the E96D mutation affected the $S_{0.5,ATP}$ for LexA cleavage in the same way as the $S_{0.5,ATP}$ for the conformational transition. This showed that the activation of LexA cleavage parallels the conformational transition.

Effect of other polynucleotides

In the presence of ATP, RecA$^+$ exclusively uses ssDNA as a cofactor for LexA cleavage. Under normal conditions RecA does not have sufficient affinity for RNA (McEntee & Weinstock, 1981; Wang *et al.*, 1988a,b), and dsDNA binding is very slow (Pugh & Cox, 1988). Because nucleic acid binding and ATP binding form a thermodynamic cycle (Kowalczykowski, 1991), RecA$^+$ can utilize RNA and dsDNA as cofactors for LexA cleavage in the presence of the tight-binding ATP analog ATPγS (DiCapua *et al.*, 1992). Because E96D binds ATP more tightly than RecA$^+$ (Figure 2), we hypothesized that the mutant might be able to utilize RNA and dsDNA as ligands for LexA cleavage. Figure 4 confirms this prediction. In the presence of ATP, RecA$^+$ was selectively activated by the ssDNA (Figure 4, lanes 1-3), which it binds rapidly (Pugh & Cox, 1987) and with high affinity (Kowalczykowski, 1991). As reported by (DiCapua *et al.* (1992), RecA$^+$ can use ssDNA, dsDNA or RNA for LexA cleavage when ATP is substituted with ATPγS (adenosine 5′-γ-(thiotriphosphate)) (Figure 4, lanes 4-6). E96D can use all three nucleic acids for LexA cleavage whether ATP or ATPγS is used in the reaction (Figure 4, lanes 8-13). Because the reaction required the addition of nucleic acids (Figure 4, lane 14) and because the E96D enzyme preparation had a high A_{280}/A_{260} ratio, we inferred that the E96D enzyme was not contaminated with nucleic acids. To eliminate the possibility that the RNA used in these experiments is contaminated with DNA, it was shown that the RNA stimulation of E96D LexA cleavage was sensitive to RNase A, but insensitive to DNaseI (data not shown).

Effect of E96D on joint molecule formation

The role of the RecA branch migration activity on *in vivo* recombination has never been tested because a mutation that separates recombinational pairing from branch migration has never been found. As presented in the accompanying paper (Campbell & Davis, 1999), two alleles of *lacZ* were used as recombination substrates in a transient

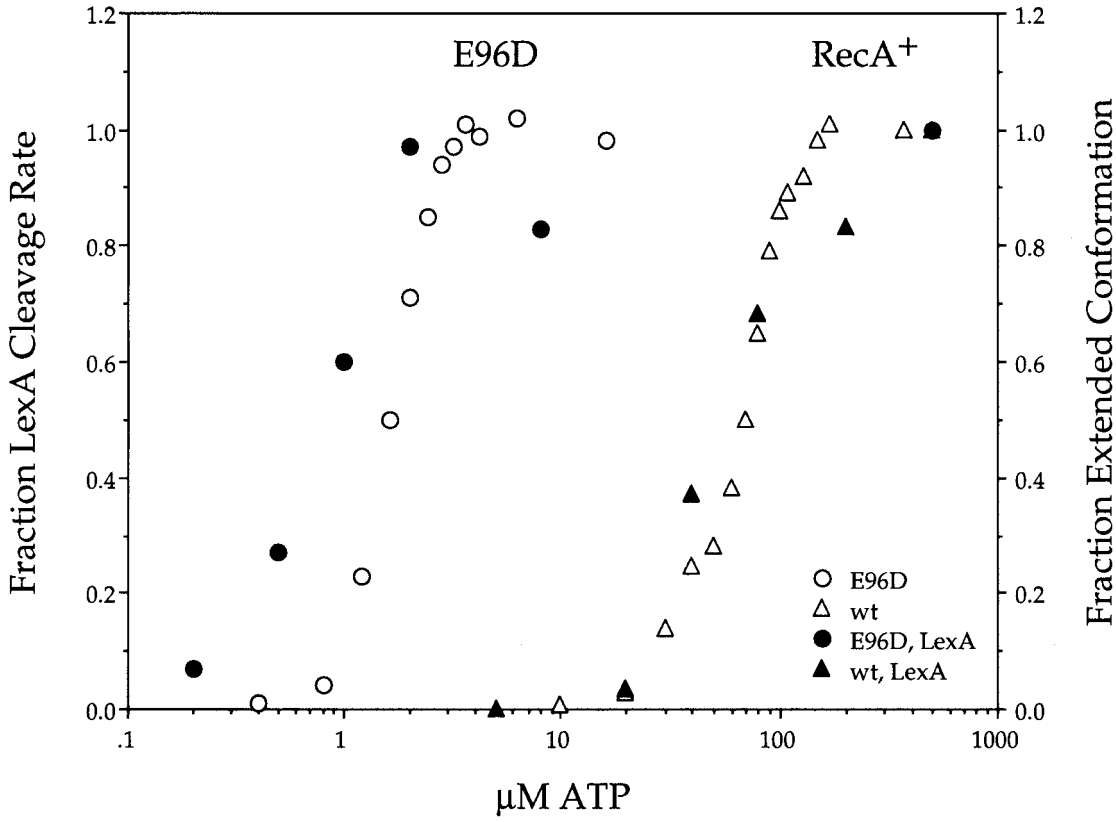

Figure 3. The effect of ATP on the LexA cleavage reaction rate. RecA-dependent LexA cleavage was performed at a variety of ATP concentrations. The rate was determined from the initial slope of the reaction, and plotted as a function of [ATP] as filled characters. The ATP-dependent conformational transition curves from Figure 2 are included as open characters for comparison. Full activation of LexA cleavage by RecA$^+$ is 0.52 μM LexA cleaved per minute per μM RecA. Full activation for E96D is 0.25 μM LexA cleaved per minute per μM RecA.

recombination assay showing that the *recA E96D* mutant could catalyze recombination *in vivo* at a significant, yet reduced, level compared with *recA*$^+$. *E96D* gave one quarter of the number of recombinants that *recA*$^+$ did, but its activity was 27-fold higher than the Δ*recA* background. Because there is a correlation between RecA's ATPase and branch migration activities (Menetski *et al.*, 1990; Rehrauer & Kowalczykowski, 1993), it seemed that the ATPase-defective E96D mutant might separate branch migration from recombinational pairing. To test this possibility, we measured the strand invasion and branch migration activities of the E96D mutant using a three-stranded joint molecule assay.

Figure 5 shows that E96D is capable of forming joint molecules *in vitro*. This confirms that polymerization, pairing and invasion are functional in this mutant, as expected from the *in vivo* recombination results. RecA$^+$ also forms joint molecules that are resolved into the RFII nicked circle product through extensive branch migration (Figure 5; Cox & Lehman, 1981). Joint molecules are rapidly formed by E96D that migrate at a distinct mobility, but are not resolved into the RFII product (Figure 5). Thus, the ATPase-defective E96D mutant is defective in extensive branch migration just like RecA$^+$ in the absence of ATP hydrolysis (Menetski *et al.*, 1990).

Discussion

RecA catalyzes recombination and LexA cleavage *in vivo* with a greatly reduced ATPase activity

The role of the ATPase activity in the *E. coli* RecA enzyme has been the subject of considerable debate over the past ten years. The protein carries out DNA pairing and strand invasion during genetic recombination and LexA cleavage when DNA is damaged. *In vitro* studies have shown that the LexA cleavage reaction, the DNA pairing activity and strand invasion activity all require ATP binding, but not ATP hydrolysis (Kowalczykowski & Eggleston, 1994). *In vitro* studies have underscored the need for ATP hydrolysis for efficient branch migration (Shan *et al.*, 1996). Despite this extensive biochemical analysis, there has been no direct test of the RecA ATPase function *in vivo*. Attempts to show the dispensability of ATP hydrolysis *in vivo* with the RecA K72R mutant failed when it was

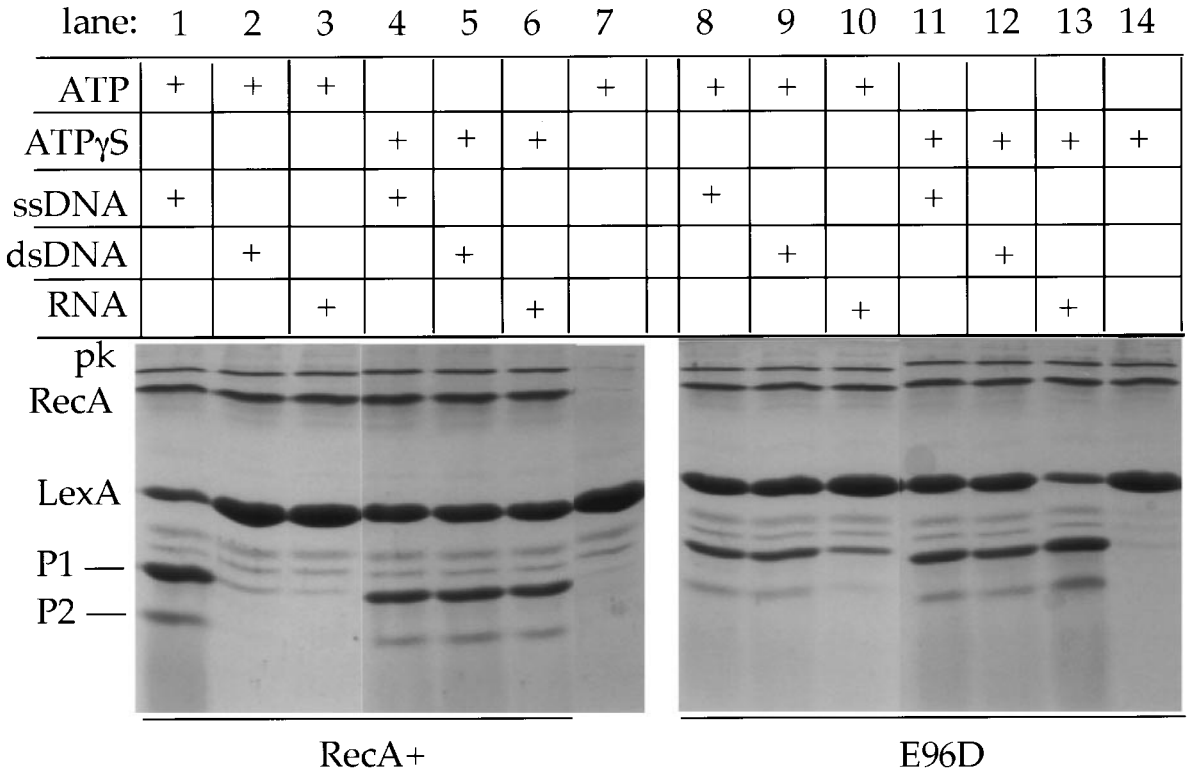

Figure 4. The nucleic acid dependence of LexA cleavage. LexA cleavage reactions were carried out for 15 minutes with ssDNA and 40 minutes with dsDNA, RNA, and no nucleic acid (lane 14). The proteins in each reaction are resolved using a SDS/15% polyacrylamide gel. pk, pyruvate kinase; P1 and P2 are the two LexA polypeptide fragments generated from proteolytic cleavage.

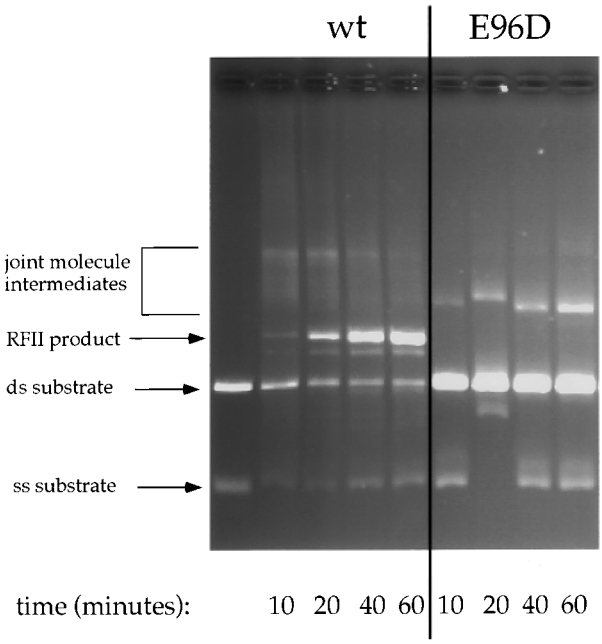

Figure 5. E96D can form joint molecules but cannot catalyze extensive branch migration. A time-course of joint molecule formation is shown in the presence of ATP and SSB protein. M13mp18 ssDNA circle and linear duplex are the substrates of the reaction, and the product is the nicked circle (RFII). The slower migration of the ssDNA substrate in lane 7 was an artifact of an incomplete denaturation of RecA.

found to have no recombinational or LexA cleavage activities *in vivo* (A. Bailone, personal communication).

Here, we investigated whether ATP hydrolysis is needed for genetic recombination *in vivo* using the RecA E96D mutant. We find that despite hydrolyzing ATP 100-fold more slowly than wild-type, the E96D mutant catalyzes one-quarter of the level of recombination and constitutively catalyzes LexA cleavage *in vivo* (Campbell & Davis, 1999).

The *in vivo* activity of E96D is in sharp contrast to that of the other ATPase-defective RecA mutant, K72R. The primary difference between the two is that E96D can attain the extended, biologically active conformation through ATP binding, whereas K72R could not unless ATP was substituted with a high concentration of dATP (Rehrauer & Kowalczykowski, 1993).

In vivo recombination with a large reduction of ATP hydrolysis has also been recently observed for the *Saccharomyces cerevisiae* RecA-homolog RAD51 (Sung & Stratton, 1996). This RAD51 mutation occurs at the same position in the Walker P-loop as the RecA K72R mutation. Unlike the RecA K72R mutant, the RAD51 mutant could use ATP as a cofactor for recombination *in vitro* and it also could catalyze recombination *in vivo*. These results imply that attaining the extended conformation through ATP binding has a greater bearing on *in vivo*

recombination (and in the case of RecA, LexA cleavage) than does ATP hydrolysis.

Effects of E96D on nucleotide binding

Besides greatly reducing the ATP hydrolysis rate, the E96D mutation affects ATP binding in a few different ways. First, E96D has lost the cooperativity in ATP hydrolysis. Secondly, ATP activates ATP hydrolysis in E96D at half the level required to activate RecA$^+$. Thirdly, ATP has a much more pronounced effect on the conformational change of E96D than on its ATP hydrolysis activation. E96D requires 44-fold less ATP to change conformations than does RecA$^+$. This conformational change remains cooperative in E96D, unlike its ATP hydrolysis.

These results strongly imply that the affinity for ATP has been enhanced by the mutation. In the presence of DNA, the K_D for ATP is 2.5 μM (Kowalczykowski, 1986). However, only 1.6 μM ATP is required for E96D to change conformations. Generally, RecA must be heavily saturated with ATP to change conformations given that ATP is needed at a much higher concentration than its K_D (Kowalczykowski, 1991). The caviat to this argument is that an ATP occupancy of less that 0.5 could potentially facilitate the conformational change of E96D.

The results also confirm that the conformational change and ATP hydrolysis act independently (Rehrauer & Kowalczykowski, 1993; Kowalczykowski, 1991). The E96D mutation seems to prevent the cooperative activation of ATP hydrolysis. Somehow the mutation allows the RecA subunits to cooperate during the conformational change but not during ATP hydrolysis.

RecA-promoted extensive branch migration is not necessary for recombination *in vivo*

For many years, recombinational pairing could only be attained *in vitro* when ATP was hydrolyzed (Cox & Lehman, 1987). It was then discovered that under competitive DNA binding conditions RecA could catalyze joint molecule formation in the absence of ATP hydrolysis (Menetski *et al*., 1990). Under highly selective conditions, RecA could catalyze longer stretches of heteroduplex DNA (Shan *et al*., 1996), but in general, extensive branch migration was substantially inhibited by using a non-hydrolyzable analog of ATP.

Genetic recombination can involve more than 20 kb of heteroduplex DNA (Huisman & Fox, 1986). Although RecA catalyzes branch migration, the RuvAB (Parsons *et al*., 1992) and RecG (Lloyd & Sharples, 1993a,b) enzymes of *E. coli* also participate in moving the Holliday junction. Despite being defective in branch migration (Figure 5), the ATPase-defective E96D enzyme was shown to catalyze recombination *in vivo*, though at a somewhat lower level than RecA$^+$ (Campbell & Davis, 1999). This shows that the extensive branch migration activity of RecA is at least partly dispensable for *in vivo* recombination. This is consistent with the recent observation that the wild-type human RAD51 protein can catalyze joint molecule formation, but not extensive branch migration (Baumann *et al*., 1996). Presumably, the dispensability of the RecA/RAD51 branch migration activity lies in the redundancy of this activity with other branch migration enzymes, RuvAB and RecG. Conversely, eliminating both the RuvAB and RecG enzymes from *E. coli* has the synergistic effect of reducing genetic recombination 1000-fold (Lloyd, 1991), implying that the RecA branch migration activity cannot effectively substitute for these more processive enzymes.

Enhanced ATP binding by E96D drives the conformational transition and is linked to DNA affinity

ATP binding and DNA binding are linked in a thermodynamic cycle (Kowalczykowski, 1991). This means that an enhanced nucleic acid affinity should give rise to an enhanced ATP affinity and *vice versa*. E96D was found to bind ssDNA more tightly than RecA$^+$ did (Campbell & Davis, 1999). Likewise, we showed that ATP and its non-hydrolyzable analog AMP-PNP are both more potent allosteric effectors for stabilizing the extended conformation in E96D compared to RecA$^+$. Thus, the enhanced nucleotide binding is sufficient to explain the enhanced allosteric effect of ATP, without needing to invoke an effect from the ATPase activity. We showed that this allosteric effect of RecA is synonymous with activating the LexA cleavage reaction.

The increased available nucleic acid binding energy of E96D allows it to substitute ssDNA with dsDNA, which normally binds slowly, and RNA, which normally binds weakly, for assembling the extended conformation of RecA. This interpretation is consistent with the observations made by DiCapua *et al*. (1992) and in Figure 4, that RecA$^+$ has an enhanced affinity for RNA and has more rapid binding kinetics with dsDNA (Pugh & Cox, 1987) in the presence of the tightly binding nucleotide ATPγS. By productively interacting with RNA and dsDNA or by effectively displacing SSB protein from ssDNA (Campbell & Davis, 1999), the need for DNA damage in activating LexA cleavage by RecA E96D is potentially eliminated. It is also consistent with the results by Wang *et al*. (Wang *et al*., 1986, 1988a,b) who showed that the coprotease constitutive mutants Q184K and E38K, which have enhanced interactions with nucleotides, can also substitute RNA for ssDNA in LexA cleavage.

The link between nucleotide affinity and nucleic acid affinity of RecA underscores the evolutionary pressures in keeping these interactions in balance. If the ATP affinity becomes too low, as in RecA430, then DNA binding is not stable enough to successfully compete with other proteins like SSB (Menetski & Kowalczykowski, 1990). If the

ATP affinty becomes too high, as in the E96D mutant, then the nucleic acid specificity is not selective enough to prevent gratuitous induction of the SOS genes in the absence of DNA damage, whether it be through competition with SSB for ssDNA, or binding dsDNA or RNA directly. The cell needs to regulate the activation of RecA because constitutive activation prevents cell division through derepression of the *sulA* gene (George *et al.*, 1975) and causes derepression of lambdoid prophages.

Two populations of RecA can explain the apparent contradiction for needing ATP hydrolysis

Despite catalyzing recombination and LexA cleavage *in vivo*, the ATPase-defective E96D mutation prevents chromosome segregation and is toxic to *E. coli* (Campbell & Davis, 1999). A model was presented in the accompanying paper in which RecA dissociates very slowly from DNA, preventing resolution of recombinational intermediates and chromosomes, and giving rise to lethal filamentation of *E. coli*. When RecA-DNA dissociation was examined in the absence of ATP hydrolysis, 40% of the RecA dissociated from DNA. This population of RecA may allow some recombinational products to dissociate while the tighter binding population prevents the resolution of chromosomes, which may have more complexes bound to them.

Materials and Methods

Chemicals and DNA

[γ-^{32}P]ATP (6000 Ci/mmol) was from New England Nuclear. Etheno-M13 ssDNA was prepared as described (Zlotnick *et al.*, 1993) and measured spectrophotometrically using an extinction coefficient of 7000 M^{-1} cm^{-1} at 260 nm. AMP-PNP, type II rabbit muscle pyruvate kinase, NADH and phosphoenolpyruvate were obtained from Sigma. ATP, yeast RNA, and rabbit muscle lactate dehydrogenase were obtained from Boehringer-Mannheim. M13mp18 ssDNA was prepared as described by Sambrook *et al.* (1989). M13mp18 dsDNA was prepared using a standard Qiaprep plasmid procedure (Qiagen Inc.), followed by equilibrium CsCl-ethidium bromide centrifugation (Sambrook *et al.*, 1989), dialysis and ethanol precipitation.

Protein purification

The histidine-tagged RecA$^+$ and RecA E96D proteins were purified as described in the accompanying paper (Campbell & Davis, 1999). LexA was expressed from strain JL652 (Little, 1984; obtained from S. Kowalczykowski) using the preparative procedure of Schnarr *et al.* (1985). Protein concentration was determined using a molar extinction coefficient of 7300 M^{-1} cm^{-1} at 280 nm. SSB protein was obtained as a gift from the A. Kornberg lab.

LexA cleavage assay

The rate of LexA cleavage was determined by performing a time-course of LexA cleavage and calculating the rate over the initial, linear portion of the reaction. Reactions contained 30 mM Tris-HCl (pH 7.5), 30 mM NaCl, 4.0 mM $MgCl_2$, 10 μM LexA, 6.0 μM etheno-ssDNA, 1.2 μM RecA, 2 mM phosphoenolpyruvate and 12 units pyruvate kinase/ml. The concentration of nucleoside triphosphate varied, and is indicated in the legends to the Figures. The products and substrate of the LexA cleavage reaction were separated on a 15% polyacrylamide gel. The gels were stained with Coomassie Brilliant Blue, destained, and quantified on an AlphaImager 2000 (Alpha Innotech Corp., San Leandro, CA).

DNA binding assay

The DNA binding assay was performed as described in the accompanying paper (Campbell & Davis, 1999) RecA was used at 1.2 μM and εssDNA was used at 3.7 μM.

ATP hydrolysis

ATP hydrolysis by the E96D mutant was measured by the time-dependent conversion of [γ-^{32}P]ATP to $^{32}P_i$, using polyethyleneimine-cellulose thin-layer chromatography developed in 0.4 M LiCl with 1 M formic acid to resolve product from substrate. Radioactivity was quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Assays were conducted in 20 mM Tris-HCl (pH 7.5), 4 mM $MgCl_2$, 100 μM etheno-M13 ssDNA, 10 μM E96D, with various levels of nucleotides at 30 °C.

The rate of RecA$^+$ ATPase was determined using a coupled assay to NADH oxidation following the time-dependent decrease in absorbance at 340 nm (Kowalczykowski & Krupp, 1987). The same conditions were used as with the radioactive assay, except that 6 μM etheno-ssDNA and 0.4 μM RecA$^+$ were used. The rate of E96D ATPase at 500 μM ATP was measured using this assay, and RecA$^+$ was measured using the radioactive assay to show that the assays were consistent with one another.

Joint molecule assay

Joint molecules were formed using the method by Lavery & Kowalczykowski (1990). RecA protein was preincubated with ssDNA for three minutes. Afterwards, SSB protein was added, followed by dsDNA three minutes later. The reaction was carried out at 37 °C.

Acknowledgements

We thank S. Kowalczykowski for many stimulating discussions. We thank K. Guillemin, R. Cho, and R. Devoret for a careful reading of the manuscript. We thank R. Rehrauer and members of the I. R. Lehman lab for advice regarding protein purification. M. J. Campbell was a Howard Hughes Medical Institute Predoctoral Fellow during some of this work. This work was supported by the NIH grant R37 HG00198-19 to R.W.D.

References

Baumann, P., Benson, F. E. & West, S. C. (1996). Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions *in vitro*. *Cell,* **87(4)**, 757-766.

Campbell, M. J. & Davis, R. W. (1999). Toxic mutations in the recA gene of *E. coli* prevent proper chromosome segregation. *J. Mol. Biol.* **286**, 417-435.

Cazenave, C., Toulme, J. J. & Helene, C. (1983). Binding of RecA protein to single-stranded nucleic acids: spectroscopic studies using fluorescent polynucleotides. *EMBO J.* **2**, 2247-2251.

Cox, M. M. & Lehman, I. R. (1981). recA protein of *Escherichia coli* promotes branch migration, a kinetically distinct phase of DNA strand exchange. *Proc. Natl Acad. Sci. USA,* **78(6)**, 3433-3437.

Cox, M. M. & Lehman, I. R. (1987). Enzymes of general recombination. *Annu. Rev. Biochem.* **56**, 229-262.

DiCapua, E., Cuillel, M., Hewat, E., Schnarr, M., Timmins, P. A. & Ruigrok, R. W. (1992). Activation of recA protein. The open helix model for LexA cleavage. *J. Mol. Biol.* **226(3)**, 707-719.

Ellouze, C., Takahashi, M., Wittung, P., Mortensen, K., Schnarr, M. & Norden, B. (1995). Evidence for elongation of the helical pitch of the RecA filament upon ATP and ADP binding using small-angle neutron scattering. *Eur. J. Biochem.* **233**, 579-583.

George, J., Castellazzi, M. & Buttin, G. (1975). Prophage induction and cell division in *E. coli*. III. Mutations sfiA and sfiB restore division in tif and lon strains and permit the expression of mutator properties of tif. *Mol. Gen. Genet.* **140(4)**, 309-332.

Huisman, O. & Fox, M. S. (1986). A genetic analysis of primary products of bacteriophage lambda recombination. *Genetics,* **112(3)**, 409-420.

Kowalczykowski, S. C. (1986). Interaction of recA protein with a photoaffinity analogue of ATP, 8-azido-ATP: determination of nucleotide cofactor binding parameters and of the relationship between ATP binding and ATP hydrolysis. *Biochemistry.* **25**, 5872-5881.

Kowalczykowski, S. C. (1991). Biochemistry of genetic recombination: energetics and mechanism of DNA strand exchange. *Annu. Rev. Biophys. Chem.* **20(539)**, 539-575.

Kowalczykowski, S. C. & Eggleston, A. K. (1994). Homologous pairing and DNA strand-exchange proteins. *Annu. Rev. Biochem.* **63(991)**, 991-1043.

Kowalczykowski, S. C. & Krupp, R. A. (1987). Effects of *Escherichia coli* SSB protein on the single-stranded DNA-dependent ATPase activity of *Escherichia coli* RecA protein. Evidence that SSB protein facilitates the binding of RecA protein to regions of secondary structure within single-stranded DNA. *J. Mol. Biol.* **193(1)**, 97-113.

Lavery, P. E. & Kowalczykowski, S. C. (1990). Properties of recA441 protein-catalyzed DNA strand exchange can be attributed to an enhanced ability to compete with SSB protein. *J. Biol. Chem.* **265(7)**, 4004-4010.

Lee, J. W. & Cox, M. M. (1990). Inhibition of recA protein promoted ATP hydrolysis. 1. ATP gamma S and ADP are antagonistic inhibitors. *Biochemistry,* **29(33)**, 7666-7676.

Little, J. W. (1984). Autodigestion of lexA and phage lambda repressors. *Proc. Natl Acad. Sci. USA,* **81**, 1375-1379.

Little, J. W., Edmiston, S. H., Pacelli, L. Z. & Mount, D. W. (1980). Cleavage of the *Escherichia coli* lexA protein by the recA protease. *Proc. Natl Acad. Sci. USA,* **77(6)**, 3225-3229.

Little, J. W. & Mount, D. W. (1982). The SOS regulatory system of *Escherichia coli*. *Cell,* **29(1)**, 11-22.

Lloyd, R. G. (1991). Conjugational recombination in resolvase-deficient *ruvC* mutants of *Escherichia coli* K-12 depends on recG. *J. Bacteriol.* **173(17)**, 5414-5418.

Lloyd, R. G. & Sharples, G. J. (1993a). Dissociation of synthetic Holliday junctions by *E. coli* RecG protein. *EMBO J.* **12(1)**, 17-22.

Lloyd, R. G. & Sharples, G. J. (1993b). Processing of recombination intermediates by the RecG and RuvAB proteins of *Escherichia coli*. *Nucl. Acids Res.* **21(8)**, 1719-1725.

MacFarland, K. J., Shan, Q., Inman, R. B. & Cox, M. M. (1997). RecA as a motor protein. Testing models for the role of ATP hydrolysis in DNA strand exchange. *J. Biol. Chem.* **272(28)**, 17675-17685.

McEntee, K. & Weinstock, G. M. (1981). tif-1 mutation alters polynucleotide recognition by the recA protein of *Escherichia coli*. *Proc. Natl Acad. Sci. USA,* **78(10)**, 6061-6065.

Menetski, J. P. & Kowalczykowski, S. C. (1990). Biochemical properties of the *Escherichia coli* recA430 protein. Analysis of a mutation that affects the interaction of the ATP-recA protein complex with single-stranded DNA. *J. Mol. Biol.* **211(4)**, 845-855.

Menetski, J. P., Bear, D. G. & Kowalczykowski, S. C. (1990). Stable DNA heteroduplex formation catalyzed by the *Escherichia coli* RecA protein in the absence of ATP hydrolysis. *Proc. Natl Acad. Sci. USA,* **87(1)**, 21-25.

Parsons, C. A., Tsaneva, I., Lloyd, R. G. & West, S. C. (1992). Interaction of *Escherichia coli* RuvA and RuvB proteins with synthetic Holliday junctions. *Proc. Natl Acad. Sci USA,* **89(12)**, 5452-5456.

Pugh, B. F. & Cox, M. M. (1987). Stable binding of recA protein to duplex DNA. Unraveling a paradox. *J. Biol. Chem.* **262(3)**, 1326-1336.

Pugh, B. F. & Cox, M. M. (1988). General mechanism for RecA protein binding to duplex DNA. *J. Mol. Biol.* **203(2)**, 479-493.

Radding, C. M. (1982). Homologous pairing and strand exchange in genetic recombination. *Annu. Rev. Genet.* **16**, 405-437.

Rehrauer, W. M. & Kowalczykowski, S. C. (1993). Alteration of the nucleoside triphosphate (NTP) catalytic domain within *Escherichia coli* recA protein attenuates NTP hydrolysis but not joint molecule formation. *J. Biol. Chem.* **268(2)**, 1292-1297.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schnarr, M., Pouyet, J., Granger-Schnarr, M. & Daune, M. (1985). Large-scale purification, oligomerization equilibria, and specific interaction of the LexA repressor of *Escherichia coli*. *Biochemistry,* **24**, 2812-2818.

Shan, Q., Cox, M. M. & Inman, R. B. (1996). DNA strand exchange promoted by RecA K72R. Two reaction phases with different $Mg2^{+}$ requirements. *J. Biol. Chem.* **271(10)**, 5712-5724.

Silver, M. S. & Fersht, A. R. (1982). Direct observation of complexes formed between recA protein and a fluorescent single-stranded deoxyribonucleic acid derivative. *Biochemistry,* **21**, 6066-6072.

Story, R. M. & Steitz, T. A. (1992). Structure of the recA protein-ADP complex. *Nature,* **355(6358)**, 374-376.

Sung, P. & Stratton, S. A. (1996). Yeast Rad51 recombinase mediates polar DNA strand exchange in the absence of ATP hydrolysis. *J. Biol. Chem.* **271(45)**, 27983-27986.

Wang, W. B. & Tessman, E. S. (1986). Location and functional regions of the *Escherichia coli* RecA protein by DNA sequence analysis of RecA protease-constitutive mutants. *J. Bacteriol.* **168**, 901-910.

Wang, W. B., Sassanfar, M., Tessman, I., Roberts, J. W. & Tessman, E. S. (1988a). Activation of protease-constitutive recA proteins of *Escherichia coli* by all of the common nucleoside triphosphates. *J. Bacteriol.* **170(10)**, 4816-4822.

Wang, W. B., Tessman, E. S. & Tessman, I. (1988b). Activation of protease-constitutive recA proteins of *Escherichia coli* by rRNA and tRNA. *J. Bacteriol.* **170**, 4823-4827.

Zlotnick, A., Mitchell, R. S., Steed, R. K. & Brenner, S. L. (1993). Analysis of two distinct single-stranded DNA binding sites on the recA nucleoprotein filament. *J. Biol. Chem.* **268(30)**, 22525-22530.

Edited by M. Gottesman

(Received 24 July 1998; received in revised form 2 November 1998; accepted 25 November 1998)