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Molecular Design and Functional Organization of the RecA Protein

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ABSTRACT: The bacterial RecA protein participates in a remarkably diverse set of functions, all of which are involved in the maintenance of genomic integrity. RecA is a central component in both the catalysis of recombinational DNA repair and the regulation of the cellular SOS response. Despite the mechanistic differences of its functions, all require formation of an active RecA/ATP/DNA complex. RecA is a classic allosterically regulated enzyme, and ATP binding results in a dramatic increase in DNA binding affinity and a cooperative assembly of RecA subunits to form an ordered, helical nucleoprotein filament. The molecular events that underlie this ATP-induced structural transition are becoming increasingly clear. This review focuses on descriptions of our current understanding of the molecular design and allosteric regulation of RecA. We present a comprehensive list of all published *recA* mutants and use the results of various genetic and biochemical studies, together with available structural information, to develop ideas regarding the design of RecA functional domains and their catalytic organization.

KEYWORDS: Homologous recombination, DNA repair, genome integrity, protein design, allosteric regulation

INTRODUCTION

The bacterial RecA protein is a central catalytic and regulatory component in the process of homologous genetic recombination, a process that is of fundamental importance to the maintenance of genomic integrity. RecA is traditionally known for its ability to catalyze the exchange of genetic information between two DNA molecules and to regulate the expression of its own gene and many other DNA repair genes in response to DNA damage. Originally discovered by Clark and Margulies (1965) in their search for mutants that showed defects in both general recombination as well as survival following exposure to UV light, *recA* has since been the subject of intensive study. RecA is a multifunctional enzyme that carries out several activities that have little mechanistic similarity, yet all are integrated to ensure efficient repair of damaged DNA. We now have a good understanding of most of the basic mechanistic features of RecA, as will be discussed in this review. Important questions remain as to how the protein coordinates the functions of its various domains to carry out its regulatory and catalytic activities.

A major catalytic function of RecA is the catalysis of recombinational DNA repair. RecA creates crossover structures between homologous DNAs, *e.g.*, sister chromatids, in which the undamaged DNA is used as a template to restore genetic information in the damaged partner. In this role the functional form of RecA is a helical nucleoprotein filament that is created by the directional polymerization (5′ to 3′) of RecA monomers bound to ATP onto single-stranded DNA. Recombinational repair comes into play in many instances where regions of single-stranded DNA arise inappropriately, either by

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virtue of direct external insults to DNA, *e.g.*, the creation of double strand breaks (DSBs) by exposure to ionizing radiation, or as a result of replication fork collapse when the polymerase complex encounters various lesions that it cannot use as template. In fact, a significant amount of work over the past several years has greatly improved our understanding of the mechanistic contribution of RecA to the reinitiation of replication following fork collapse (reviewed in Cox, 1998; Kowalczykowski, 2000; Cox *et al*., 2000; Courcelle *et al.*, 2001; Lusetti and Cox, 2002a). A simplified general model of the steps in the strand exchange mechanism for the repair of a DSB is shown in Figure 1. DSBs are processed by exonucleases, *e.g.*, RecBCD, leaving ssDNA ends. In the presence of single-stranded DNA binding protein (SSB), which binds to these ends and removes regions of secondary structure, RecA will efficiently polymerize on ssDNA to form a RecA nucleoprotein filament, also referred to as the presynaptic filament. These events are referred to as the "initiation phase." The active nucleoprotein filament will then incorporate a duplex DNA molecule and begin the next phase of the reaction, "homologous pairing and DNA strand exchange," resulting in formation of heteroduplex DNA. This step can involve accessory proteins such as RecF, RecO, and RecR that appear to assist in the regulation of RecA filament assembly and disassembly (see below), although the RecFOR complex is widely considered to have its major role in RecA-mediated repair of DNA gaps that can result when a replication complex stalls at a lesion but reinitiates further downstream (Kowalczykowski, 2000). The crossover structures, or Holliday junctions, will migrate ("branch migration") in a directional fashion as a result of either the motor function of RecA and/or the helicase activity of the RuvB protein complexed with RuvA. Resolution of the crossover structure is catalyzed by the RuvC protein in complex with RuvAB.

Prior to publication of the *E. coli* RecA protein structure (Story *et al*., 1992), electron microscopic studies had shown that the oligomeric filament structure of RecA assumed two general forms,

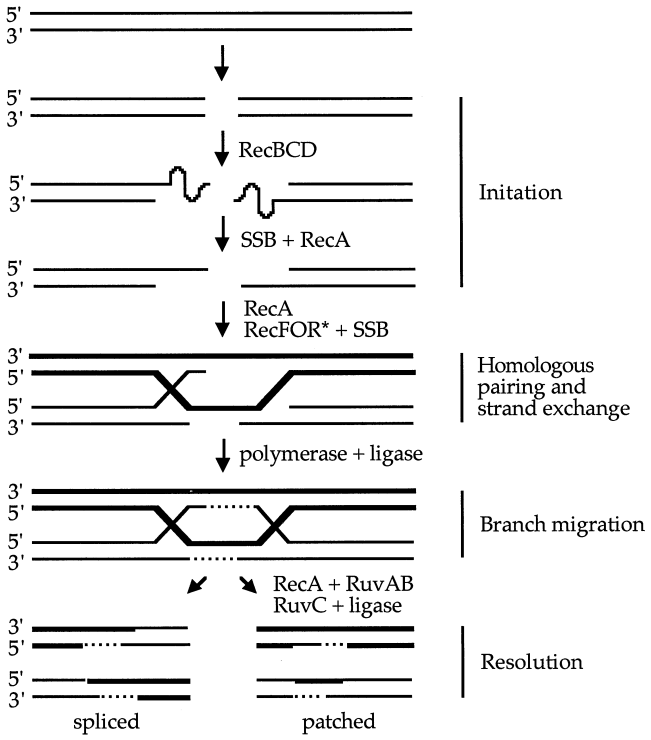

FIGURE 1. Model of recombinational repair of DNA double-strand breaks. This model shows only a basic image of DSB repair via homologous recombination and includes proteins important to each step. The pathway is divided into 4 general steps, and those proteins involved in the initiation" and homologous pairing" steps are discussed in this review. The RecFOR* complex plays a role in regulating the assembly and disassembly of the RecA nucleoprotein lament (see text), but is widely considered to have its major role in gap repair rather than DSB repair.

a compressed "inactive" filament and an extended "active" filament. The inactive filament is defined as that formed by protein alone, by protein with DNA but in the absence of any nucleotide, or by a protein/DNA complex with bound ADP. The inactive filament has ≈6.2 subunits/turn and the helical pitch (distance between helical turns) is approximately 68 Å. The active filament is defined as that formed by a complex of RecA, ss- or dsDNA, and ATP or an ATP analog. This filament also has ≈6.2 subunits/turn, but the pitch is approximately 95 Å (Egelman and Stasiak, 1993). In the crystal structure of *E. coli* RecA the filament has 6 subunits/turn and a helical pitch of 82.7 Å for the protein alone (Story *et al.*, 1992), and 83.1 Å in the presence of Mg^{2+}-ADP (Story and Steitz, 1992). Because this pitch is intermediate between the inactive and active forms, and because the structure contains ADP, it has been considered to be more representative of the inactive form of RecA. An image of the *E. coli* RecA crystal structure is shown in Figure 2. An unfortunate problem that persists to this day is that no group—and many have tried—has been successful in solving a structure of a RecA/ATP/DNA complex, the active form. Several additional X-ray structures of the *Mycobacterium tuberculosis* and *M. smegmatis* RecA proteins in either the absence or presence of NTP analogs are now available (Datta *et al.*, 2003a, 2003b), but in each case the helical pitch approximates 72.5 Å and more closely resembles the inactive filament form. Therefore, numerous mutational, biochemical, and electron microscopic studies using the current structures as models have been directed at providing a detailed molecular description of how the active RecA filament carries out its various activities. Models regarding the dynamic nature of the protein and its allosteric mechanism have been proposed based on these studies and will be discussed in this review.

We also present the first comprehensive listing of all known *recA* mutants. Using a number of these mutants and various published analyses we focus our discussions on a current understanding of the design and mechanistic abilities of the RecA protein. We begin with descriptions of individual protein domains. Models of the molecular mechanisms by which the functions of these domains are coordinated are then discussed in an attempt to provide

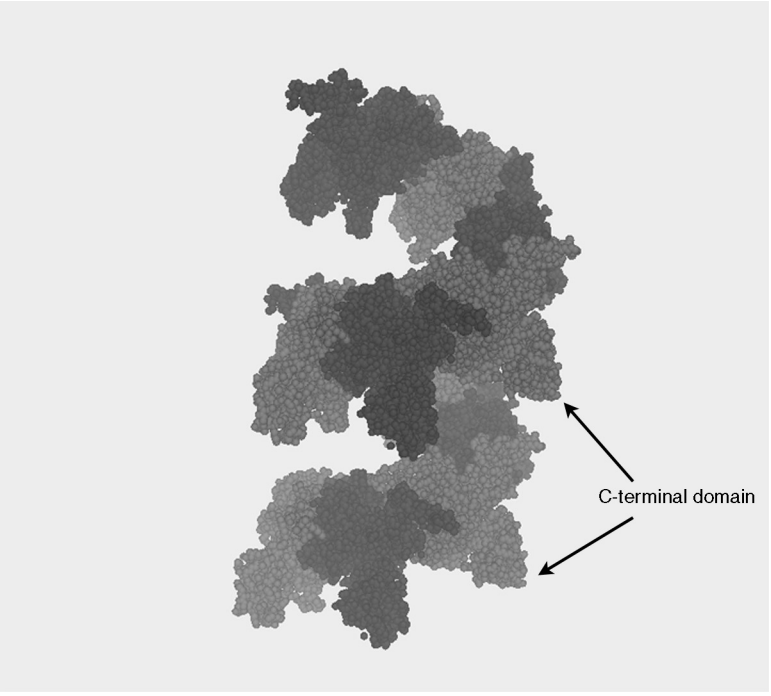

FIGURE 2. Two turns of helical RecA protein lament. Twelve subunits in this image are colored in alternating teal and beige. This lament is oriented such that the 5′ end of bound DNA is at the top and the 3′ end at the bottom (Story *et al.*, 1992). The C-terminal domain is de ned as residues 270—352, and the part visible in the structure (residues 270—328) is indicated. This image was created using main chain and side chain atoms in 2REB (Story *et al.*, 1992). (Figure appears in color online at www.crbmb.com)

some insight into the allosteric regulation of RecA activity. Finally, we describe work that now provides a better understanding of the physical interactions between RecA and other proteins involved in homologous recombination and damage-inducible DNA repair, and how this contributes to RecA function and the overall process of DNA repair.

DNA BINDING DOMAINS

The DNA binding domains remain undefined in specific structural terms despite the number of RecA structures currently available (Story *et al.*, 1992; Datta *et al.*, 2000, 2003a, 2003b). Residues which make up the primary and secondary DNA binding sites, as well as a region in the C-terminal part of the protein that may assist in binding the secondary DNA substrate, have been proposed based on these structures as well as mutational and biochemical studies. Also, recent structures of *M. tuberculosis* RecA reveal for the first time the general location of regions of the protein likely to make up the DNA binding domains (Datta *et al.*, 2003a). In discussing these domains we will use the terms "primary" and "single-stranded" DNA binding sites interchangeably; similarly, we use the terms "secondary" and "double-stranded" DNA binding sites interchangeably as well. This derives from a traditional mechanistic description of RecA strand exchange activity in which a RecA/ssDNA/ATP nucleoprotein filament serves to initiate strand invasion of the secondary DNA substrate, a homologous DNA duplex.

Early efforts to identify DNA binding domains using sequences of protein mutants defective for particular activities gave way to a somewhat clearer identification of these domains when the first crystal structure of *E. coli* RecA was published by Story *et al*. (1992). Despite the fact that the regions most likely to interact with DNA were unresolved in this structure, the authors proposed that two of the disordered regions, loop 2 (L2; residues 195–209) and loop 1 (L1; residues 157–164), are likely to be the primary and secondary DNA binding sites, respectively. This proposal was supported by the fact that these regions lie close to the polymer axis, and previous electron microscopic studies had shown that DNA lies on the inside surface of the RecA filament (Egelman and Yu, 1989). Also, a Gly204Ser substitution in the RecA430 protein, now known to be in the L2 region, had been shown to have a lower affinity for ssDNA than wild-type RecA (Menetski and Kowalczykowski, 1990). Very recently, the Vijayan lab has published a series of structures of *M. tuberculosis* RecA in complex with various nucleotides, and these reveal the positions of the L1 and L2 regions for the first time (Datta *et al.*, 2003a). In the MtRecA-ATPγS structure the main chain within L2 is now visible, as are the positions of the following side chains: Gln194, Ile195, Pro206, Thr208, Thr209, and the Cβ of Glu207 (numbering in terms of *E. coli* RecA). Only main chain atoms are visible for other positions in L2 (Figure 3). Despite the relatively high B-factors and absence of DNA in this structure, it is clear that the loop is positioned within the inner core of the filament in a position to make contact with DNA (Figure 4). In the MtRecA-(Mg^{2+}-ATPγS) structure the main chain of L1 is visible, as are the following side chains: Glu156, Gly157, Glu158, Gly160, His163, Val164 (Met in *E. coli* RecA), Gly165, and the Cβ of residues Met159 (Ile in *E. coli* RecA), Asp161, and Ser162 (numbering in terms of *E. coli* RecA; see Figure 3). The authors note that His163 is oriented toward the central groove such that it can make contact with DNA phosphates. However, this proposed interaction is unlikely to be important because both Trp and Leu substitutions are tolerated (see Table 1; Stole and Bryant, 1994; Nastri and Knight, 1994). As for the L2 region in the MtRecA-ATPγS structure, the B-factors are high but, as expected from the *E. coli* RecA structure, L1 assumes a general position that would allow interaction with DNA along the top edge of the filament surface (Figure 4). Given that Figures 3 and 4 were made as composites of two different but very similar structures that show either L1 or L2—1MO5 (MtRecA/Mg^{2+}-ATPγS) and 1MO4 (MtRecA-ATPγS), respectively—it is possible that the positions of L1 and L2 may be somewhat different in a structure in which they are both visible. However, their general position is in agreement with that discerned from the *E. coli* RecA structure. Comparison of the various MtRecA structures shows that NTP binding induces subtle conformational changes in L2 and surrounding residues, whereas no conformational differences are observed in L1. This is consistent with idea that binding of NTP induces a high affinity DNA binding conformation of the primary DNA binding site (Silver and Fersht, 1982; Menetski and Kowalczykowski, 1985).

An extensive mutagenesis study of a DNA binding region has been conducted by Hortnagel *et al*. (1999) in which they created all possible single substitution mutants at positions within L2 (residues 195–209) and flanking residues 193, 194, 210, 211, and 212. Using genetic screens for phage

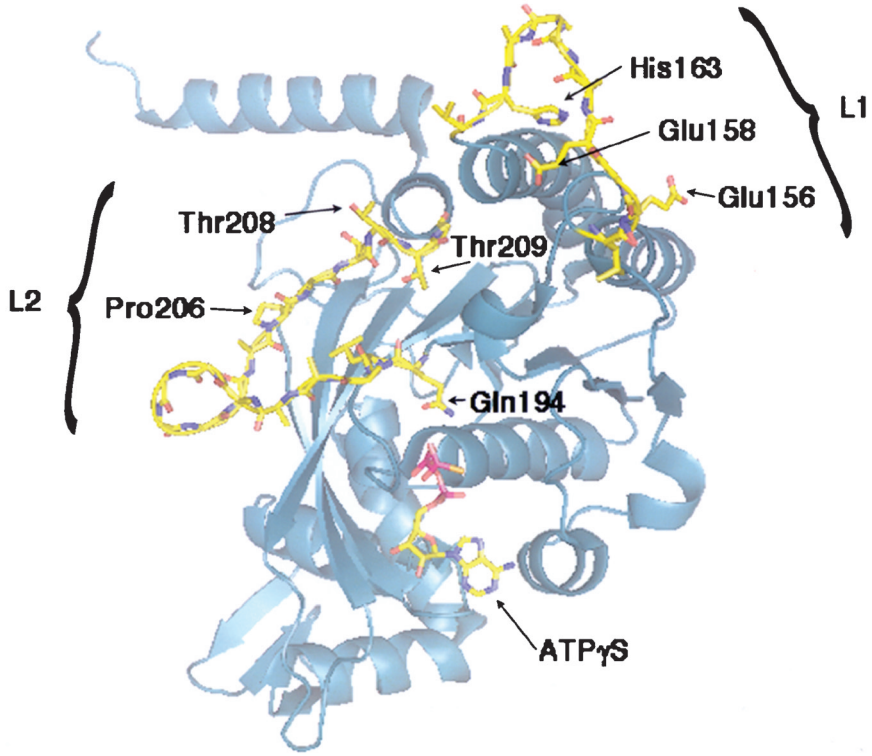

FIGURE 3. Structure of *M. tuberculosis* RecA protein showing the L1 and L2 regions. Main chain atoms of RecA are shown in teal, in complex with ATPγS. The α-carbons and side chains of L1 (residues 156—164) and L2 (194—210) are colored by atom (C, yellow; O, red; N, blue). This image was made using a composite of 1MO4 and 1MO5 from Datta *et al.* (2003a). (Figure appears in color online at www.crbmb.com)

λ recombination and recombinational DNA repair, they identified the following residues as nonmutable and, therefore, critical to these functions: Asn193, Gln194, Arg196, Thr209, Gly211, and Gly212. This coincides with a very high degree of sequence identity for these residues across 64 bacterial RecA sequences, with each being identical in at least 63 of the 64 sequences (Karlin and Brocchieri, 1996; Roca and Cox, 1997).

Using a series of peptides derived from various regions of the RecA protein sequence, Camerini-Otero's group defined a 20-residue peptide containing the L2 region (positions 193–212) as the minimal unit capable of binding ssDNA from this region of the protein (Gardner *et al.*, 1995). They went on to demonstrate that this peptide bound ssDNA cooperatively and promoted joint molecule formation between single-stranded oligonucleotides and a homologous site on dsDNA (Voloshin *et al.*, 1996). Additionally, the peptide assumes a β-structure on binding DNA or ATP (Wang *et al.*, 1998; Voloshin *et al.*, 2000). The authors proposed that the biochemical functions and ATP/DNA-induced structural transitions observed for the L2 peptide simulates what occurs in the intact protein, and that through interpeptide interactions the β-hairpin structure formed by neighboring L2 regions may result in an extended β-sheet throughout the length of the RecA filament core (Wang *et al.*, 1998; Voloshin *et al.*, 2000). The arrangement of the polypeptide chain within L2 in the *M. tuberculosis* RecA structures supports this idea (Datta *et al.*, 2003a). In other work, Sugimoto (2000) has shown a related 24-residue peptide to bind ssDNA and not dsDNA, thus supporting the model in which the L2 region is the primary DNA binding site.

To this point few studies have been published analyzing the effects on DNA binding by mutations in the L1 region. Most accounts support the idea that L1 is part of the secondary DNA binding site (see section called "Coprotease Activity" below), but several studies also suggest that it plays some role in binding of the primary DNA substrate. For example, crosslinking of photoreactive oligonucleotides shows contacts with residues in both the L1 and L2 regions (Malkov and Camerini-Otero, 1995; Wang and Adzuma, 1996) and studies of two mutant proteins with either a Glu207Gln or Glu207Lys

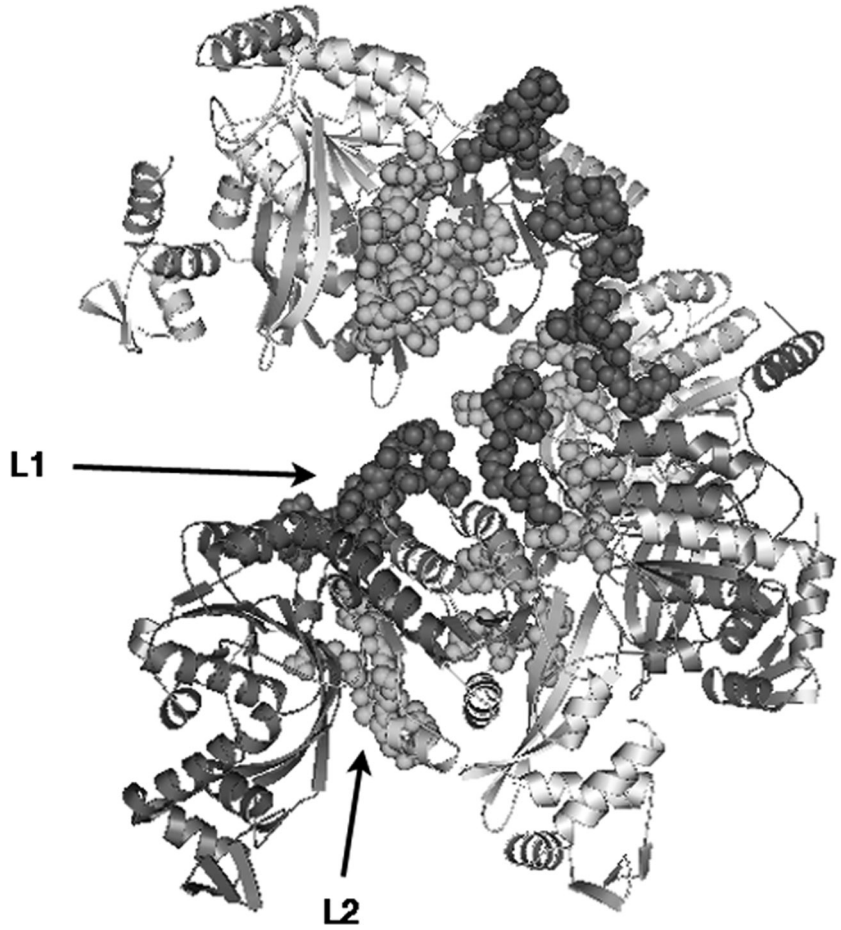

FIGURE 4. *M. tuberculosis* RecA protein lament showing L1 and L2 regions. This image shows one turn of a RecA lament with the six subunits colored in alternating teal and beige. L1 residues are shown in red and L2 in green. This image was created using a composite of 1MO4 and 1MO5 from Datta *et al.* (2003a). (Figure appears in color online at www.crbmb.com)

mutation suggest that the major effect is on binding of the secondary DNA (Cazaux *et al.*, 1998). Recent biochemical studies of a double mutant with changes in L1 residues (Glu156Leu/Gly157Val) suggest that L1 may participate in binding to both the primary and secondary substrates (Mirshad and Kowalczykowski, 2003a). Therefore, it appears that the L1 region has either a direct effect on binding of the primary DNA or that there may be more crosstalk between the two DNA binding domains that previously appreciated.

Shibata and colleagues have proposed that the C-terminal domain of RecA (residues 270–352) forms part of a "gateway" through which dsDNA gains access to the core of the presynaptic filament (Kurumizaka *et al.*, 1996). They showed that mutations at several basic residues in this region prevented homologous pairing by specifically blocking binding of dsDNA. Using a series of truncation mutants, Cox and colleagues have recently provided strong evidence that the extreme C-terminus of RecA (residues 329–352), which is unresolved in all current RecA structures, plays an important role in regulating binding of dsDNA to the secondary DNA binding site (Eggler *et al.*, 2003; Lusetti *et al.*, 2003a). Their data suggest that in the absence of Mg^{2+} a network of salt bridges forms between negatively charged side chains at the C-terminus (Asp at positions 336, 340, 341, and 351, and Glu at 343, 347, and 350) and basic residues elsewhere in the structure, perhaps those studied by the Shibata group (Kurumizaka *et al.*, 1996). This results in what they refer to as a "closed" conformation of the C-terminus. In the presence of 6–10 mM Mg^{2+} these salt bridges are disrupted, resulting in an "open" conformation that promotes access of dsDNA to the presynaptic filament and hence strand exchange activity (Lusetti *et al.*, 2003a).

TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*AE4K8	4	E → A	rec^{+}, prt^{i},	63	*rec*A6608	66	G → Q	rec^{-}	16
	8	K → A	cI$^{\pm}$, D^{i}		*rec*A6609	66	G → H	rec^{-}	16
*rec*AK6A	6	K → A	rec^{+}	38	*rec*A6610	66	G → D	rec^{-}	16
*rec*AK6D	6	K → D	rec^{+}	38	*rec*A6611	66	G → E	rec^{-}	16
*rec*AK6D/D139R	6	K → D	rec$^{\pm}$	38	*rec*A6612	66	G → R	rec^{-}	16
	139	D → R			*rec*A6613	66	G → A	rec^{-}	16
*rec*A453	7	operator	ND	7		68	E → D		
		base			*rec*A6614	66	G → A	rec^{-}	16
		change				74	T → A		
*rec*A99	7	Q → amb	rec^{-}	7, 11	*rec*A6615	66	G → A	rec^{-}	16
*rec*AQ16/K19	16	Q → A	rec^{+}, prt$^{\pm}$,	63		68	E → G		
	19	K → A	cI^{i}, D^{i}			70	S → F		
*rec*A727	18	E → K	prt^{i}	24	*rec*A6616	66	G → P	rec^{-}	16
	204	G → S				70	S → A		
*rec*A1222	25	S → F	rec^{+}, prt^{c}	10, 45	*rec*A6617	66	G → S	rec^{-}	16
*rec*AR28A	28	R → A	rec^{+}	38		67	P → L		
*rec*AR28D	28	R → D	rec^{+}	38	*rec*A6618	66	G → T	rec^{-}	16
*rec*AR18D/D112R	28	R → D	rec$^{\pm}$	38		70	S → A		
	112	D → R			*rec*A6619	66	G → N	rec^{-}	16
*rec*AR28N	28	R → N	rec$^{\pm}$	38		76	T → A		
*rec*AR28N/N113R	28	R → N	rec$^{\pm}$	38	*rec*A6620	66	G → Q	rec^{-}	16
	113	N → R				72	K → T		
*rec*A629	32	D → G	rec^{+}, cold	9, 10	*rec*A6621	66	G → R	rec^{-}	16
	38	E → K	sens			70	S → F		
	298	I → V			*rec*A6622	66	G → R	rec^{-}	16
*rec*AR33D36	33	R → A	rec^{+}, prt^{i},	63		72	K → T		
	36	D → A	cI^{i}, D^{i}			76	T → A		
*rec*A803	37	V → M	rec^{+}, srf	56	*rec*A114	67	P → L	rec^{+}	17
*rec*A730	38	E → K	rec$^{\pm}$, prt^{c},	1, 11, 30		69	S → A		
			srf		*rec*ApolyA1	67	P → A	rec^{+}	76
*rec*A85	38	E → K	rec^{+}, prt^{c}	15		69	S → A		
*rec*A1211	38	E → K	rec^{+}, prt^{c}	10		70	S → A		
*rec*A441 (*tif*)	38	E → K	rec^{+}, prt^{ts+},	2, 9, 44, 8		74	T → S		
	298	I → V	srf		*rec*ApolyA2	67	P → A	rec$^{\pm}$	76
*rec*A718	38	E → K	rec^{+}, prt^{i}	55		69	S → A		
	126	L → V				70	S → A		
*rec*A1235	39	T → I	rec^{+}, prt^{c}	10		74	T → A		
*rec*A720	39	T → I	rec^{+}, prt^{i},	24	*rec*ApolyA3	67	P → A	rec$^{\pm}$	76
			srf			68	E → A		
	204	G → S				69	S → A		
*rec*AS44L	44	S → L	rec$^{\pm}$,	67		70	S → A		
			UmuR			74	T → A		
*rec*A13	51	L → F	rec^{-}	11	*rec*ApolyA4	67	P → A	rec$^{\pm}$	76
*rec*A56	60	R → C	rec^{-}	2, 11, 57		68	E → A		
*rec*A207	66	G → A	rec^{-}	17		69	S → A		
	67	P → S				70	S → A		
*rec*A208	66	G → R	rec^{-}	17		74	T → F		
	72	K → I			*rec*ApolyA5	67	P → A	rec$^{\pm}$	76
*rec*A229	66	G → V	rec^{-}	17		68	E → A		
	72	K → R				69	S → A		
	73	T → S				70	S → A		
*rec*A6601	66	G → T	rec^{-}	16		74	T → S		
*rec*A6602	66	G → F	rec^{-}	16	*rec*AHF1	67	P → H	rec^{-}	76
*rec*A6603	66	G → A	rec^{-}	16		69	S → M		
*rec*A6604	66	G → L	rec^{-}	16		70	S → C		
*rec*A6605	66	G → C	rec^{-}	16		74	T → F		
*rec*A6606	66	G → S	rec^{-}	16	*rec*A115	67	P → A	rec^{+}	17
*rec*A6607	66	G → N	rec^{-}	16		70	S → A		

(Continued on next page)

TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*A116	67	P → S	rec^+	17	*rec*A6714	67	P → K	$rec^{\pm}$, prt^-, D^i	16, 78, 79
	70	S → A			*rec*A6715	67	P → E	$rec^{\pm}$, prt^c, D^-	16, 78, 79
*rec*A117	67	P → S	rec^+	17	*rec*A6716	67	P → R	$rec^{\pm}$, prt^-, D^i	16, 78, 79
	75	L → A							
*rec*A126	67	P → A	rec^+	17	*rec*A6717	67	P → V	rec^-	16
	70	S → A			*rec*A6718	67	P → I	rec^-	16
	74	T → S			*rec*A6719	67	P → A	$rec^{\pm}$	16
*rec*A209	67	P → K	rec^-	17		68	E → P		
	71	G → D			*rec*AP67G/E68A	67	P → G		64
*rec*A210	67	P → K	rec^-	17		68	E → A		
	73	T → S			*rec*A6721	67	P → S	rec^-	16
*rec*A211	67	P → K	rec^-	17		68	E → L		
	73	T → P			*rec*A216	68	E → D	rec^-	17
*rec*A212	67	P → K	rec^-	17		70	S → Y		
	73	T → L			*rec*A6720	67	P → S	rec^-	16
*rec*A213	67	P → R	rec^-	17		71	G → D		
	74	T → M			*rec*A217	68	E → Q	rec^-	17
*rec*A214	67	P → R	rec^-	17		75	L → M		
	74	T → R			*rec*A234	68	E → G	rec^-	17
*rec*A215	67	P → R	rec^-	17		72	K → E		
	75	L → R				73	T → A		
*rec*A230	67	P → Q	rec^-	17	*rec*A301	68	E → A	rec^-	17
	68	E → K			*rec*A302	68	E → D	rec^-	17
	73	T → P			*rec*A312	68	E → A	$rec^{\pm}$	17
*rec*A231	67	P → T	rec^-	17		75	L → M		
	69	S → A			*rec*A313	68	E → D	$rec^{\pm}$	17
	73	T → S				75	L → V		
*rec*A232	67	P → T	rec^-	17	*rec*A6801	68	E → N	$rec^{\pm}$, prt^+	16, 78
	69	S → Y			*rec*A6802	68	E → P	$rec^{\pm}$, prt^+	16, 78
	74	T → M			*rec*A6803	68	E → A	$rec^{\pm}$, prt^+	16, 78
*rec*A233	67	P → Q	rec^-	17	*rec*A6804	68	E → S	$rec^{\pm}$, prt^+	16, 78
	70	S → Y			*rec*A6805	68	E → H	$rec^{\pm}$, prt^+	16, 78
	72	K → T			*rec*A6806	68	E → T	$rec^{\pm}$, prt^+	16, 78
*rec*A308	67	P → A	$rec^{\pm}$	17	*rec*A6807	68	E → M	rec^-	16
	68	E → A			*rec*A6808	68	E → C	rec^-	16
*rec*A309	67	P → Q	$rec^{\pm}$	17	*rec*A6809	68	E → L	rec^-	16
	70	S → C			*rec*A6810	68	E → F	rec^-	16
*rec*A311	67	P → S	$rec^{\pm}$	17	*rec*A6811	68	E → G	rec^-	16
	74	T → V			*rec*A6812	68	E → R	rec^-	16
*rec*A315	67	P → T	$rec^{\pm}$	17	*rec*A6813	68	E → Q	rec^-	16
	74	T → G			*rec*A6814	68	E → V	rec^-	16
	75	L → M			*rec*A6815	68	E → I	rec^-	16
*rec*A316	67	P → R	$rec^{\pm}$	17	*rec*A6816	68	E → W	rec^-	16
	74	T → S			*rec*A6817	68	E → Y	rec^-	16
	75	L → M			*rec*A6818	68	E → K	rec^-	16
*rec*A6701	67	P → H	rec^+, prt^+	16, 78	*rec*A6819	68	E → H	$rec^{\pm}$	16
*rec*A6702	67	P → N	rec^+, prt^+	16, 78		76	T → A		
*rec*A6703	67	P → T	rec^+, prt^+	16, 78	*rec*A6820	68	E → S	$rec^{\pm}$	16
*rec*A6704	67	P → Q	rec^+, prt^+	16, 17, 78		69	S → A		
*rec*A6705	67	P → A	rec^+, prt^+	16, 17, 78		70	S → A		
*rec*A6706	67	P → S	rec^+, prt^+	16, 17, 78	*rec*A6821	68	E → L	rec^-	16
*rec*A6707	67	P → L	rec^+, prt^+	16, 17, 78		75	L → M		
*rec*A6708	67	P → G	$rec^{\pm}$, prt^c, D^i	16, 78	*rec*A6822	68	E → C	rec^-	16
*rec*A6709	67	P → C	$rec^{\pm}$, prt^+	16,78		76	T → R		
*rec*A6710	67	P → W	$rec^{\pm}$, prt^c, D^c	16, 78, 79	*rec*A6823	68	E → S	rec^-	16
*rec*A6711	67	P → F	$rec^{\pm}$, prt^+	16, 78		76	T → R		
*rec*A6712	67	P → Y	$rec^{\pm}$, prt^+	16, 78	*rec*A6824	68	E → T	rec^-	16
*rec*A6713	67	P → D	$rec^{\pm}$, prt^c, D^-	16, 78, 79		74	T → K		

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TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*A6825	68	E → D	rec$^-$	16	*rec*A6923	69	S → A	rec$^-$	16
	69	S → F				76	T → R		
*rec*A6826	68	E → K	rec$^-$	16	*rec*A6924	69	S → P	rec$^-$	16
	70	S → A				76	T → R		
*rec*A6827	68	E → K	rec$^-$	16	*rec*A6925	69	S → T	rec$^-$	16
	75	L → P				70	S → C		
*rec*A6828	68	E → R	rec$^-$	16	*rec*A6926	69	S → E	rec$^-$	16
	72	K → T				70	S → F		
*rec*A118	69	S → C	rec$^+$	17	*rec*A6927	69	S → K	rec$^-$	16
	70	S → A				70	S → A		
*rec*A119	69	S → A	rec$^+$	17	*rec*A7001	70	S → A	rec$^+$, prt$^+$	16, 17, 78
	74	T → S			*rec*A7002	70	S → C	rec$^+$, prt$^+$	16, 17, 78
*rec*A120	69	S → A	rec$^+$	17	*rec*A7003	70	S → G	rec$^+$, prt$^+$	16, 78
	75	L → V			*rec*A7004	70	S → T	rec$^+$, prt$^+$	16, 17, 78
*rec*A218	69	S → A	rec$^-$	17	*rec*A7005	70	S → N	rec$^-$	16
	72	K → R			*rec*A7006	70	S → V	rec$^-$	16
*rec*A219	69	S → F	rec$^-$	17	*rec*A7007	70	S → Q	rec$^-$	16
	73	T → S			*rec*A7008	70	S → I	rec$^-$	16
*rec*A220	69	S → F	rec$^-$	17	*rec*A7009	70	S → L	rec$^-$	16
	75	L → V			*rec*A7010	70	S → F	rec$^-$	16
*rec*A221	69	S → T	rec$^-$	17	*rec*A7011	70	S → P	rec$^-$	16
	75	L → P			*rec*A7012	70	S → M	rec$^-$	16
*rec*A235	69	S → T	rec$^-$	17	*rec*A7013	70	S → Y	rec$^-$	16
	70	S → F			*rec*A7014	70	S → D	rec$^-$	16
	73	T → P			*rec*A7015	70	S → E	rec$^-$	16
*rec*A236	69	S → A	rec$^-$	17	*rec*A7016	70	S → R	rec$^-$	16
	72	K → T			*rec*A121	70	S → C	rec$^+$	17
	73	T → I				74	T → S		
*rec*A237	69	S → A	rec$^-$	17	*rec*A122	70	S → A	rec$^+$	17
	72	K → D				75	L → V		
	73	T → P			*rec*A123	70	S → T	rec$^+$	17
*rec*A6901	69	S → A	rec$^+$, prt$^+$	16, 17, 78		75	L → V		
*rec*A6902	69	S → G	rec$^+$, prt$^+$	16, 65, 78	*rec*A124	70	S → T	rec$^+$	17
*rec*A6903	69	S → M	rec$^+$, prt$^+$	16, 78		75	L → Q		
*rec*A6904	69	S → C	rec$^\pm$, prt$^+$	16, 78	*rec*A222	70	S → C	rec$^-$	17
*rec*A6905	69	S → D	rec$^-$	16		72	K → G		
*rec*A6906	69	S → F	rec$^-$	16	*rec*A223	70	S → C	rec$^-$	17
*rec*A6907	69	S → E	rec$^-$	16		73	T → I		
*rec*A6908	69	S → L	rec$^-$	16	*rec*A224	70	S → F	rec$^-$	17
*rec*A6909	69	S → P	rec$^-$	16		73	T → S		
*rec*A6910	69	S → K	rec$^-$	16	*rec*A225	70	S → A	rec$^-$	17
*rec*A6911	69	S → V	rec$^-$	16		73	T → S		
*rec*A6912	69	S → I	rec$^-$	16	*rec*A238	70	S → C	rec$^-$	17
*rec*A6913	69	S → W	rec$^-$	16		73	T → I		
*rec*A6914	69	S → T	rec$^-$	16		75	L → M		
*rec*A6915	69	S → N	rec$^-$	16	*rec*A239	70	S → C	rec$^-$	17
*rec*A6916	69	S → Q	rec$^-$	16		72	K → A		
*rec*A6917	69	S → Y	rec$^-$	16		73	T → F		
*rec*A6918	69	S → H	rec$^-$	16	*rec*A314	70	S → C	rec$^\pm$	17
*rec*A6919	69	S → A	rec$^+$	16		75	L → E		
	70	S → A			*rec*A202	71	G → V	rec$^-$	17
*rec*A6920	69	S → A	rec$^+$	16	*rec*A203	71	G → D	rec$^-$	17
	74	T → M			*rec*A227	72	K → N	rec$^-$	17
*rec*A6921	69	S → A	rec$^+$	16		73	T → P		
	74	T → G			*rec*AK72R	72	K → R	rec$^-$	16, 39, 59, 60
*rec*A6922	69	S → A	rec$^-$	16					
	72	K → N			*rec*AK72T	72	K → T	rec$^-$	16

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TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.
*rec*A204	72	K → N	rec$^-$	17
*rec*A7202	72	K → T	rec$^-$	16
	74	T → K		
*rec*A7203	72	K → T	rec$^-$	16
	74	T → R		
*rec*A7204	72	K → N	rec$^-$	16
	74	T → S		
*rec*A205	73	T → S	rec$^-$	17
*rec*AT73A	73	T → A	rec$^-$	16
*rec*A304	74	T → M	rec$^\pm$	17
*rec*A7401	74	T → S	rec$^+$, prt$^+$	16, 17, 78
*rec*A7402	74	T → F	rec$^+$, prt^c, D^i	16, 78, 79
*rec*A7403	74	T → C	rec$^\pm$, prt$^+$	16, 78
*rec*A7404	74	T → L	rec$^\pm$, prt$^+$	16, 17, 78
*rec*A7405	74	T → G	rec$^\pm$, prt^c, D$^-$	16, 17, 78, 79
*rec*A7406	74	T → A	rec$^\pm$, prt$^+$	16, 17, 78
*rec*A7407	74	T → Y	rec$^\pm$, prt$^+$	16, 78
*rec*A7408	74	T → D	rec$^-$	16
*rec*A7409	74	T → R	rec$^-$	16, 17
*rec*A7410	74	T → K	rec$^-$	16
*rec*A7411	74	T → V	rec$^-$	16
*rec*A7412	74	T → I	rec$^-$	16
*rec*A7413	74	T → P	rec$^-$	16
*rec*A7414	74	T → N	rec$^-$	16
*rec*A7415	74	T → H	rec$^-$	16
*rec*A7417	74	T → A	rec$^-$	16
	75	L → P		
*rec*A7418	74	T → W	rec$^-$	16
	75	L → P		
*rec*A125	74	T → S	rec$^+$	17
	75	L → Q		
*rec*A228	74	T → M	rec$^-$	17
	75	L → I		
*rec*A7416	74	T → S	rec$^+$	16
	76	T → A		
*rec*A110	75	L → Q	rec$^+$	17
*rec*A111	75	L → S	rec$^+$	17
*rec*A112	75	L → F	rec$^+$	17
*rec*A113	75	L → M	rec$^+$	17
*rec*AR85E86	85	R → A	rec$^+$, prt^i, cI^i, D^i	63
	86	E → A		
*rec*AE86GH97L	86	E → G	rec$^-$	77
	97	H → L		
*rec*AT89A	89	T → A	rec$^\pm$	38
*rec*AT89V	89	T → V	rec$^\pm$	38
*rec*AC90A	90	C → A	rec$^+$	23
*rec*AC90T	90	C → T	rec$^+$	23
*rec*AC90S	90	C → S	rec$^+$, prt^i	23
*rec*AC90Q	90	C → Q	rec$^\pm$, prt^i	23
*rec*AC90F	90	C → F	rec$^\pm$	23
*rec*AC90L	90	C → L	rec$^-$, prt$^-$	23
*rec*AC90V	90	C → V	rec$^-$	23
*rec*AC90I	90	C → I	rec$^-$	23
*rec*AC90E	90	C → E	rec$^-$	23
*rec*AC90Y	90	C → Y	rec$^-$, prt$^-$	23
*rec*AC90K	90	C → K	rec$^-$, prt$^-$	23
*rec*AI93C	93	I → C	rec$^+$, prt^i	37
*rec*A93C/F217C	93	I → C	rec$^\pm$, prt^c	37
	217	F → C		
*rec*AA95C	95	A → C	rec$^\pm$, prt$^-$	37
*rec*AH95C/F217C	95	A → C	rec$^\pm$, prt$^-$	37
	217	F → C		
*rec*AE96D	96	E → D	rec$^\pm$, prt^c	45
*rec*AH97A	97	H → A	rec$^-$	34
*rec*AH97R	97	H → R	rec$^\pm$, prt^c	45
*rec*AH97W	97	H → W	rec$^-$	77
*rec*AH97G	97	H → G	rec$^\pm$	77
*rec*AH97P	97	H → P	rec$^-$	77
*rec*AH97E	97	H → E	rec$^-$	77
*rec*AH97S	97	H → S	rec$^\pm$	77
*rec*AH97I	97	H → I	rec$^-$	77
*rec*AH97M	97	H → M	rec$^\pm$	77
*rec*AH97F	97	H → F	rec$^\pm$	77
*rec*AH97Q	97	H → Q	rec$^\pm$	77
*rec*AH97N	97	H → N	rec$^\pm$	77
*rec*AH97D	97	H → S	rec$^-$	77
*rec*AH97L	97	H → L	rec$^-$	77
*rec*AD100N	100	D → N	ND	27
*rec*AI102Y103	102	I → A	rec$^-$, prt$^\pm$, cI$^\pm$, D$^\pm$	63
	103	Y → A		
*rec*AY103W	103	Y → W	rec$^+$, prt^i	31
*rec*AY103A	103	Y → A	rec$^-$	31
*rec*AR105/K106	105	R → A	rec$^+$, prt$^\pm$, cI$^\pm$, D^i	63
	106	K → A		
*rec*A1622	111	I → M	rec$^+$, prt^i	3
	184	Q → K		
*rec*AD112R	112	D → R	rec$^\pm$	38
*rec*AD112A	112	D → A	rec$^+$	38
*rec*AD112G	112	D → G	rec$^\pm$, UmuR	67
*rec*AN113A	113	N → A	rec$^\pm$	38
*rec*AN113K	113	N → K	rec$^\pm$, UmuR	67
*rec*AL114V	114	L → V	rec$^\pm$, UmuR	67
*rec*AC116L	116	C → L	rec$^+$, prt^i	23
*rec*AC116M	116	C → M	rec$^+$	23
*rec*AC116I	116	C → I	rec$^+$	23
*rec*AC116Q	116	C → Q	rec$^\pm$, prt$^-$	23
*rec*AC116S	116	C → S	rec$^+$, prt^i	23
*rec*AC116T	116	C → T	rec$^\pm$	23
*rec*AC116Y	116	C → Y	rec$^-$, prt$^-$	23
*rec*AC116F	116	C → F	rec$^-$	23
*rec*AC116P	116	C → P	rec$^-$	23
*rec*AC116H	116	C → H	rec$^-$	23
*rec*AC116K	116	C → K	rec$^-$, prt$^-$	23
*rec*AC116R	116	C → R	rec$^-$	23
*rec*A1730	117	S → F	rec$^-$, prt$^\pm$	7,36
*rec*AS117P	117	S → P	rec$^\pm$, prt^c	45
*rec*A432	119	P → S	prt^c, D^c	15
*rec*AD120C	120	D → C	unstable	37
*rec*A2020	121	T → I	rec$^+$, srf	58

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TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*AT121C	121	T → C	rec$^+$, prt^c	37	*rec*AN4	152	K → S	rec$^+$, prt^i	19
*rec*AT121C/F217C	121	T → C	rec$^\pm$, prt^c	37	*rec*AN5	152	K → H	rec$^+$, prt^i	19
	217	F → C			*rec*AN6	152	K → T	rec$^+$, prt^i	19
*rec*A142-6	124	Q → L	rec$^+$	29	*rec*AN7	152	K → N	rec$^+$, prt^i	19
	225	I → V			*rec*AN41	152	K → R	rec$^+$, prt^i	19
*rec*AA125C	125	A → C	rec$^\pm$, prt^c	37		153	A → G		
*rec*AA125C/F217C	125	A → C	rec$^\pm$, prt$^-$	37	*rec*AN126	152	K → N	rec$^-$, prt$^-$	19
	217	F → C				153	A → V		
*rec*AI128T	128	I → T	rec$^+$, prt^c	45	*rec*AN127	152	K → N	rec$^-$, prt$^-$	19
*rec*A1203	128	I → I	rec$^-$, prt^c	10		154	E → G		
	169	R → C			*rec*AN128	152	K → T	rec$^-$, prt$^-$	19
*rec*A1601	128	I → I	rec$^-$, prt^c	10		155	I → T		
	301	G → S			*rec*AN42	152	K → R	rec$^+$, prt^i	19
*rec*AC129L	129	C → L	rec$^+$, prt^i	23		155	I → L		
*rec*AC129I	129	C → I	rec$^+$	23	*rec*AN43	152	K → E	rec$^+$, prt^c	19
*rec*AC129V	129	C → V	rec$^+$	23		155	I → K		
*rec*AC129Q	129	C → Q	rec, prt$^\pm$	23	*rec*AN44	152	K → N	rec$^+$, prt^i	19
*rec*AC129S	129	C → S	rec$^+$, prt^i	23		155	I → L		
*rec*AC129Y	129	C → Y	rec$^-$, prt$^-$	23	*rec*AN112	152	K → R	rec$^\pm$, prt^c	19
*rec*AC129E	129	C → E	rec$^-$	23		153	A → D		
*rec*AC129K	129	C → K	rec$^-$, prt$^-$	23		163	H → Y		
*rec*AC129M	129	C → M	rec$^-$	21	*rec*AN134	152	K → A	rec$^-$, prt$^-$	19
*rec*A1636	132	L → Q	rec$^\pm$, prt^i	3		153	A → P		
	184	Q → K				154	E → A		
*rec*AD139A	139	D → A	rec$^+$	38	*rec*AN135	152	K → Y	rec$^-$, prt$^-$	19
*rec*AD139K	139	D → K	rec$^\pm$	38		153	A → S		
*rec*A1626	139	D → G	rec$^+$, prt^c	3		155	I → M		
	184	Q → K			*rec*AN136	152	K → T	rec$^-$, prt$^-$	19
*rec*AS145C	145	S → C	unstable	37		153	A → D		
*rec*A1647	145	S → T	rec$^+$, prt^c	3		166	L → A		
	184	Q → K			*rec*AN137	152	K → Q	rec$^-$, prt$^-$	19
*rec*AS145C/F217C	145	S → C	no protein	37		154	E → V		
	217	F → C				155	I → S		
*rec*AN125	147	A → V	rec$^-$, prt$^-$	19	*rec*AN138	152	K → I	rec$^-$, prt$^-$	19
	153	A → V				154	E → D		
*rec*AA148C	148	A → C	unstable	37		155	I → L		
*rec*AA148C/F217C	148	A → C	no protein	37	*rec*AN139	152	K → I	rec$^-$, prt$^-$	19
	217	F → C				154	E → V		
*rec*AT150C	150	T → C	rec$^+$, prt^i	37		163	H → Y		
*rec*AN91	150	T → S	rec$^\pm$, prt^i	19	*rec*AN146	152	K → Y	rec$^-$, prt$^-$	19
	155	I → F				153	A → T		
*rec*AT150C/F217C	150	T → C	rec$^\pm$, prt^c	37		154	E → K		
	217	F → C				155	I → M		
*rec*AN40	151	P → S	rec$^+$, prt^i	19	*rec*AN149	152	K → N	rec$^-$, prt$^-$	19
	153	A → S				153	A → T		
*rec*AN108	151	P → Q	rec$^\pm$, prt^i	19		154	E → G		
	152	K → S				155	I → V		
	158	E → V			*rec*A1641	152	K → I	rec$^+$, prt^i	3
*rec*AN148	151	P → A	rec$^-$, prt$^-$	19		184	Q → K		
	152	K → E			*rec*AN8	153	A → S	rec$^+$, prt^i	19
	154	E → K			*rec*AN9	153	A → P	rec$^+$, prt^i	19
	155	I → M			*rec*AN10	153	A → T	rec$^+$, prt^i	19
	156	E → V			*rec*AN11	153	A → V	rec$^+$, prt^i	19
*rec*AN1	152	K → A	rec$^+$, prt^i	19	*rec*AN12	153	A → L	rec$^+$, prt^i	19
*rec*AN2	152	K → R	rec$^+$, prt^i	19	*rec*AN13	153	A → Q	rec$^+$, prt^i	19
*rec*AN3	152	K → Q	rec$^+$, prt^i	19	*rec*AN14	153	A → G	rec$^+$, prt^i	19

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TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*AN84	153	A → E	rec$^{\pm}$, prt^{c}	19	*rec*AN57	155	I → L	rec^{+}, prt^{i}	19
*rec*AN129	153	A → T	rec^{-}, prt^{-}	19		162	S → F		
	154	E → Q			*rec*AN96	155	I → M	rec$^{\pm}$, prt^{c}	19
*rec*AN48	153	A → S	rec^{+}, prt^{i}	19		164	M → I		
	155	I → V			*rec*AI155C/F217C	155	I → C	rec$^{\pm}$, prt^{c}	37
*rec*AN49	153	A → T	rec^{+}, prt^{i}	19		217	F → C		
	155	I → V			*rec*AN27	156	E → R	rec^{+}, prt^{c}, D^{c}	19, 20
*rec*AN93	153	A → V	rec$^{\pm}$, prt^{c}	19	*rec*AN29	156	E → K	rec^{+}, prt^{c}, D^{c}	19, 20
	155	I → M			*rec*AN23	156	E → I	rec^{+}, prt^{i}, D^{i}	19, 20
*rec*AN94	153	A → S	rec$^{\pm}$, prt^{i}	19	*rec*AN24	156	E → V	rec^{+}, prt^{-}, D^{i}	19, 20
	155	I → F			*rec*AN25	156	E → A	rec^{+}, prt^{i}, D^{c}	19, 20
*rec*AN50	153	A → T	rec^{+}, prt^{i}	19	*rec*AN26	156	E → G	rec^{+}, prt^{i}, D^{i}	19, 20
	156	E → D			*rec*AN28	156	E → D	rec^{+}, prt^{i}, D^{c}	19, 20
*rec*AN51	153	A → T	rec^{+}, prt^{i}	19	*rec*AN97	156	E → G	rec$^{\pm}$, prt^{c}	19
	156	E → V				157	G → A		
*rec*AN52	153	A → V	rec$^{\pm}$, prt^{i}, D^{c}	19, 20	*rec*AN98	156	E → V	rec$^{\pm}$, prt^{c}	19
	157	G → S				157	G → D		
*rec*AN53	153	A → T	rec^{+}, prt^{i}	19	*rec*AN99	156	E → L	rec$^{\pm}$, prt^{c}	19
	158	E → N				157	G → V		
*rec*AN54	153	A → S	rec^{+}, prt^{i}	19	*rec*AN113	156	E → V	rec$^{\pm}$, prt^{i}, D^{c}	19, 20
	161	D → N				157	G → A		
*rec*AN140	153	A → T	rec^{-}, prt^{-}	19		158	E → D		
	154	E → Y			*rec*AN58	156	E → A	rec^{+}, prt^{i}	19
	155	I → F				158	E → D		
*rec*AN141	153	A → P	rec^{-}, prt^{-}	19	*rec*AN59	156	E → R	rec^{+}, prt^{c}	19
	154	E → D				158	E → V		
	155	I → L			*rec*AN60	156	E → Q	rec^{+}, prt^{c}	19
*rec*AN142	153	A → V	rec^{-}, prt^{-}	19		158	E → A		
	154	E → A			*rec*AN61	156	E → L	rec^{+}, prt^{c}	19
	156	E → V				158	E → R		
*rec*AN85	154	E → D	rec$^{\pm}$, prt^{i}, D^{i}	19, 20	*rec*AN62	156	E → L	rec^{+}, prt^{i}	19
*rec*AN121	154	E → Q	rec^{-}, prt^{-}, D^{c}	19, 20		158	E → I		
*rec*AN122	154	E → K	rec^{-}, prt^{-}, D^{-}	19, 20	*rec*AN63	156	E → G	rec^{+}, prt^{-}	19
*rec*AN123	154	E → R	rec^{-}, prt^{-}, D^{-}	19, 20		158	E → Q		
*rec*AN95	154	E → D	rec$^{\pm}$, prt^{i}	19, 20	*rec*AN64	156	E → V	rec^{+}, prt^{-}	19
	155	I → M				158	E → V		
*rec*AN130	154	E → G	rec^{-}, prt^{-}	19	*rec*AN100	156	E → D	rec$^{\pm}$, prt^{i}	19
	155	I → F				158	E → Y		
*rec*AN131	154	E → V	rec^{-}, prt^{-}	19	*rec*AN101	156	E → D	rec$^{\pm}$, prt^{i}	19
	158	E → V				158	E → I		
*rec*AN171	154	E → D	rec^{+}, prt^{c}	19, 20	*rec*AN65	156	E → Q	rec^{+}, prt^{i}	19
	158	E → G				159	I → W		
*rec*AN55	154	E → D	rec^{+}, prt^{i}	19, 20	*rec*AN66	156	E → L	rec^{+}, prt^{c}	19
	162	S → F				159	I → Q		
*rec*AN132	154	E → Q	rec^{-}, prt^{-}	19	*rec*AN67	156	E → G	rec^{+}, prt^{i}	19
	163	H → L				159	I → M		
*rec*AN18	155	I → Y	rec^{+}, prt^{i}, D^{c}	19, 20	*rec*AN68	156	E → I	rec^{+}, prt^{i}	19
*rec*AN19	155	I → M	rec^{+}, prt^{c}, D^{c}	19, 20		159	I → M		
*rec*AN15	155	I → C	rec^{+}, prt^{i}, D^{i}	19, 20, 37	*rec*AN69	156	E → D	rec^{+}, prt^{i}	19
*rec*AN16	155	I → H	rec^{+}, prt^{i}, D^{i}	19, 20		159	I → F		
*rec*AN17	155	I → F	rec^{+}, prt^{i}, D^{i}	19, 20	*rec*AN102	156	E → D	rec$^{\pm}$, prt^{i}	19
*rec*AN20	155	I → V	rec^{+}, prt^{i}, D^{i}	19, 20		159	I → Y		
*rec*AN21	155	I → A	rec^{+}, prt^{i}, D^{i}	19, 20	*rec*AN103	156	E → V	rec$^{\pm}$, prt^{i}	19
*rec*AN22	155	I → L	rec^{+}, prt^{i}, D^{i}	19, 20		159	I → T		
*rec*AN86	155	I → N	rec$^{\pm}$, prt^{i}, D^{i}	19, 20	*rec*AN114	156	E → A	rec$^{\pm}$, prt^{c}	19
*rec*AN56	155	I → A	rec^{+}, prt^{i}	19,20		157	G → V		
	158	E → D				158	E → L		

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TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*AN77	156	E → Q	rec^{+}, prt^{c}	19	*rec*AN119	157	G → V	rec$^{\pm}$, prt^{c}	19
	157	G → S				158	E → K		
	159	I → M				159	I → M		
*rec*AN143	156	E → H	rec^{-}, prt^{-}	19	*rec*AN144	157	G → V	rec^{-}, prt^{-}	19
	157	G → V				158	E → V		
	159	I → H				159	I → L		
*rec*AN78	156	E → V	rec^{+}, prt^{i}	19	*rec*AN120	157	G → C	rec$^{\pm}$, prt^{i}	19
	158	E → L				158	E → D		
	159	I → V				163	H → L		
*rec*AN79	156	E → V	rec^{+}, prt^{i}	19	*rec*A1201	157	G → D	rec^{-}, prt^{c}	10
	158	E → L				301	G → S		
	159	I → L			*rec*AN30	158	E → K	rec^{+}, prt^{c}, D^{c}	19, 20
*rec*AN80	156	E → Q	rec^{+}, prt^{i}	19	*rec*AN31	158	E → Q	rec^{+}, prt^{c}, D^{c}	19, 20
	158	E → V			*rec*AN32	158	E → L	rec^{+}, prt^{i}, D^{i}	19, 20
	159	I → V			*rec*AN33	158	E → N	rec^{+}, prt^{c}, D^{c}	19, 20
*rec*AN81	156	E → G	rec^{+}, prt^{i}	19	*rec*AN162	158	E → R	rec^{+}, prt^{c}, D^{c}	20
	158	E → V			*rec*AN163	158	E → A	rec^{+}, prt^{c}, D^{c}	20
	159	I → V			*rec*AN164	158	E → D	rec^{+}, prt^{i}, D^{i}	20
*rec*AN115	156	E → V	rec$^{\pm}$, prt^{i}	19	*rec*AN165	158	E → V	rec^{+}, prt^{i}, D^{c}	20
	158	E → F			*rec*AN166	158	E → F	rec^{+}, prt^{i}, D^{i}	20
	159	I → V			*rec*AN167	158	E → I	rec^{+}, prt^{i}, D^{c}	20
*rec*AN116	156	E → D	rec$^{\pm}$, prt^{i}	19	*rec*AN168	158	E → Y	rec^{+}, prt^{i}, D^{i}	20
	158	E → D			*rec*AN169	158	E → W	rec$^{\pm}$, prt^{i}, D^{c}	20
	159	I → L			*rec*AN170	158	E → T	rec^{+}, prt^{i}, D^{c}	20
*rec*AN147	156	E → K	rec^{-}, prt^{-}	19	*rec*AN74	158	E → Q	rec^{+}, prt^{i}	19
	157	G → C				159	I → V		
	158	E → K			*rec*A1219	158*	E → K	rec^{+}, prt^{c}	10
	159	I → K			*rec*AN71	158	E → S	rec^{+}, prt^{c}	19
*rec*A1602	157	G → D	rec$^{\pm}$, prt^{c}	10		159	I → M		
*rec*AN87	157	G → C	rec$^{\pm}$, prt^{c}, D^{c}	19, 20	*rec*AN72	158	E → D	rec^{+}, prt^{i}	19
*rec*AN88	157	G → D	rec$^{\pm}$, prt^{c}, D^{c}	19, 20		159	I → F		
*rec*AN150	157	G → R	rec$^{\pm}$, prt^{c}, D^{c}	20	*rec*AN73	158	E → A	rec^{+}, prt^{c}	19
*rec*AN151	157	G → A	rec$^{\pm}$, prt^{c}, D^{c}	20		159	I → M		
*rec*AN152	157	G → K	rec$^{\pm}$, prt^{c}, D^{c}	20	*rec*AN107	158	E → G	rec$^{\pm}$, prt^{c}	19
*rec*AN153	157	G → S	rec$^{\pm}$, prt^{c}, D^{c}	20		160	G → S		
*rec*AN154	157	G → V	rec$^{\pm}$, prt^{c}, D^{c}	20	*rec*AN145	158	E → Q	rec^{-}, prt^{-}	19
*rec*AN155	157	G → L	rec$^{\pm}$, prt^{c}, D^{c}	20		159	I → L		
*rec*AN156	157	G → T	rec$^{\pm}$, prt^{c}, D^{c}	20		160	G → R		
*rec*AN157	157	G → Q	rec$^{\pm}$, prt^{c}, D^{c}	20	*rec*AN34	159	I → V	rec^{+}, prt^{i}	19
*rec*AN158	157	G → N	rec$^{\pm}$, prt^{i}, D^{c}	20	*rec*AN35	159	I → M	rec^{+}, prt^{i}	19
*rec*AN159	157	G → H	rec$^{\pm}$, prt^{i}, D^{c}	20	*rec*AN36	159	I → F	rec^{+}, prt^{i}	19
*rec*AN160	157	G → W	rec$^{\pm}$, prt^{-}, D^{i}	20	*rec*AN75	159	I → V	rec^{+}, prt^{i}	19
*rec*AN161	157	G → P	rec^{-}, prt^{-}, D^{-}	20		164	M → I		
*rec*AN105	157	G → A	rec^{+}, prt^{i}	20	*rec*AN133	159	I → V	rec^{-}, prt^{-}	19
	158	E → Q				165	G → S		
*rec*AN70	157	G → C	rec^{+}, prt^{c}	19	*rec*A1	160	G → D	rec^{-}	11, 12, 61
	158	E → K							
*rec*AN106	157	G → S	rec^{+}, prt^{c}	19	*rec*AG160N	160	G → N	rec^{-}	6
	158	E → V			*rec*AN124	160	G → A	rec^{-}, prt^{-}	19
*rec*AN104	157	G → A	rec$^{\pm}$, prt^{i}	19	*rec*AN37	161	D → Q	rec^{+}, prt^{i}	19
	158	E → D			*rec*AN45	161	D → V	rec$^{\pm}$, prt^{i}	19
*rec*AN117	157	G → W	rec$^{\pm}$, prt^{i}	19	*rec*AN38	162	S → F	rec^{+}, prt^{i}	19
	158	E → Q			*rec*AH163A	163	H → A	rec^{-}	5
	159	I → V			*rec*AN90	163	H → L	rec$^{\pm}$, prt^{i}	19
*rec*AN118	157	G → R	rec$^{\pm}$, prt^{i}, D^{c}	19, 20	*rec*AH163W	163	H → W	rec^{+}	35
	158	E → D			*rec*AN39	164	M → L	rec^{+}, prt^{i}	19
	159	I → V			*rec*A423	169	R → H	rec^{-}	33

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TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*AR176K177	176	R → A	rec$^-$, prt$^-$, cI$^-$, D$^-$	63	*rec*AQ194L	194	Q → L	rec$^-$, prt$^-$	28, 40
					*rec*AQ194C	194	Q → C	rec$^-$, prt$^-$	28, 40
	177	K → A			*rec*AQ194M	194	Q → M	rec$^-$	28
*rec*A1630	177	K → Q	rec$^\pm$, prt^i	3	*rec*AQ194H	194	Q → H	rec$^-$, prt$^-$	28, 40
	184	Q → K			*rec*AQ194R	194	Q → R	rec$^-$, prt$^-$	28, 40
*rec*A1206	177	K → K	rec$^-$, prt^c	4, 10	*rec*AQ194K	194	Q → K	rec$^-$, prt$^-$	28, 40
	301	G → D			*rec*AQ194D	194	Q → D	rec$^-$, prt$^-$	28, 40
*rec*A1212	179	A → V	rec$^+$, prt^c	10	*rec*AQ194E	194	Q → E	rec$^-$, prt$^-$	28, 40
*rec*A1213	179	A → V	rec$^+$, prt^c	10	*rec*AQ194S	194	Q → S	rec$^-$, prt$^-$	28, 40
*rec*A1623	182	L → E	rec$^-$, prt$^-$	3	*rec*AQ194N	194	Q → N	rec$^-$, prt$^-$	28, 40
	184	Q → K			*rec*AQ194A	194	Q → A	rec$^-$, prt$^-$	28, 40
*rec*A2183	183	K → M	rec$^-$, prt$^-$	26	*rec*AQ194P	194	Q → P	rec$^-$, prt$^-$	28, 40
*rec*A1202	184	Q → K	rec$^+$, prt^c	4, 10	*rec*AQ194G	194	Q → G	rec$^-$, prt$^-$	28, 40
*rec*A1633	184*	Q → K	rec$^-$, prt$^-$	3	*rec*AI195F	195	I → F	rec$^-$	28
*rec*A1625	184	Q → K	rec$^+$, prt^c	3	*rec*AI195V	195	I → V	rec$^{-\S}$	28
	187	T → A			*rec*AI195Y	195	I → Y	rec$^+$	28
*rec*A1624	184	Q → K	rec$^+$, prt^c	3	*rec*AI195T	195	I → T	rec$^\pm$	28
	208	T → N			*rec*AI195W	195	I → W	rec$^-$	28
*rec*A1634	184	Q → K	rec$^+$, prt^c	3	*rec*AI195L	195	I → L	rec$^+$	28
	213	N → K			*rec*AI195C	195	I → C	rec$^-$	28
*rec*A1649	184	Q → K	rec$^\pm$, prt^i	3	*rec*AI195M	195	I → M	rec$^\pm$	28
	214	A → S			*rec*AI195H	195	I → H	rec$^-$	28
*rec*A1628	184	Q → K	rec$^\pm$, prt^i	3	*rec*AI195R	195	I → R	rec$^-$	28
	231	V → E			*rec*AI195K	195	I → K	rec$^-$	28
*rec*A1642	184	Q → K	rec$^+$, prt^i	3	*rec*AI195D	195	I → D	rec$^-$	28
	238	V → E			*rec*AI195E	195	I → E	rec$^-$	28
*rec*A1627	184	Q → K	rec$^\pm$, prt^i	3	*rec*AI195Q	195	I → Q	rec$^-$	28
	244	V → E			*rec*AI195S	195	I → S	rec$^-$	28
*rec*A1620	184	Q → K	rec$^-$, prt^c	3	*rec*AI195N	195	I → N	rec$^-$	28
	275	V → D			*rec*AI195A	195	I → A	rec$^-$	28
*rec*A1631	184	Q → K	rec$^-$, prt$^-$	3	*rec*AI195P	195	I → P	rec$^-$	28
	284	I → N			*rec*AI195G	195	I → G	rec$^-$	28
*rec*AN193I	193	N → I	rec$^-$	28	*rec*AR196I	196	R → I	rec$^-$	28
*rec*AN193F	193	N → F	rec$^-$	28	*rec*AR196F	196	R → F	rec$^-$	28
*rec*AN193V	193	N → V	rec$^-$	28	*rec*AR196V	196	R → V	rec$^-$	28
*rec*AN193Y	193	N → Y	rec$^-$	28	*rec*AR196Y	196	R → Y	rec$^-$	28
*rec*AN193T	193	N → T	rec$^-$	28	*rec*AR196T	196	R → T	rec$^-$	28
*rec*AN193W	193	N → W	rec$^-$	28	*rec*AR196W	196	R → W	rec$^-$	28
*rec*AN193L	193	N → L	rec$^-$	28	*rec*AR196L	196	R → L	rec$^-$	28
*rec*AN193C	193	N → C	rec$^-$	28	*rec*AR196C	196	R → C	rec$^-$	28
*rec*AN193M	193	N → M	rec$^-$	28	*rec*AR196M	196	R → M	rec$^-$	28
*rec*AN193H	193	N → H	rec$^-$	28	*rec*AR196H	196	R → H	rec$^-$	28
*rec*AN193R	193	N → R	rec$^-$	28	*rec*AR196K	196	R → K	rec$^-$	28
*rec*AN193K	193	N → K	rec$^-$	28	*rec*AR196D	196	R → D	rec$^-$	28
*rec*AN193D	193	N → D	rec$^-$	28	*rec*AR196E	196	R → E	rec$^-$	28
*rec*AN193E	193	N → E	rec$^-$	28	*rec*AR196Q	196	R → Q	rec$^-$	28
*rec*AN193Q	193	N → Q	rec$^-$	28	*rec*AR196S	196	R → S	rec$^-$	28
*rec*AN193S	193	N → S	rec$^-$	28	*rec*AR196N	196	R → N	rec$^-$	28
*rec*AN193A	193	N → A	rec$^-$	28	*rec*AR196A	196	R → A	rec$^-$	28
*rec*AN193P	193	N → P	rec$^-$	28	*rec*AR196P	196	R → P	rec$^-$	28
*rec*AN193G	193	N → G	rec$^-$	28	*rec*AR196G	196	R → G	rec$^-$	28
*rec*AQ194I	194	Q → I	rec$^-$, prt$^-$	28, 40	*rec*AM197I	197	M → I	rec$^+$	28
*rec*AQ194F	194	Q → F	rec$^-$, prt$^-$	28, 40	*rec*AM197F	197	M → F	rec$^\pm$	28
*rec*AQ194V	194	Q → V	rec$^-$, prt$^-$	28, 40	*rec*AM197V	197	M → V	rec$^\pm$	28
*rec*AQ194Y	194	Q → Y	rec$^-$	28	*rec*AM197Y	197	M → Y	rec$^\pm$	28
*rec*AQ194T	194	Q → T	rec$^-$	28	*rec*AM197T	197	M → T	rec$^\pm$	28
*rec*AQ194W	194	Q → W	rec$^-$, prt$^-$	28, 40	*rec*AM197W	197	M → W	rec$^\pm$	28

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TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*AM197L	197	M → L	rec^{+}	28	*rec*AG200W	200	G → W	rec^{-}	28
*rec*AM197C	197	M → C	rec$^{\pm}$	28	*rec*AG200L	200	G → L	rec^{+}	28
*rec*AM197H	197	M → H	rec$^{\pm}$	28	*rec*AG200C	200	G → C	rec$^{\pm}$	28
*rec*AM197R	197	M → R	rec^{-}	28	*rec*AG200M	200	G → M	rec$^{\pm}$	28
*rec*AM197K	197	M → K	rec^{-}	28	*rec*AG200H	200	G → H	rec$^{\pm}$	28
*rec*AM197D	197	M → D	rec^{-}	28	*rec*AG200R	200	G → R	rec$^{\pm}$	28
*rec*AM197E	197	M → E	rec$^{-\S}$	28	*rec*AG200K	200	G → K	rec$^{\pm}$	28
*rec*AM197Q	197	M → Q	rec^{-}	28	*rec*AG200D	200	G → D	rec^{-}	28
*rec*AM197S	197	M → S	rec^{-}	28	*rec*AG200E	200	G → E	rec^{-}	28
*rec*AM197N	197	M → N	rec^{-}	28	*rec*AG200Q	200	G → Q	rec$^{\pm}$	28
*rec*AM197A	197	M → A	rec$^{-\S}$	28	*rec*AG200S	200	G → S	rec$^{\pm}$	28
*rec*AM197P	197	M → P	rec^{-}	28	*rec*AG200N	200	G → N	rec$^{\pm}$	28
*rec*AM197G	197	M → G	rec^{-}	28	*rec*AG200A	200	G → A	rec$^{\pm}$	28
*rec*AK198I	198	K → I	rec$^{-\S}$	28	*rec*AG200P	200	G → P	rec$^{\pm}$	28
*rec*AK198F	198	K → F	rec^{-}	28	*rec*AV201I	201	V → I	rec^{+}	28
*rec*AK198V	189	K → Y	rec$^{-\S}$	28	*rec*AV201F	201	V → F	rec$^{\pm}$	28
*rec*AK198Y	198	K → Y	rec^{-}	28	*rec*AV201Y	201	V → Y	rec$^{\pm}$	28
*rec*AK198T	198	K → T	rec$^{-\S}$	28	*rec*AV201T	201	V → T	rec$^{\pm}$	28
*rec*AK198W	198	K → W	rec^{-}	28	*rec*AV201W	201	V → W	rec^{-}	28
*rec*AK198L	198	K → L	rec$^{-\S}$	28	*rec*AV201L	201	V → L	rec$^{\pm}$	28
*rec*AK198C	198	K → C	rec^{-}	28	*rec*AV201C	201	V → C	rec$^{\pm}$	28
*rec*AK198M	198	K → M	rec$^{-\S}$	28	*rec*AV201M	201	V → M	rec$^{\pm}$	28
*rec*AK198H	198	K → H	rec$^{-\S}$	28	*rec*AV201H	201	V → H	rec$^{\pm}$	28
*rec*AK198R	198	K → R	rec^{+}	28	*rec*AV201R	201	V → R	rec$^{-\S}$	28
*rec*AK198D	198	K → D	rec^{-}	28	*rec*AV201K	201	V → K	rec$^{-\S}$	28
*rec*AK198E	198	K → E	rec^{-}	28	*rec*AV201D	201	V → D	rec$^{-\S}$	28
*rec*AK198Q	198	K → Q	rec$^{-\S}$	28	*rec*AV201E	201	V → E	rec$^{-\S}$	28
*rec*AK198S	198	K → S	rec$^{-\S}$	28	*rec*AV201Q	201	V → Q	rec$^{\pm}$	28
*rec*AK198N	198	K → N	rec$^{-\S}$	28	*rec*AV201S	201	V → S	rec$^{\pm}$	28
*rec*AK198A	198	K → A	rec$^{-\S}$	28	*rec*AV201N	201	V → N	rec$^{\pm}$	28
*rec*AK198P	198	K → P	rec^{-}	28	*rec*AV201A	201	V → A	rec$^{\pm}$	28
*rec*AK198G	198	K → G	rec$^{-\S}$	28	*rec*AV201P	201	V → P	rec$^{\pm}$	28
*rec*AI199F	199	I → F	rec$^{\pm}$	28	*rec*AV201G	201	V → G	rec^{-}	28
*rec*AI199Y	199	I → Y	rec$^{-\S}$	28	*rec*AM202I	202	M → I	rec^{-}	28
*rec*AI199T	199	I → T	rec^{-}	28	*rec*AM202F	202	M → F	rec$^{-\S}$	28
*rec*AI199W	199	I → W	rec^{-}	28	*rec*AM202V	202	M → V	rec^{+}	28
*rec*AI199L	199	I → L	rec^{+}	28	*rec*AM202Y	202	M → Y	rec^{+}	28
*rec*AI199C	199	I → C	rec$^{-\S}$	28	*rec*AM202T	202	M → T	rec^{+}	28
*rec*AI199M	199	I → M	rec$^{\pm}$	28	*rec*AM202W	202	M → W	rec$^{\pm}$	28
*rec*AI199H	199	I → H	rec^{-}	28	*rec*AM202L	202	M → L	rec$^{-\S}$	28
*rec*AI199R	199	I → R	rec^{-}	28	*rec*AM202C	202	M → C	rec$^{\pm}$	28
*rec*AI199K	199	I → K	rec^{-}	28	*rec*AM202H	202	M → H	rec$^{\pm}$	28
*rec*AI199D	199	I → D	rec^{-}	28	*rec*AM202R	202	M → R	rec^{-}	28
*rec*AI199E	199	I → E	rec^{-}	28	*rec*AM202K	202	M → K	rec$^{\pm}$	28
*rec*AI199Q	199	I → Q	rec^{-}	28	*rec*AM202D	202	M → D	rec$^{-\S}$	28
*rec*AI199S	199	I → S	rec^{-}	28	*rec*AM202E	202	M → E	rec$^{-\S}$	28
*rec*AI199N	199	I → N	rec^{-}	28	*rec*AM202Q	202	M → Q	rec$^{\pm}$	28
*rec*AI199A	199	I → A	rec^{-}	28	*rec*AM202S	202	M → S	rec^{+}	28
*rec*AI199P	199	I → P	rec^{-}	28	*rec*AM202N	202	M → N	rec^{-}	28
*rec*AI199G	199	I → G	rec^{-}	28	*rec*AM202A	202	M → A	rec$^{\pm}$	28
*rec*AI199V	199	I → V‡	rec^{-}	28	*rec*AM202P	202	M → P	rec^{-}	28
*rec*A694	199	I → V‡	rec^{+}, prt^{i}	13, 32	*rec*AM202G	202	M → G	rec$^{\pm}$	28
*rec*AG200I	200	G → I	rec^{-}	28	*rec*AF203I	203	F → I	rec$^{\pm}$	28
*rec*AG200F	200	G → F	rec^{-}	28	*rec*AF203V	203	F → V	rec$^{\pm}$	28
*rec*AG200V	200	G → V	rec^{+}	28	*rec*AF203Y	203	F → Y	rec$^{\pm}$	28
*rec*AG200Y	200	G → Y	rec^{+}	28	*rec*AF203T	203	F → T	rec$^{-\S}$	28
*rec*AG200T	200	G → T	rec^{+}	28	*rec*AF203W	203	F → W	rec$^{\pm}$	28

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TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*AF203L	203	F → L	rec$^{\pm}$	28	*rec*AN205P	205	N → P	rec^{-}	28
*rec*AF203C	203	F → C	rec$^{\pm}$	28	*rec*AN205G	205	N → G	rec^{-}	28
*rec*AF203M	203	F → M	rec$^{\pm}$	28	*rec*AN205S/Y218G	205	N → S	rec^{+}	77
*rec*AF203H	203	F → H	rec$^{\pm}$	28		218	Y → G		
*rec*AF203R	203	F → R	rec^{-}	28	*rec*AP206I	206	P → I	rec$^{\pm}$	28
*rec*AF203K	203	F → K	rec$^{-\S}$	28	*rec*AP206F	206	P → F	rec$^{\pm}$	28
*rec*AF203D	203	F → D	rec^{-}	28	*rec*AP206V	206	P → V	rec$^{\pm}$	28
*rec*AF203E	203	F → E	rec^{-}	28	*rec*AP206Y	206	P → Y	rec$^{\pm}$	28
*rec*AF203Q	203	F → Q	rec$^{-\S}$	28	*rec*AP206T	206	P → T	rec^{+}	28
*rec*AF203S	203	F → S	rec$^{-\S}$	28	*rec*AP206W	206	P → W	rec^{-}	28
*rec*AF203N	203	F → N	rec^{-}	28	*rec*AP206L	206	P → L	rec$^{\pm}$	28
*rec*AF203A	203	F → A	rec$^{-\S}$	28	*rec*AP206C	206	P → C	rec$^{\pm}$	28
*rec*AF203P	203	F → P	rec^{-}	28	*rec*AP206M	206	P → M	rec$^{\pm}$	28
*rec*AF203G	203	F → G	rec^{-}	28	*rec*AP206H	206	P → H	rec$^{\pm}$	28
*rec*AF204I	204	G → I	rec^{-}	28	*rec*AP206R	206	P → R	rec$^{-\S}$	28
*rec*AG204F	204	G → F	rec^{-}	28	*rec*AP206K	206	P → K	rec^{-}	28
*rec*AG204V	204	G → V	rec^{-}	28	*rec*AP206D	206	P → D	rec^{-}	28
*rec*AG204Y	204	G → Y	rec^{-}	28	*rec*AP206E	206	P → E	rec$^{-\S}$	28
*rec*AG204T	204	G → T	rec$^{-\S}$	28	*rec*AP206Q	206	P → Q	rec$^{\pm}$	28
*rec*AG204W	204	G → W	rec^{-}	28	*rec*AP206S	206	P → S	rec$^{\pm}$	28
*rec*AG204L	204	G → L	rec^{-}	28	*rec*AP206N	206	P → N	rec$^{\pm}$	28
*rec*AG204C	204	G → C	rec$^{\pm}$	28	*rec*AP206A	206	P → A	rec$^{\pm}$	28
*rec*AG204M	204	G → M	rec^{-}	28	*rec*AP206G	206	P → G	rec$^{\pm}$	28
*rec*AG204H	204	G → H	rec^{+}	28	*rec*AE207I	207	E → I	rec^{-}	28
*rec*AG204R	204	G → R	rec^{-}	28	*rec*AE207F	207	E → F	rec^{-}	28
*rec*AG204K	204	G → K	rec$^{-\S}$	28	*rec*AE207V	207	E → V	rec^{-}	28
*rec*AG204D	204	G → D	rec^{-}	28	*rec*AE207Y	207	E → Y	rec^{-}	28
*rec*AG204E	204	G → E	rec^{-}	28	*rec*AE207T	207	E → T	rec^{-}	28
*rec*AG204Q	204	G → Q	rec$^{-\S}$	28	*rec*AE207W	207	E → W	rec$^{\pm}$	28
*rec*AG204S	204	G → S	rec$^{\pm}$	28	*rec*AE207L	207	E → L	rec^{-}	28
*rec*A430	204	G → S	rec$^{\pm}$, prt$^{\pm}$	8, 13	*rec*AE207C	207	E → C	rec^{-}	28
					*rec*AE207M	207	E → M	rec^{-}	28
*rec*AG204N	204	G → N	rec^{-}	28	*rec*AE207H	207	E → H	rec^{-}	28
*rec*AG204A	204	G → A	rec^{-}	28	*rec*AE207R	207	E → R	rec^{-}	28
*rec*AG604	204	G → A	rec$^{\pm}$, prt$^{\pm}$	13, 62	*rec*AE207K	207	E → K	rec$^{\pm}$, prt^{i}	28, 32
					*rec*AE207D	207	E → D	rec^{-}	28
*rec*AG204P	204	G → P	rec^{-}	28	*rec*AE207Q	207	E → Q	rec$^{\pm}$	28
*rec*AG204V	204	G → V‡	rec^{-}	28	*rec*A659	207	E → Q	rec$^{\pm}$, prt^{-}	13, 32
*rec*A605	204	G → V‡	rec$^{\pm}$, prt^{++}	13	*rec*AE207S	207	E → S	rec^{-}	28
					*rec*AE207N	207	E → N	rec^{-}	28
*rec*AN205I	205	N → I	rec^{-}	28	*rec*AE207A	207	E → A	rec^{-}	28
*rec*AN205F	205	N → F	rec^{-}	28	*rec*AE207P	207	E → P	rec^{-}	28
*rec*AN205V	205	N → V	rec^{-}	28	*rec*AE207G	207	E → G	rec^{-}	28
*rec*AN205Y	205	N → Y	rec^{-}	28	*rec*AT208I	208	T → I	rec^{+}	28
*rec*AN205T	205	N → T	rec$^{-\S}$	28	*rec*AT208F	208	T → F	rec^{-}	28
*rec*AN205W	205	N → W	rec^{-}	28	*rec*AT208V	208	T → V	rec^{+}	28
*rec*AN205L	205	N → L	rec^{-}	28	*rec*AT208Y	208	T → Y	rec^{-}	28
*rec*AN205C	205	N → C	rec$^{\pm}$	28	*rec*AT208W	208	T → W	rec^{-}	28
*rec*AN205M	205	N → M	rec^{-}	28	*rec*AT208L	208	T → L	rec^{-}	28
*rec*AN205H	205	N → H	rec^{-}	28	*rec*AT208C	208	T → C	rec$^{\pm}$	28
*rec*AN205R	205	N → R	rec^{-}	28	*rec*AT208M	208	T → M	rec^{-}	28
*rec*AN205K	205	N → K	rec^{-}	28	*rec*AT208H	208	T → H	rec^{-}	28
*rec*AN205D	205	N → D	rec^{-}	28	*rec*AT208R	208	T → R	rec^{-}	28
*rec*AN205E	205	N → E	rec^{-}	28	*rec*AT208K	208	T → K	rec^{-}	28
*rec*AN205Q	205	N → Q	rec^{-}	28	*rec*AT208D	208	T → D	rec^{-}	28
*rec*AN205S	205	N → S	rec$^{\pm}$	28	*rec*AT208E	208	T → E	rec^{-}	28
*rec*AN205A	205	N → A	rec$^{\pm}$	28	*rec*AT208Q	208	T → Q	rec^{-}	28

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TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*AT208S	208	T → S	rec^{-}	28	*rec*AG211D	211	G → D	rec^{-}	28
*rec*AT208N	208	T → N	rec$^{\pm}$	28	*rec*AG211E	211	G → E	rec^{-}	28
*rec*AT208A	208	T → A	rec^{-}	28	*rec*AG211Q	211	G → Q	rec^{-}	28
*rec*AT208P	208	T → P	rec^{-}	28	*rec*AG211S	211	G → S	rec^{-}	28
*rec*AT208G	208	T → G	rec^{-}	28	*rec*AG211N	211	G → N	rec^{-}	28
*rec*AT208I/F217A	208	T → I	rec^{-}	77	*rec*AG211A	211	G → A	rec^{-}	22, 28
	217	F → A			*rec*A611	211	G → A	rec^{-}	13, 32
*rec*AT209I	209	T → I	rec^{-}	28	*rec*AG211P	211	G → P	rec^{-}	28
*rec*AT209F	209	T → F	rec^{-}	28	*rec*AG212I	212	G → I	rec^{-}	28
*rec*AT209V	209	T → V	rec^{-}	28	*rec*AG212F	212	G → F	rec^{-}	28
*rec*AT209Y	209	T → Y	rec^{-}	28	*rec*AG212V	212	G → V	rec^{-}	28
*rec*AT209W	209	T → W	rec^{-}	28	*rec*AG212Y	212	G → Y	rec^{-}	28
*rec*AT209L	209	T → L	rec^{-}	28	*rec*AG212T	212	G → T	rec^{-}	28
*rec*AT209C	209	T → C	rec^{-}	28	*rec*AG212W	212	G → W	rec^{-}	28
*rec*AT209M	209	T → M	rec^{-}	28	*rec*AG212L	212	G → L	rec^{-}	28
*rec*AT209H	209	T → H	rec^{-}	28	*rec*AG212C	212	G → C	rec^{-}	28
*rec*AT209R	209	T → R	rec^{-}	28	*rec*AG212M	212	G → M	rec^{-}	28
*rec*AT209K	209	T → K	rec^{-}	28	*rec*AG212H	212	G → H	rec^{-}	28
*rec*AT209D	209	T → D	rec^{-}	28	*rec*AG212R	212	G → R	rec^{-}	28
*rec*AT209E	209	T → E	rec^{-}	28	*rec*AG212K	212	G → K	rec^{-}	28
*rec*AT209Q	209	T → Q	rec^{-}	28	*rec*AG212D	212	G → D	rec^{-}	28
*rec*AT209S	209	T → S	rec^{-}	28	*rec*AG212E	212	G → E	rec^{-}	28
*rec*AT209N	209	T → N	rec^{-}	28	*rec*AG212Q	212	G → Q	rec^{-}	28
*rec*AT209A	209	T → A	rec^{-}	28	*rec*AG212S	212	G → S	rec^{-}	28
*rec*AT209P	209	T → P	rec^{-}	28	*rec*AG212N	212	G → N	rec^{-}	28
*rec*AT209G	209	T → G	rec^{-}	28	*rec*AG212A	212	G → A	rec^{-}	28
*rec*AT210I	210	T → I	rec^{-}	28	*rec*AG212P	212	G → P	rec^{-}	28
*rec*AT210F	210	T → F	rec^{-}	28	*rec*AG212S/Y218L	212	G → S	rec^{-}	77
*rec*AT210V	210	T → V	rec$^{\pm}$	28		218	Y → L		
*rec*AT210Y	210	T → Y	rec^{-}	28	*rec*AN213F	213	N → F	rec^{+}, prt^{i†}	18
*rec*AT210W	210	T → W	rec^{-}	28					
*rec*AT210L	210	T → L	rec^{-}	28	*rec*AN213K	213	N → K	rec^{+}, prt^{i†}	18
*rec*AT210C	210	T → C	rec$^{\pm}$	28					
*rec*AT210M	210	T → M	rec^{-}	28	*rec*AN213T	213	N → T	rec^{+}, prt^{i†}	18
*rec*AT210H	210	T → H	rec^{-}	28	*rec*AN213C	213	N → C	rec^{+}, prt^{i†}	18
*rec*AT210R	210	T → R	rec^{-}	28	*rec*AN213S	213	N → S	rec^{+}, prt^{i†}	18
*rec*AT210K	210	T → K	rec^{-}	28	*rec*AN213G	213	N → G	rec^{+}, prt^{i†}	18
*rec*AT210D	210	T → D	rec^{-}	28	*rec*AN213H	213	N → H	rec^{+}, prt^{i†}	18
*rec*AT210E	210	T → E	rec^{-}	28	*rec*AN213M	213	N → M	rec^{+}, prt^{c†}	18
*rec*AT210Q	210	T → Q	rec^{-}	28	*rec*AN213A	213	N → A	rec^{+}, prt^{i†}	18
*rec*AT210S	210	T → S	rec$^{\pm}$	28	*rec*AN213L	213	N → L	rec^{+}, prt^{c†}	18
*rec*AT210N	210	T → N	rec^{-}	28	*rec*AN213I	213	N → I	rec^{+}, prt^{i†}	18
*rec*AT210A	210	T → A	rec^{-}	28	*rec*AN213V	213	N → V	rec^{+}, prt^{i†}	18
*rec*AT210P	210	T → P	rec$^{\pm}$	28	*rec*AN213R	213	N → R	rec$^{\pm}$, prt^{i†}	18
*rec*AT210G	210	T → G	rec^{-}	28	*rec*AN213G	213	N → E	rec$^{\pm}$, prt^{c†}	18
*rec*AG211I	211	G → I	rec^{-}	28	*rec*AN213P	213	N → P	rec$^{\pm}$, prt$^{-\dagger}$	18
*rec*AG211F	211	G → F	rec^{-}	28	*rec*AN213K/Y218L	213	N → K	rec$^{\pm}$	77
*rec*AG211V	211	G → V	rec^{-}	28		218	Y → L		
*rec*AG211Y	211	G → Y	rec^{-}	28	*rec*AK216R	216	K → R	rec$^{\pm}$, prt^{c†}	18
*rec*AG211T	211	G → T	rec^{-}	28	*rec*AK216H	216	K → H	rec$^{\pm}$, prt^{c†}	18
*rec*AG211W	211	G → W	rec^{-}	28	*rec*AK216C	216	K → C	rec$^{\pm}$, prt^{c†}	18
*rec*AG211L	211	G → L	rec^{-}	28	*rec*AK216M	216	K → M	rec$^{\pm}$, prt^{c†}	18
*rec*AG211C	211	G → C	rec^{-}	28	*rec*AK216S	216	K → S	rec$^{\pm}$, prt^{c†}	18
*rec*AG211M	211	G → M	rec^{-}	28	*rec*AK216A	216	K → A	rec$^{\pm}$, prt^{c†}	18
*rec*AG211H	211	G → H	rec^{-}	28	*rec*AK216Q	216	K → Q	rec$^{\pm}$, prt^{c†}	18
*rec*AG211R	211	G → R	rec^{-}	28	*rec*A616	216	K → Q	rec^{+}, prt^{i}	32
*rec*AG211K	211	G → K	rec^{-}	28	*rec*AK216I	216	K → I	rec$^{\pm}$, prt^{c†}	18

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TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*AK216L	216	K → L	rec$^{\pm}$, prt^{c†}	18	*rec*AR222Q/L223V	222	R → Q	rec^{+}	77
*rec*AK216Y	216	K → Y	rec$^{\pm}$, prt^{i†}	18		223	L → V		
*rec*AK216G	216	K → G	rec$^{\pm}$, prt^{c†}	18	*rec*AR222G/L223V	222	R → G	rec^{-}	95
*rec*AK216V	216	K → Y	rec$^{\pm}$, prt^{c†}	18		223	L → V		
*rec*AK216F	216	K → F	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*AR222T/L223V	222	R → T	rec^{-}	95
*rec*AK216D	216	K → D	rec^{-}, prt$^{-\dagger}$	18		223	L → V		
*rec*AK216E	216	K → E	rec^{-}, prt$^{-\dagger}$	18	*rec*A2223	223	L → M	rec$^{\pm ts}$, prt$^{\pm ts}$	26
*rec*AK216T	216	K → T	rec^{-}, prt^{i†}	18	*rec*A142	225	I → V	rec$^{\pm}$, prt$^{\pm}$	7, 29
*rec*AK216N/F217M	216	K → N	rec^{-}	77	*rec*A91	229	G → S	prt$^{\pm}$	41
	217	F → M			*rec*AK232E235	232	K → A	rec^{+}, prt$^{\pm}$,	63
*rec*AF217Y	217	F → Y	rec^{+}, prt^{c†}	18		235	E → A	cI$^{\pm}$, D^{i}	
*rec*AF217C	217	F → C	rec$^{\pm}$, prt^{c}	18, 37	*rec*AE233N236	233	E → A	rec^{+}, prt$^{\pm}$,	63
*rec*AF217P	217	F → P	rec$^{\pm}$, prt$^{-\dagger}$	18		236	N → A	cI^{i}, D^{i}	
*rec*AF217R	217	F → R	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*AT242R243	242	T → A	rec$^{\pm}$, prt^{i},	63
*rec*AF217K	217	F → K	rec$^{\pm}$, prt$^{-\dagger}$	18		243	R → A	cI$^{\pm}$, D^{-}	
*rec*AF217L	217	F → L	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*A433	243	R → H	prt^{i}, D^{-}	15
*rec*AF217S	217	F → S	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*A435	243	R → H	prt^{i}, D^{-}	15
*rec*AF217E	217	F → E	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*A1734	243	R → L	rec$^{\pm}$, prt$^{\pm}$	7
*rec*AF217T	217	F → T	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*A44	246	V → M	rec^{-}, prt^{-}, ts	12
*rec*AF217G	217	F → G	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*AV247M	247	V → M	rec$^{\pm}$, UmuR	67
*rec*AF217Q	217	F → Q	rec$^{\pm}$	18	*rec*AK248A	248	K → A	rec^{-}	34
*rec*AF217A	217	F → A	rec^{-}, prt$^{-\dagger}$	18	*rec*AN249T	249	N → T	rec$^{\pm}$, prt^{c}	45
*rec*AF217M	217	F → M	rec^{-}, prt$^{-\dagger}$	18	*rec*AK250N	250	K → N	rec$^{\pm}$, prt^{c}	45
*rec*AF217W	217	F → W	rec^{-}, prt$^{-\dagger}$	18	*rec*AK255S	255	F → N	rec$^{\pm}$, prt^{c}	45
*rec*AF217V	217	F → V	rec^{-}, prt$^{-\dagger}$	18	*rec*AK256Q257	256	K → A	rec^{+}, prt^{i},	63
*rec*AT218G	218	Y → G	rec^{+}, prt^{i†}	18		257	Q → A	cI^{c}, D^{i}	
*rec*AY218S	218	Y → S	rec^{+}, prt^{i†}	18	*rec*A801	257	Q → P	rec^{+}, srf	58
*rec*AY218A	218	Y → A	rec^{+}, prt^{c†}	18	*rec*AE259Q261	259	E → A	rec^{+}, prt^{i},	63
*rec*AY218W	218	Y → W	rec^{+}, prt^{c†}	18		261	Q → A	cI^{c}, D^{i}	
*rec*AY218H	218	Y → H	rec$^{\pm}$, prt^{c†}	18	*rec*AY264F	264	Y → F	rec^{-}, prt^{+}	14
*rec*AY218C	218	Y → C	rec$^{\pm}$, prt^{i†}	18	*rec*AY264S	264	Y → S	rec^{-}, prt^{+}	14
*rec*AY218P	218	Y → P	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*AY264G	264	Y → G	rec^{-}, prt^{-}	14
*rec*AY218K	218	Y → K	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*AY264W	264	Y → W	rec^{-}, prt^{+}	31
*rec*AY218D	218	Y → D	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*AY264A	264	Y → A	rec^{-}	31
*rec*AY218L	218	Y → L	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*A664	264	Y → E	rec^{-}, prt^{-}	66
*rec*AY218R	218	Y → R	rec$^{\pm}$, prt^{i†}	18	*rec*A665	264	Y → H	rec^{-}, prt^{-}	66
*rec*AY218E	218	Y → E	rec^{-}, prt$^{-\dagger}$	18	*rec*A2277	277	L → N	rec$^{\pm ts}$, prt$^{\pm ts}$	26
*rec*AY218N	218	Y → N	rec^{-}, prt$^{-\dagger}$	18	*rec*A2278	278	G → P	rec$^{\pm ts}$, prt$^{\pm ts}$	26
*rec*AY218C/A219R	218	Y → C	rec$^{\pm}$	77	*rec*A2278-5	278	G → T	rec$^{\pm}$, prt$^{\pm}$	26
	219	A → R				275	V → F		
*rec*AY218G/A219R	218	Y → G	rec$^{\pm}$	77	*rec*AK280N/K282N	280	K → N	rec$^{\pm}$	25
	219	A → R				282	K → N		
*rec*AA219R	219	A → R	rec^{+}	77	*rec*AK280E281K282	280	K → A	rec^{+}, prt$^{\pm}$,	63
*rec*AR222K	222	R → K	rec$^{\pm}$, prt^{c†}	18		281	E → A	cI$^{\pm}$, D$^{\pm}$	
*rec*AR222M	222	R → M	rec$^{\pm}$, prt^{c†}	18		282	K → A		
*rec*AR222I	222	R → I	rec$^{\pm}$, prt^{c†}	18	*rec*A2283	283	L → P	rec$^{\pm ts}$,	26
*rec*AR222Q	222	R → Q	rec$^{\pm}$, prt^{c†}	18				prt$^{\pm ts}$	
*rec*AR222V	222	R → V	rec$^{\pm}$, prt^{c†}	18	*rec*A2283E	283	L → E	rec^{+}, prt^{+}	26
*rec*AR222L	222	R → L	rec$^{\pm}$, prt^{i†}	18	*rec*A2284	284	I → D	rec^{-}, prt^{-}	26
*rec*AR222N	222	R → N	rec$^{\pm}$, prt^{i†}	18	*rec*AE285K286	285	E → A	rec$^{\pm}$, prt$^{\pm}$,	63
*rec*AR222A	222	R → A	rec^{-}, prt^{c†}	18				cI$^{\pm}$, D$^{\pm}$	
*rec*AR222D	222	R → D	rec$^{\pm}$, prt$^{-\dagger}$	18		286	K → A	protein	
*rec*AR222G	222	R → G	rec$^{\pm}$, prt^{c†}	18				unstable	
*rec*AR222E	222	R → E	rec$^{\pm}$, prt$^{-\dagger}$	18	*rec*AK286N	286	K → N	rec$^{\pm}$	25
*rec*AR222P	222	R → P	rec^{-}, prt$^{-\dagger}$	18	*rec*AW290T	290	W → T	rec$^{\pm}$, prt$^{\pm}$	68
*rec*A1735	222	R → C	prt$^{\pm}$	7	*rec*AW290F	290	W → F	rec$^{\pm}$, prt$^{\pm}$	68

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TABLE 1
Comprehensive Listing of All Known *E. coli recA* Mutants *(Continued)*

Mutant	Res	Change	Phenotype	Ref.	Mutant	Res	Change	Phenotype	Ref.
*rec*AW290H	290	W → H	rec$^{\pm}$, prt$^{\pm}$	68	*rec*AQ300N304	300	Q → A	rec$^{\pm}$, prt^{c},	63
*rec*AW290T/W308F	290	W → T	rec$^{\pm}$, prt^{c}	68		304	N → A	cI^{c}, D^{c}	
	308	W → F			*rec*AI207	301	G → D	rec^{-}, prt^{c}	10
*rec*AW290H/W308F	290	W → H	rec$^{\pm}$, prt^{i}	68	*rec*AK302N	302	K → N	rec$^{\pm}$	25
	308	W → F			*rec*AW308F	308	W → F	rec^{+}, prt^{i}	68
*rec*AW290F/W308F	290	W → F	rec$^{\pm}$, prt^{i}	68	*rec*AK310D311	310	K → A	rec^{+}, prt$^{\pm}$,	63
	308	W → F				311	D → A	cI$^{\pm}$, D^{i}	
*rec*AK294E318	294	K → A	rec$^{\pm}$, prt$^{\pm}$, cI$^{\pm}$, D^{+}	63	*rec*AE314K317	314	E → A	rec^{+}, prt^{i},	63
						317	K → A	cI$^{\pm}$, D^{i}	
	318	E → A	protein unstable		*rec*AK317N	317	K → N	rec^{+}	25
					*rec*AK321E325	321	K → A	rec^{+}, prt^{i},	63
*rec*AE296K297	296	E → A	rec^{+}, prt$^{\pm}$,	63		325	E → A	cI^{i}, D^{i}	
	297	K → A	cI^{i}, D^{i}		*rec*AK324Q	324	R → Q	rec^{+}	25
*rec*AK297N	297	K → N	rec$^{\pm}$	25	*rec*A750	Not	determ.	rec^{+}, prt^{i}	24

Key: **Ref**, citation in Appendix A; **amb**, translation termination codon; **rec^{+}**, activity similar to wild type; **rec^{-}**, no detectable activity; **rec$^{\pm}$**, intermediate activity; **prt^{-}**, negative LexA coprotease activity; **prt^{i}**, LexA coprotease inducible, similar to wild type; **prt$^{\pm}$**, intermediate, inducible LexA coprotease activity; **prt^{c}**, LexA coprotease constitutive; **D^{-}**, UmuD prt^{-}; **D^{i}**, UmuD prt^{i}; **D$^{\pm}$**, UmuD prt$^{\pm}$; **D^{c}**, UmuD prt^{c}; **cI^{-}**, λ cI prt^{-}; **cI^{i}**, λ cI prt^{i}; **cI$^{\pm}$**, λ cI coprt$^{\pm}$; **cI^{c}**, λ cI coprt^{c}; **srf**, suppressor of *recF*; **ts**, temperature sensitive (typically function is less at 42°C than 32°C); **ts+**, activated at elevated temp., see *recA441*; **tif**, temperature induced filamentation; **UmuR**, these mutations block UmuD'C-mediated inhibition of recombination activity; *, additional base change in 3′ UTR; **, additional base change in the promoter; §, positive for λ recombination proficiency but negative for repair activity; †, unpublished result (M.C. Skiba and K.L.K.); ‡, results differ between sources; **ND**, Phenotype not determined. Mutants are listed according to codon number. Those with multiple substitutions are listed only in the order of the N-terminal most codon with a mutation. rec phenotypes are taken from the citation indicated and in some cases derive from DNA damage survival assays, *e.g.*, survival following exposure to UV light or mitomycin C, and in other cases from general recombination assays, *e.g.*, host RecA-mediated plaque formation by a *red^{-} gam^{-}* λ phage. We do not distinguish between these assays unless specific comparisons are made in the citation indicated, *e.g.*, Hortnagel *et al.* (1999). Other phenotypes, *e.g.*, coprotease activity (LexA, UmuD and phage λ cI), suppressor of *recF* mutations (srf), suppressor of $UmuD'_2C$ inhibition (UmuR), etc. are listed as described in the key. "Unstable" in the phenotype column indicates that the steady-state cellular level of the mutant protein was far below that seen for wild-type RecA and a phenotype could not be scored. "No protein" indicates that no detectable amount of protein was visible in cell extracts, presumably due to extreme instability, and no phenotype was scored.

Related studies of these truncation mutants show that the ΔC6, ΔC13, and ΔC17 proteins are resistant to UV damage. However, the ΔC13 and ΔC17 proteins show a marked sensitivity to DNA damage induced by mitomycin C (Lusetti *et al.*, 2003b). Given that the repair of MMC-induced interstrand crosslinks requires functions of RecA, UvrABC, and pol I, the authors raise the possibility that the C-terminus of RecA may play a specialized role for this type of repair, perhaps through specific protein-protein interactions, a hypothesis previously developed by Devoret and colleagues (Ishimori *et al.*, 1996).

Together, the currently available data suggests that ssDNA within the active RecA nucleoprotein filament interacts specifically with residues in the L2 region, and that subsequent binding of the homologous duplex DNA to the L1 region involves transfer of dsDNA through a "gateway" within the C-terminal domain of RecA. There is also evidence that the L1 and L2 domains interact in a functional and perhaps physical sense. The regulated interaction of these domains as a result of ATP-induced allosteric changes is discussed further in the sections below.

ATP BINDING AND HYDROLYSIS DOMAINS

Walker A Motif

The Walker A motif, defined as GXXXXGK(T/S) (Walker *et al.*, 1982), spans residues 66–73 in *E. coli* RecA. The sequence, GPESSGKT, is very highly conserved and identical in 61 of 64 bacterial RecA proteins (Karlin and

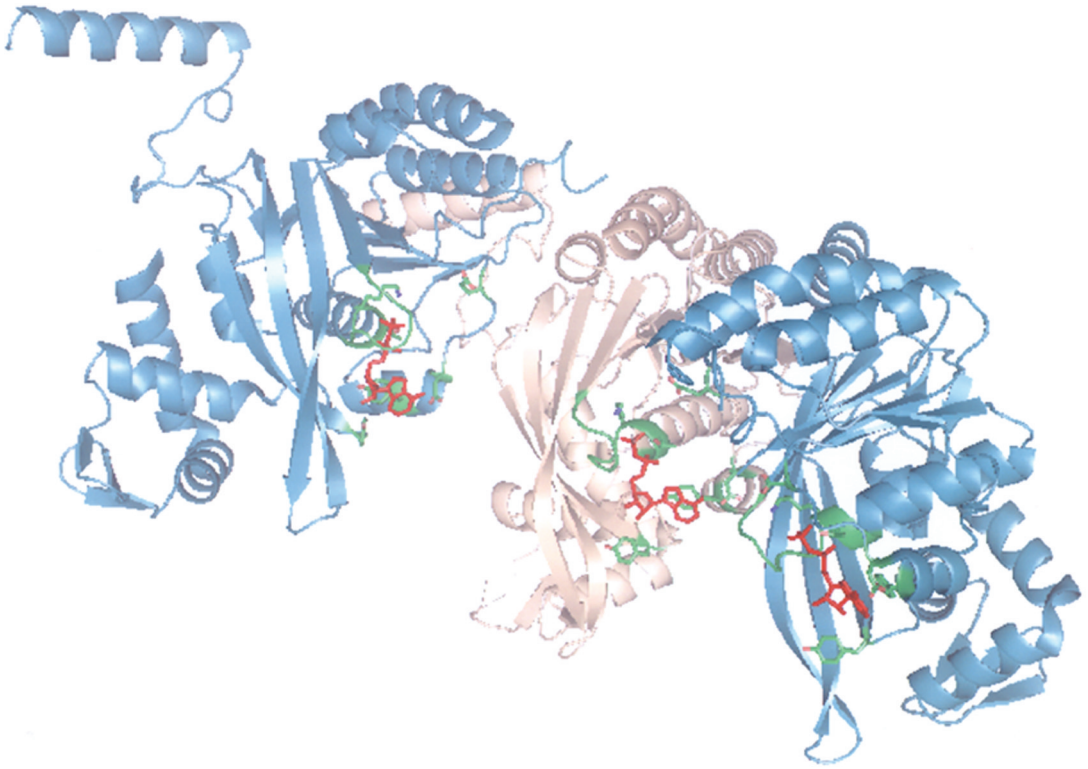

FIGURE 5. RecA trimer showing ATP binding sites. This view of three subunits shows the inside surface of the RecA lament. Subunits are colored in alternating teal and beige. Main chain of the Walker A motif (P-loop; residues 66—72) is shown in green. Main chain and side chain of residues 72, 73, 96, 100, 103, and 264 are colored by atom (C, green; O, red; N, blue). ADP is in red. This image was created using 1REA from Story and Steitz (1992). (Figure appears in color online at www.crbmb.com)

Brocchieri, 1996; Roca and Cox, 1997). This motif is frequently observed in ATP and GTP binding proteins, and its function typically involves stable binding of NTP. It is also referred to as the P-loop motif, named for its looplike secondary structure and specific interaction with the phosphate groups of bound NTPs (Saraste *et al.*, 1990). In the RecA structure, the ATP binding site, in particular the Walker A motif, lies on the inside surface of the protein filament (Figures 5 and 6).

Not surprisingly, extensive mutagenesis studies show that for the maintenance of any level of enzyme function the identity of the consensus residues, Gly66, Gly71, Lys72, and Thr73 must be maintained (Logan and Knight, 1993; Konola *et al.*, 1994). However, despite the high level of sequence identity for the other residues in this motif across 64 bacterial RecA sequences there is a surprising lack of mutational stringency. For example, although identical in 61 of 64 RecA sequences, substitution of Pro67 with any of 13 different polar, nonpolar, or charged amino acid side chains still allows between 25% and 100% of recombinational repair and general recombination functions (Konola *et al.*, 1994). Biochemical studies of Ser69Gly and Pro67Gly/Glu68Ala mutant proteins have provided mechanistic insights into the coupling of ATP hydrolysis and various steps in the strand exchange process catalyzed by RecA (Nayak and Bryant, 1999; Nayak *et al.*, 2001). Functions other than ATP binding have also been suggested by mutagenesis studies. For example, Pro67 has been identified as a likely residue for specific interaction with coprotease substrates (Nastri *et al.*, 1997; Konola *et al.*, 1998; also see the section called "Coprotease activity" below), and other studies suggest a role in DNA binding for Glu68 (Konola *et al.*, 1994). Despite the implications of these studies, however, the relative functional importance of the wild-type side chains at nonconsensus P-loop residues must be minimal. This point is made particularly clear in studies of mutants containing multiple substitutions. In fact, relative to wild-type RecA, a mutant with four contiguous Ala substitutions (GAAAAGKT) shows 90% recombinational

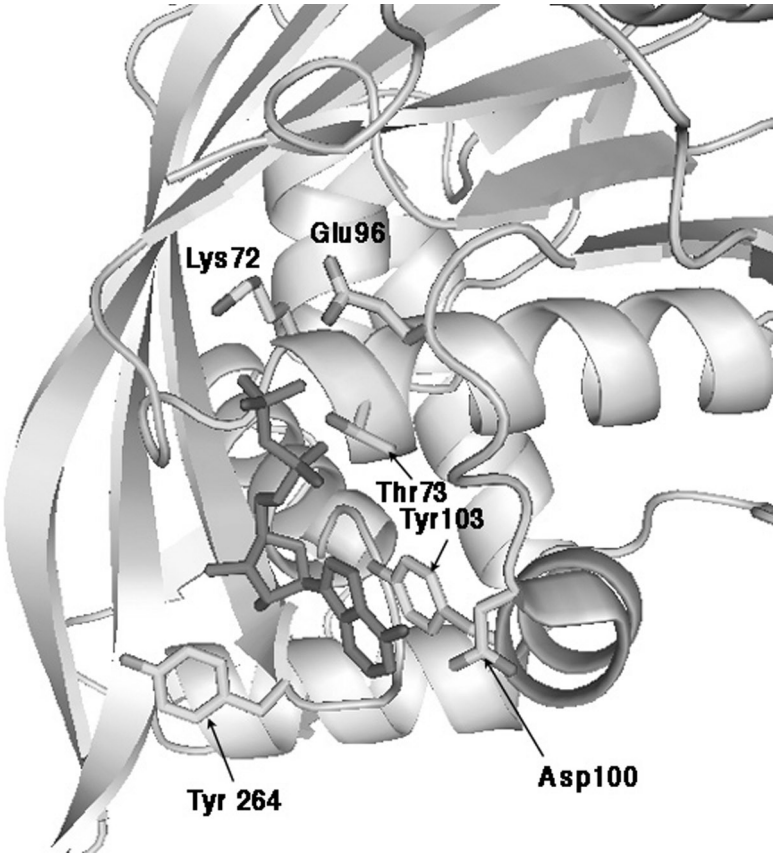

FIGURE 6. Detailed view of RecA ATP binding site. A close-up of the ATP binding site in a RecA monomer is shown in a similar orientation and same coloring as the right-most subunit in Figure 5. This was created using 1REA from Story and Steitz (1992). (Figure appears in color online at www.crbmb.com)

repair and 60% LexA coprotease activities *in vivo* and *in vitro* (J. T. Konola and K. L. Knight, unpublished).

Studies of the Lys72Arg mutant have revealed important mechanistic insights into RecA's use of a nucleoside triphosphate cofactor. While this conservative substitution inhibits all RecA activities *in vivo* (Konola *et al.*, 1994) the purified mutant protein is competent for activities *in vitro* that require NTP binding and formation of an active nucleoprotein filament, but not activities that require NTP hydrolysis. The initial idea that RecA requires only NTP binding and not hydrolysis to promote DNA strand exchange (Menetski and Kowalczykowski, 1990) was clearly established when Rehrauer and Kowalczykowski (1993) showed that the Lys72Arg mutant protein carried out strand exchange up to 1.5 kilobase pairs but could not complete the reaction. This occurred despite an approximate 700-fold decrease in the rate of ATP hydrolysis. Curiously, the mutant performed this limited strand exchange only in the presence of dATP and not ATP, a result for which there is still no clear structural explanation. This result demonstrated that the mutant protein still responds allosterically to NTP binding to form a catalytically competent nucleoprotein filament. Further studies using this mutant revealed that ATP hydrolysis allows wild-type RecA to carry out the strand exchange reaction in a unidirectional fashion as well as through regions of DNA heterology (Shan *et al.*, 1996; Shan and Cox, 1997). Use of this particular mutant also assisted in developing a model of RecA serving as a motor protein during the catalysis of strand exchange (Cox, 2000; Lusetti and Cox, 2002a).

Walker B Motif

The Walker B-motif is defined by residues 139–144, with Asp139 and Asp144 surrounding 4 hydrophobic residues (Karlin and Brocchieri, 1996). Asp144 is identical across all 64 bacterial RecA sequences (Karlin and Brocchieri, 1996; Roca and

Cox, 1997) and the structure (Story and Steitz, 1992) shows it to be positioned for interaction with the Mg^{2+} associated with bound ADP (Figure 6). Although Asp139 is identical in 62 of 64 RecA sequences (Karlin and Brocchieri, 1996; Roca and Cox, 1997), and the structure shows a well defined ionic bond between Lys6 in one subunit and Asp139 in the neighboring subunit, mutational studies show this interaction and the identity of residue 139 to be unimportant for RecA function (Eldin *et al.*, 2000; also see the "Oligomeric Interface Domains" section below).

In addition to the Walker A and B motifs, Karlin and Brocchieri (1996) observed that residues 145–149, which are immediately C-terminal to the B motif, are identical in 63 RecA sequences. They suggested that this region has some functional or structural importance regarding ATP hydrolysis. The recent structures of the MtRecA/NTP complexes reveal that main chain atoms of residues Val146 and Ala147 form hydrogen bonds with positions flanking L2, and Datta *et al*. (2003a) propose that this "connector" segment is involved in the transmission of NTP-induced structural changes in L2 to L1, thereby coordinating DNA binding to the primary and secondary sites.

MAW MOTIF

Roca and Cox (1997) also noted a structural motif defined by residues 42–65 that is conserved in all RecA homologs (Figure 7). Eight of these 24 residues are identical among the 64 bacterial RecA sequences (Ala50, Gly52, Gly54, Gly55, Pro57, Gly59, Arg60, and Glu63), nonpolar residues Leu47, Leu51, Ile61, and Ile64 are part of the hydrophobic protein core (Story *et al.*, 1992), and many other residues are chemically conserved. Interactions are seen between residues in this motif and regions of the structure on either side of the ATP binding site. Based on these observations, Roca and Cox (1997) speculated that this region may be involved in communicating ATP-induced conformational changes within the structure and have referred to it as the MAW motif, which is short for "make ATP work". It has been noted that Glu63 forms an ionic bond with Arg222 (Figure 7), a residue at the

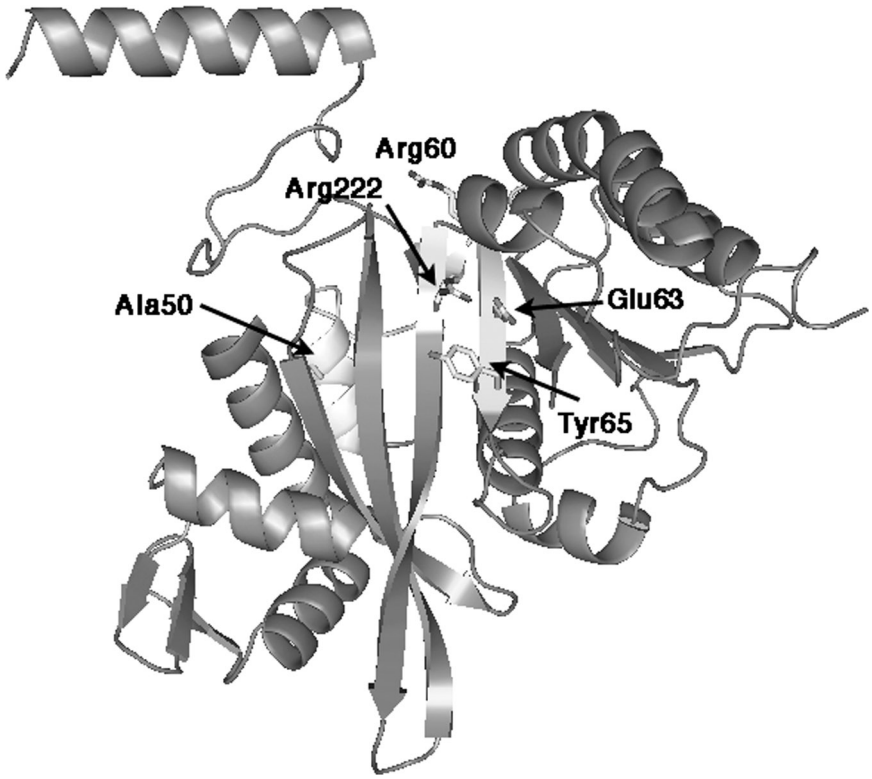

FIGURE 7. Proposed MAW motif of RecA. The MAW motif (*M*ake *A*TP *W*ork) is de ned by the αB/β1 regions (residues 45—65).Side chains are colored by atom (C, yellow; O, red; N, blue) as shown in 1REA (Story and Steitz, 1992). (Figure appears in color online at www.crbmb.com)

subunit interface that makes important cross-subunit contacts (see the "Oligomeric Interface Domains" section below) (Skiba and Knight, 1994). Together, this information suggests that residues in this region may play a role in the allosteric mechanism. However, only 3 mutants have ever been characterized with substitutions in this region, two of which are *rec*$^-$ (*recA13*, Leu51Phe; *recA56*, Arg60Cys; see Table 1) and the *rec*AS44L allele, which has close to wild-type recombination proficiency (Sommer *et al.*, 1998). Additional mutagenesis studies will be required to test the idea that this motif serves a specific function.

Residues Outside the Consensus ATP Site Domains

Mutational studies suggest important roles for other residues that are close to the ATP binding site but not part of a defined motif. In the structures of *E. coli* and *M. tuberculosis* RecA with bound nucleotide, Asp100 is in position to make a hydrogen bond with the amino group at the 6 position of the adenine ring (Figure 6). Stole and Bryant (1996) found that a D100N substitution permits the use of ITP as well as ATP as a cofactor for strand exchange, demonstrating that this residue is important in determining NTP specificity. These same structures also show a stacking interaction between bound ATP analogs or ADP and Tyr103, a result supported by fluorescence quenching experiments using a Tyr103Trp mutant protein (Morimatsu *et al.*, 1995). Other interactions that may provide some stabilization of nucleotide binding are observed in the *M. tuberculosis* RecA structures and include the following: H-bonds between the side chain hydroxyl of Thr74 and the ribose oxygen and α-phosphate; the side chain of Asn240 (Ser in *E. coli* RecA) and ribose 3′ OH; and the main chain amide proton at Gly265 and the purine N3 (Story and Steitz, 1992; Datta *et al.*, 2000, 2003a).

The identification of Tyr264 as the exclusive site of photolabeling by 8-azidoATP suggested a role for this residue in ATP binding (Knight *et al.*, 1984). However, the available structures show that although this residue is close to the ATP binding site, no contacts are seen between the bound ADP or ATP analogs and any atom in this residue. Mutant proteins carrying substitutions of Tyr264 to either Gly, Ser, or Phe are unable to carry out strand exchange (Freitag and McEntee, 1991), an inhibition that was attributed in large part to ATP binding and hydrolysis defects. Although fluorescence studies of a Tyr264Trp mutant protein supported the observation that Tyr264 lies near to or within the ATP binding site (Morimatsu *et al.*, 1995) any specific role in optimizing ATP binding or hydrolysis remains unclear. However, given that all current structures of RecA, even those with bound ATP analogs (Datta *et al.*, 2000, 2003a), represent the inactive form of the RecA filament, subtle structural changes that undoubtedly occur within the ATP binding site during the catalytic cycle of ATP hydrolysis may position the adenine C8 atom (the azido-modified position in 8-azidoATP) close to Tyr264. In fact, this would occur with only a slight rotation of the ribose C5-phosphoester bond and minimal adjustments within the ATP binding site to maintain Asp100 and Tyr103 in their appropriate positions (see Figure 6).

The position of Glu96 in the *E. coli* RecA structure suggests that it is in position to activate a water molecule for in-line attack of the γ-phosphate of ATP (Story and Steitz, 1992). The biochemical properties of the Glu96Asp mutant protein support this idea (Campbell and Davis, 1999a, 1999b). This mutant still shows cooperative ATP-mediated formation of an active nucleoprotein filament that is constitutively activated for LexA cleavage; and, although it can initiate strand exchange *in vitro*, the reaction does not go to completion, a result very similar to that seen with the Lys72Arg mutant. In addition to the roles proposed for Glu96 and Lys72 in ATP hydrolysis, Voloshin *et al.* (2000) have proposed direct roles for Gln194 and Arg196 in this activity. This idea, and discussion of the role of Gln194 as an allosteric switch, will be developed below in the "Allosteric Mechanism of RecA" section.

OLIGOMERIC INTERFACE DOMAINS

Early genetic studies using point mutants and various truncation mutants revealed that the functional form of RecA is oligomeric. Ogawa *et al.* (1979) found that all wild-type RecA activities were inhibited by coexpression of a *rec*$^-$ allele, *recA1*. Sedgewick and Yarranton (1982) found that the DNA repair activity of wild-type RecA was inhibited by coexpression of a truncation mutant that carried only the N-terminal 77 residues (of 352 total), and a similar result was observed by Kiselev *et al.* (1988) using a peptide corresponding to the N-terminal 50 residues (Table 3). These studies not only supported the idea that RecA functioned as an oligomer, they also suggested that important determinants of subunit self-association exist within a

folded N-terminal domain. Dutreix *et al*. (1989) isolated a Ser117Phe mutant (*recA1730*) whose genetic and biochemical properties suggested that it has a specific defect in filament formation. All of these results were readily explained when the structure revealed numerous cross-subunit contacts made by a clearly defined N-terminal domain. The structure showed that Ser117 lies within an interface region opposing this N-terminal domain in the neighboring monomer, and a Ser117Phe mutation would result in the loss of a hydrogen bonding interaction between the Ser–OH in one subunit and the main chain carbonyl oxygen at position 25 in the neighboring subunit (Story *et al.*, 1992).

The complex oligomeric character of RecA first became apparent from electron microscopic studies of RecA in the absence and presence of ATP analogs and either ss- or dsDNA. These images showed that monomers of RecA assembled into a helical, head-to-tail polymer that wrapped around the DNA (reviewed in Egelman and Stasiak, 1993; Egelman, 1993). RecA was observed to bind cooperatively to gapped dsDNA when incubated in the presence of ATPγS (West *et al.*, 1980). Stasiak *et al*. (1981) made the important observation that RecA binding induces elongation of dsDNA by 50%, from an axial rise per base pair of 3.4 Å for B-form DNA to 5.2 Å for RecA/dsDNA complexes. A similar RecA-induced elongation was also observed for ssDNA (Koller *et al.*, 1983). These results led to the idea that one of the primary roles of RecA is to reveal DNA bases in a way that facilitated strand switching during the catalysis of DNA strand exchange. Subsequent EM studies defined the structural parameters of both the "active" and "inactive" forms of the RecA filament, each having a helical filament pitch of 68 and 95 Å, respectively (see the "Introduction" above). Given that the structure of *E. coli* RecA shows a filament with an approximate 83 Å pitch (Story *et al.*, 1992) and different structures of the *M. tuberculosis* RecA each have a helical pitch of approximately 72.5 Å (Datta *et al.*, 2000, 2003a), some differences are likely to exist regarding cross-subunit interactions seen in these crystal structures and those that exist in the active filament form. The *E. coli* and *M. tuberculosis* RecA proteins share 62% sequence identity, and their X-ray structures show a very high degree of similarity. Superposition of all α-carbons gives a root mean square deviation of 1.1 Å (Datta *et al.*, 2000), and many of the intersubunit contacts in the *E. coli* RecA structure are retained in the *M. tuberculosis* RecA structures (Datta *et al.*, 2000, 2003a). Residues involved in cross-subunit contacts account for a significant percentage of the conserved positions among 64 bacterial RecA sequences (Karlin and Brocchieri, 1996; Roca and Cox, 1997). In fact, a RecA "signature sequence" is noted by Roca and Cox (1997) and is defined by a very highly conserved string of residues, 214–222, with 5 of the 9 residues being identical in 64 RecA sequences. Alignments show the consensus to be ^{214}Ala-Leu-Lys-Phe-[Phe/Tyr]-[Ala/Ser]-[Asp/Ser/Thr]-[Ile/Leu/Met/Gln/Val]-Arg^{222}, and mutagenesis studies have revealed important structural and functional roles for residues in this group (see below and Skiba and Knight, 1994; Kelley De Zutter *et al.*, 2001). Given that the overall pitch of the filament in the crystal structure is somewhat less than that of the active filament determined by EM, it is expected that some cross-subunit interactions important to the active filament will not be seen in the structure. Therefore, a significant amount of mutational and biochemical work has been performed aimed at identifying residues located at the subunit interface that play important roles in either stabilizing the filament structure or transmitting allosteric information across the interface. Of course, these two categories are not mutually exclusive.

The polymeric nature of the RecA filament requires that each subunit has two opposing surfaces that participate in oligomeric contacts. Residues whose solvent accessible surface area decreases by 15 Å^2 or more upon polymer formation were predicted to play a role in subunit interactions (Story *et al.*, 1992). The 55 residues that fit this criterion are shown in Figures 8 and 9. The area that shows the largest decrease in solvent accessibility following the monomer to oligomer transition is defined by residues 213–222, and the structure shows that five side chains in this area, Asn213, Lys216, Phe217, Tyr218, and Arg222 make specific contact with positions in the neighboring subunit. The surface in the neighboring subunit contacted by the residues is made up of three regions: residues 94–98, 118–123, and 148–156. Mutational analyses of these five residues showed that a number of substitutions are allowed at positions 213 and 218, indicating that the contacts seen in the structure are not important determinants of oligomer stability and function. Of the other three, Phe217 showed the least tolerance to mutation, with a Tyr substitution as the only one that retained significant function. However, the critical need for a benzyl ring at position 217 was not at all clear from the structure. The neighboring subunit provides only a minimal hydrophobic surface opposite Phe217 that is largely defined by Ile155, but a number of substitutions are tolerated here, both polar and nonpolar, with no detriment to

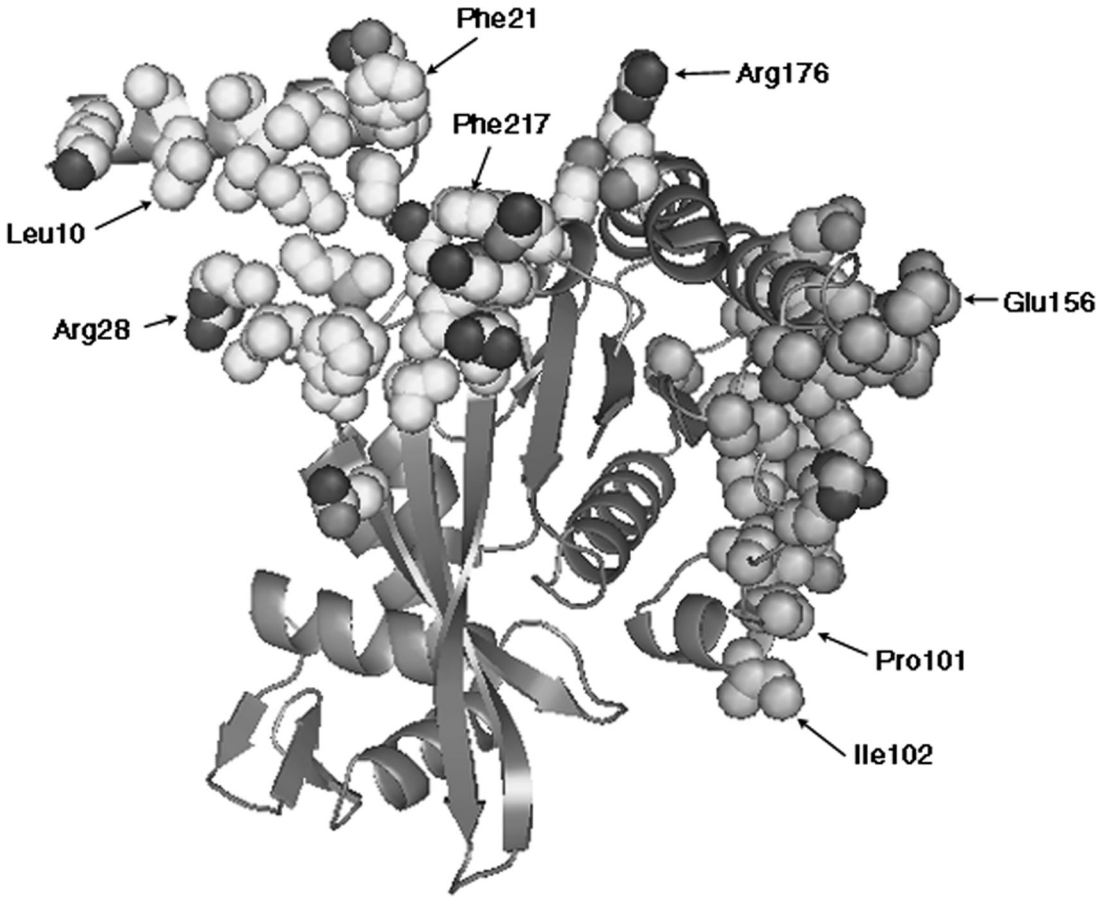

FIGURE 8. RecA monomer showing both surfaces of the subunit-subunit interface. Formation of RecA laments involves two distinct surfaces of the protein. On one surface side chain atoms are colored by atom (C, yellow; O, red; N, blue); on the other surface carbons have been shown in pink. Labeled residues are for orientation purposes only and are not meant to indicate any functional or structural importance. This image was created using 2REB in Story *et al.* (1992). (Figure appears in color online at www.crbmb.com)

RecA function. Therefore, these results suggested that important interactions between Phe217 and positions in the neighboring subunit are not apparent in the structure. In fact, further study revealed that Phe217 is important for transmitting ATP-mediated allosteric information across the subunit interface, and that its position in the active form of the RecA filament is likely different from its position in the structure (Skiba *et al.*, 1999; Kelley De Zutter *et al.*, 2001; see also the "Allosteric Mechanism" of RecA section below).

Substitutions at other positions in this region of the interface, His97Ala in one subunit and Lys248Ala in the neighboring subunit, each create inactive proteins that are defective in filament formation (Nguyen *et al.*, 1993). Although mutational information for residues in this region of the interface are in general agreement with contacts seen in the structure, it is clear that some interactions important to the function of the filament are not observed in the structure.

Extensive intersubunit contacts are seen in two other regions of the RecA structure. These are defined by α-helix A and β-strand 0 in one subunit (residues 3–30), and β-strand 3 and α-helix E in the neighboring subunit (residues 111–140; Figures 8 and 9). These two regions form complementary surfaces in which the cross-subunit interactions are largely hydrophobic, including 6 nonpolar side chains (Leu10, Ala13, Leu14, Ile17, Phe21, and Leu29) that are within van der Waals distance ($\leq$ 4 Å) of 7 nonpolar side chains (Ile111, Leu115, Ile128, Ala131, Leu132, Ala137, and Val138) in the neighboring subunit. Several ionic and polar

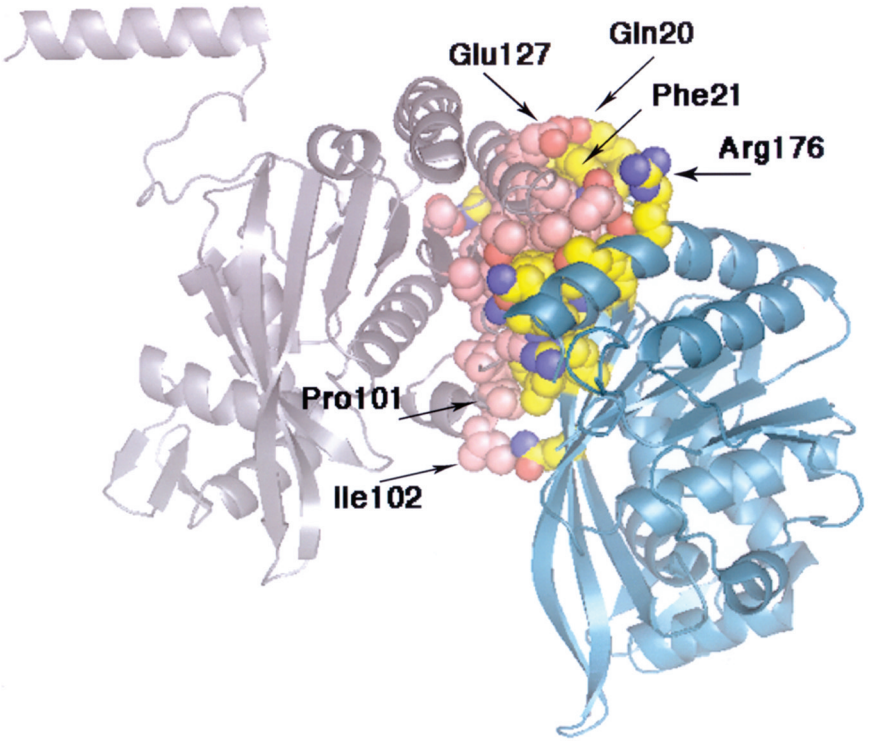

FIGURE 9. RecA dimer showing all residues involved in subunit-subunit interactions. Subunit 1 is shown in teal with yellow interface residues; subunit 2 is in gray with pink interface residues and at the same orientation as the monomer in Figure 8. This image was created using 2REB in Story *et al.* (1992). (Figure appears in color online at www.crbmb.com)

interactions that flank this hydrophobic area are also observed. Lys6 forms an ionic bond with Asp139, and Arg28 is within hydrogen bonding distance of positions in Asp112 and Asn113. Mutagenesis studies show that the Lys6-Asp139 and Arg28-Asp112/Asn113 interactions are only minimally important regarding formation and function of an active RecA nucleoprotein filament; however, they are far more important to the stability of free RecA filaments, the inactive form (Eldin *et al.*, 2000). Pairwise Cys substitutions are tolerated at positions 6/139, 28/112, and 28/113, and Logan *et al.* (2001) showed that the efficiency of cross-subunit disulfide formation for these pairs is different for the active versus inactive filament forms. These data provide a direct biochemical demonstration of ATP-induced changes in cross-subunit interactions in this region of the interface.

The above results supported earlier work by Zaitsev and Kowalczykowski (1999) that suggested cross-subunit hydrophobic interactions might account for most of the oligomeric stability in this region of the interface. They found that a Gln124Leu substitution suppressed the *rec*$^-$ phenotype of the *recA142* allele, which carries an Ile225Val mutation. Gln124 is within van der Waals distance of Phe21 across the subunit interface, and substitution with a Leu would create a new cross-subunit hydrophobic interaction. Ile225 is part of the hydrophobic core of the RecA monomer structure (Story *et al.*, 1992), and mutation to Val apparently results in a deleterious rearrangement of cross-subunit contacts, a defect that is compensated by enhancing intersubunit hydrophobic interactions. Remarkably, the Gln124Leu mutation also suppressed the *rec*$^-$ phenotype of a mutant missing the N-terminal 9 residues (*recA*Δ9; Table 2). Not only does this result support the idea that oligomeric stability in this region occurs predominantly through hydrophobic interactions but also that cross-subunit interactions mediated by residues 1–9, *e.g.*, Lys6/Asp139, do not play any significant role in either the structure or function of the RecA filament (see the "Allosteric Mechanism in RecA" section below).

Masui *et al.* (1997) have shown that the folding transition of the N-terminal region defined by residues 1–33 are coupled to the filament association-dissociation process. Using a truncated mutant protein missing the N-terminal 33 residues and a synthetic peptide corresponding to residues

TABLE 2
RecA Peptides and Mutant Peptides

Peptide	# res	Region	Residues	Function	Ref.
rec24	24	N term	1-24	$ssDNA^+$	50
rec24E	24	N term	1-24 A12E	$ssDNA^\pm$	50
rec34	34	N term	1-34	$ssDNA^+$	50
1	34	N term	6-39	$ssDNA^-$	49
12	18	L1 Loop	152-169		49
2	35	L2 Loop	185-219	$ssDNA^+$	49
4	35	L2 Loop	185-219 G211A	$ssDNA^-$	49
5	35	L2 Loop	185-219 G204S	$ssDNA^-$	49
6	35	L2 Loop	185-219 E207Q	$ssDNA^-$	49
FECO	20	L2 Loop	193-212	$ssDNA^+$, $dsDNA^+$	49, 48, 53,52
WECO	20	L2 Loop	193-212 F203W	$ssDNA^+$, $dsDNA^+$, ATP^+	48, 52
YECO	20	L2 Loop	193-212 F203Y	$ssDNA^+$, $dsDNA^+$	48, 52
HECO	20	L2 Loop	193-212 F203H	$ssDNA^-$	48, 52
AECO	20	L2 Loop	193-212 F203A	$ssDNA^-$	48, 52
IECO	20	L2 Loop	193-212 F203I		52
WR196K	20	L2 Loop	193-212 R196K	ATP^+	47
WR196G	20	L2 Loop	193-212 R196G	ATP^-	47
L2-G	24	L2-G helix	195-218	$ssDNA^+$, $deDNA^-$	46
WT-14	14	L2 Loop	196-209	$ssDNA^-$	49, 48, 52
wtw	15	L2 Loop	196-210	$ssDNA^-$	46
T-31	24		257-280	ATP^+	51
3	28	C term	302-329	$ssDNA^-$	49

Key: $ssDNA^+$, binds to single stranded DNA; $ssDNA^\pm$, binds to single stranded DNA; $ssDNA^-$, no binding to ssDNA; $dsDNA^+$, binds to double stranded DNA; $dsDNA^-$, no binding to dsDNA; ATP^+, binds ATP; ATP^-, does not bind ATP.
Peptide fragments of *E. coli* RecA are listed according to their name as used by the authors in the citation indicated (Peptide), the region of the RecA protein from which they are derived (Region), and specific residue numbers contained in the peptide (Residues). Biochemical functions, *e.g.*, ability to bind ssDNA, dsDNA, and ATP, are indicated.

1–33, their work suggested that RecA subunit dissociation is coupled to a helix-coil transition in this region. They also demonstrated that this N-terminal region is involved in the kinetic regulation of RecA self-association and proposed that this local folding transition is relevant to RecA filament assembly and disassembly *in vivo*.

Allosteric Mechanism of RecA

In the crystal structure of *E. coli* RecA the position of Gln194 suggests that it may play a key role in sensing the presence of the γ-phosphate of bound ATP (Figures 10 and 11). Story *et al.* (1992) have proposed that this interaction triggers

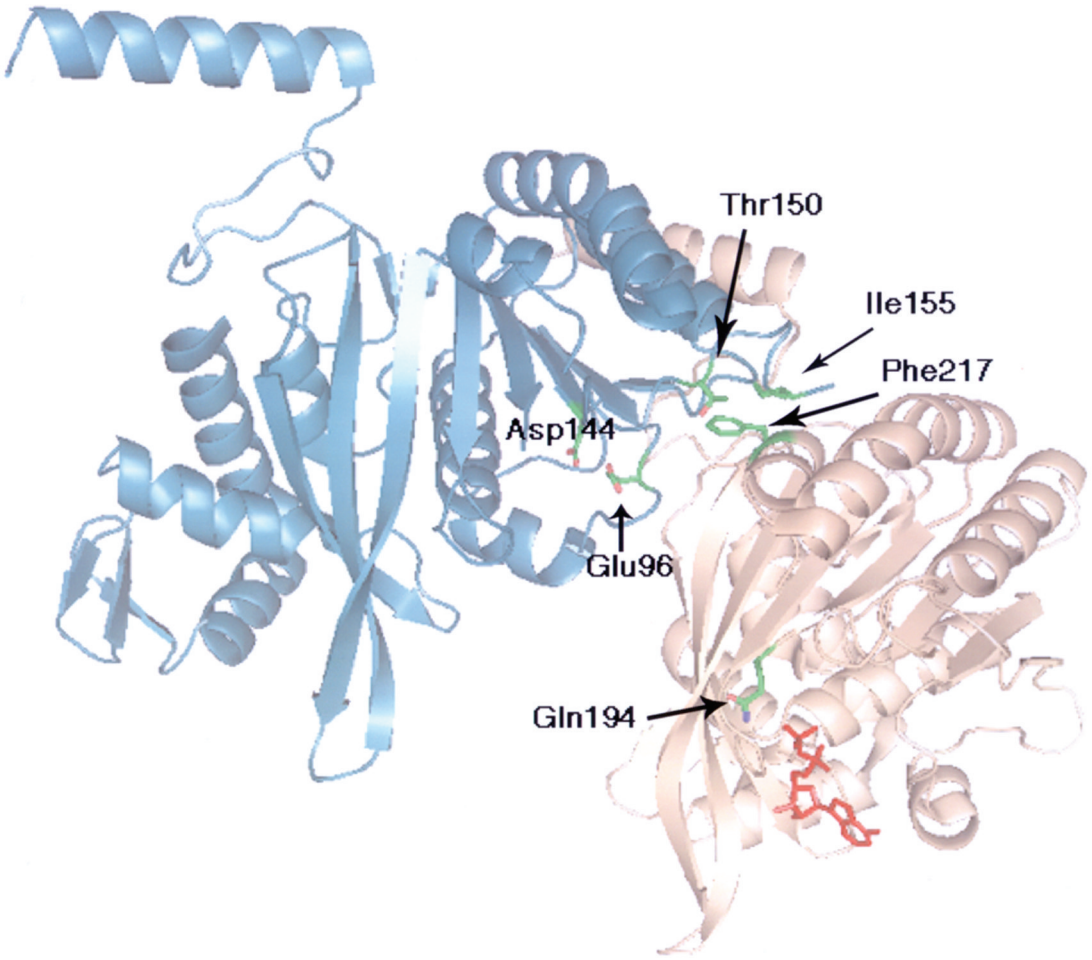

FIGURE 10. RecA dimer showing residues involved in a proposed model for the allosteric mechanism. Main chain of subunit 1 is in beige with side chains of Q194 and F217 colored by atom (C, green; O, red; N, blue); subunit 2 is colored teal with side chains of E96, D144, T150, and I155 colored the same as subunit 1. This image was created using 1REA in Story and Steitz (1992). (Figure appears in color online at www.crbmb.com)

allosteric activation of the L2 DNA binding region. Indeed, genetic analysis showed Gln194 to be nonmutable (Kelley and Knight, 1997; Hortnagel *et al.*, 1999), and biochemical studies of Gln194Asn, Gln194Glu, and Gln194Ala mutant proteins showed that each maintained basal RecA activities reminiscent of those carried out by the inactive form of wild-type RecA, *e.g.*, low affinity ssDNA binding and slow ATP turnover. Each of these 3 mutant proteins bound ATP with an affinity similar to wild-type RecA, but showed no ATP-induced activation of RecA functions (Kelley and Knight, 1997). This data supported the idea that Gln194 acts exclusively as an "allosteric switch" that signals ATP-mediated increases in RecA function. However, subsequent work by Voloshin *et al*. (2000) suggested that, in addition to the allosteric role played by Gln194, both Gln194 and Arg196 play a direct role in the catalysis of ATP hydrolysis by stabilizing a transition state complex. Their data suggest that these side chains act in ways very similar to the conserved Gln and Arg residues in members of the G-protein family (Sprang, 1997a, 1997b) and therefore serve as both catalytic residues and regulators of an allosteric transition. While these two studies differ in some ways regarding the biochemical properties of mutant enzymes, especially Gln194Asn, together they have clearly identified Gln194 as a key allosteric switch. Again, a more precise definition of the mechanistic roles played by other residues in this region awaits a structure of the active form of RecA.

Further investigation into the allosteric mechanism led to the identification of Phe217 as a critical residue at the subunit interface that regulates the

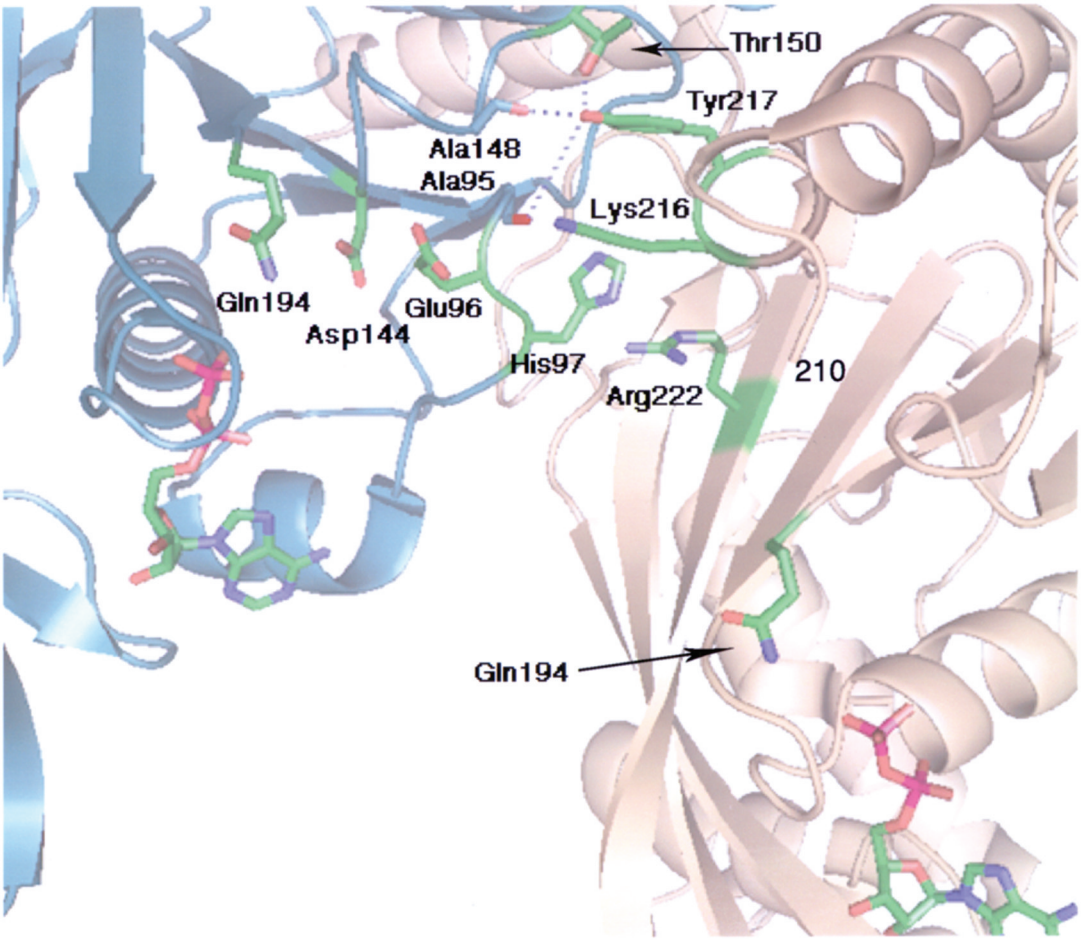

FIGURE 11. Detail of region of the subunit interface involved in ATP-mediated transfer of allosteric information. Phe217 is replaced here with Tyr, which is within hydrogen bonding distance of the carbonyl oxygen of Ala95 and Ala148, and the —OHgroup of Thr150 in the neighboring subunit. The Tyr side chain was repositioned slightly relative to the position of Phe217 in the coordinate le to accommodate these three potential hydrogen bonds. No steric clashes resulted. This image shows other cross-subunit interactions that may be involved in oligomeric stabilization, *e.g.*, Lys216-Ala95 and Arg222-His97. The image was created using 1REA in Story and Steitz (1992). This model derives from work described in DeZutter *et al.* (2001). Coloring of side chains and overall orientation of the subunit interface is the same as in Figure 10. (Figure appears in color online at www.crbmb.com)

flow of ATP-mediated information throughout the RecA filament. This position exhibits a high mutational stringency, with only Phe or Tyr allowed (Skiba and Knight, 1994). Subsequently, Kelley De Zutter *et al*. (2001) demonstrated that the Phe217Tyr substitution created a hypercooperativity mutant. Remarkably, the simple addition of an–OH group to Phe217 resulted in greater than a 250-fold increase in the ability of RecA monomers to assemble into an active filament in the presence of ATP and ssDNA. The authors proposed a model in which ATP binding adjusts the conformation of this region of the interface such that Phe217 interacts stably with positions in the neighboring subunit. This conformational event is an important step in propagating related changes throughout the RecA filament. Together with other data, the following series of events were suggested as depicted in Figures 10 and 11. The interaction of Gln194 with the γ-phosphate of bound ATP serves to trigger a conformational change in L2. Phe217 is within a small α-helix (residues 211–218) that sits immediately downstream of L2. Therefore, ATP-mediated conformational changes in L2 are propagated through this helix, resulting in the movement of Phe217 toward the neighboring subunit. Residues within the neighboring subunit that could

TABLE 3
Truncation Mutants of *E. coli* RecA

recA mutant	Residues	Phenotype	Ref.
pMH1	1-77	rec^+	72
pDR1461	1-258	$rec^{\pm}$	72
*rec*AΔ9	9-352	rec^-	29
*rec*AΔ9/Q124L	9-352 Q124L	rec^+	29
pTH6337	MKAF + 16-352	rec^-	54
pTH6324	MKA + 29-352	rec^-	54
pTH7299	MKA + 29-327	rec^-	54
Δ33RecA	33-352	ND	42
pTH6311	MKAF + 42-352	rec^-	54
pTH7248	MKAF + 42-327	rec^-	54
pTH6293	MKAF + 60-352	rec^-	54
pTH7268	MKAF + 60-327	rec^-	54
pTH5076	1-76 + Q	rec^-	54
pTH6221	MKA + 132-352	rec^-	54
*rec*A99	Q7 → amb	rec^-	11
pTH5143	1-143 + KNH	rec^-	54
pTH5328D	Deletion of 180-203	rec^-	54
pTH5203	1-204 + E	rec^-	54
*rec*A16	≈1-210	ND	70
pTH6131	MKAF + 222-352	rec^-	54
*rec*A214	1-214	rec^-, prt^-	74
pTH5259	1-259	rec^-	54
pTH5275	1-275 + KNH	rec^-	54
pTH5290	1-290	rec^-	54
*rec*A268-330	fM + 268-330		43
*rec*AΔC50	≈1-300	ND	69
*rec*A5327	1-327 + Q	rec^+, prt^c	54, 73
*rec*AΔC18	1-334	ND	71
RecA335	1-335	rec^{pm}, $prt^{\pm}$	74, 75*
*rec*AΔC17/E38K	1-335 E 38 K	ND	80
*rec*AΔC13	1-339	$rec^{\pm}$	75
*rec*AΔC6	1-346	rec^+	75

Key: amb, translation termination codon; rec^+, activity similar to wild type; rec^-, no detectable activity; $rec^{\pm}$, intermediate activity; prt^-, negative LexA coprotease activity; prt^i, LexA coprotease inducible, similar to wild type; $prt^{\pm}$, intermediate, inducible LexA coprotease activity; prt^c, LexA coprotease constitutive; D^-, UmuD prt^-; D^i, UmuD prt^i; $D^{\pm}$, UmuD $prt^{\pm}$; D^c, UmuD prt^c; cI^-, λ cI prt^-; cI^i, λ cI prt^i; $cI^{\pm}$, λ cI $coprt^{\pm}$; cI^c, λ cI $coprt^c$; srf, suppressor of *recF*; ts, temperature sensitive (typically function is less at 42°C than 32°C); ts+, activated at elevated temp., see *recA441*; tif, temperature induced filamentation; UmuR, these mutations block UmuD'C-mediated inhibition of recombination activity; *, additional base change in 3′ UTR; **, additional base change in the promoter; ˣ, positive for λ recombination proficiency but negative for repair activity; †, unpublished result (M.C. Skiba and K.L.K.); ‡, results differ between sources; ND, Phenotype not determined.

Mutants are described in terms of the residues contained in each truncation mutant (Residues). The mutants listed as pTH. . . . refer to the plasmid name carrying the indicated truncated *recA* gene under control of the lacUV5 promoter. The rec phenotype is indicated as described in the legend to Table 1.

accommodate a transient interaction with Phe217 include Ala95, Ala148, and Ile93. Story *et al*. (1992) predicted that the Gly residues at 211 and 212, both invariant in all RecA sequences and nonmutable (Roca and Cox, 1997; Hortnagel *et al.*, 1999), may be directly involved in DNA binding or in mediating ATP-induced conformational changes. This model supports the idea that the flexibility of the polypeptide backbone at these positions is critical for the propagation of conformational changes transmitted from the ATP binding site to the subunit surface. The increase in cooperative subunit interactions by a Tyr substitution most likely results from new hydrogen bonding opportunities, and several possibilities are shown in Figure 11. Phe217 lies very close to the ATP binding site of the neighboring subunit, and cross-subunit interactions in this region would undoubtedly effect the position of residues within this site involved in ATP binding and hydrolysis. This idea is supported by the observation that ATP binding affinity and the rate of hydrolysis is increased by the Phe217Tyr mutation (Kelley De Zutter *et al.*, 2001).

Recent work from the Egelman group using a novel method for image analysis of electron micrographs provides an alternative view of conformational differences between the active and inactive filament forms of RecA within this region of the subunit interface. Reconstructions of electron micrographs of a RecA filament containing the Glu96Asp mutant protein, dsDNA and ATP, were used by VanLoock *et al*. (2003) to show that in the active filament the C-terminal domain (residues 270–352) is in a significantly different position from that seen in the RecA crystal structure, although residues 329–352 are not visible in the crystal structure. In the crystal structure this domain extends outward from the main body of the filament (see Figure 2), whereas in the EM reconstructions the C-terminal domain is closer to the rest of the filament. To generate this model the authors performed a rigid body rotation of a RecA subunit in the crystal structure and manually docked the subunit volume into the EM reconstruction while trying to match the shape of the crystal subunit to that in the reconstruction. The resulting model shows the general orientation of the subunit in the active filament and also predicts that a number of residues will assume positions in the active filament very different from what is seen in the crystal structure. For example, in the crystal structure Lys216, Phe217 and Arg222 make specific cross-subunit contacts as described above. However, in this new model these residues make contact with ATP in the neighboring subunit, and the authors propose that these interactions play direct roles in transmitting allosteric information across the oligomeric interface (Figure 12). While this is an intriguing possibility, the rigid body rotation used to generate this model cannot explain the atomic details of interactions at the subunit interface. As stated by the authors, this is due to the fact the rigid body rotation cannot account for conformational differences that undoubtedly exist between the subunit seen in the crystal structure and that in the active filament. Despite the lack of resolution at the atomic level, this new model sets the stage for further investigation of how NTP-induced movements of the C-terminal domain are linked to the overall allosteric mechanism of RecA.

In summary, the activation of RecA involves a complex allosteric transformation of individual subunits into an ordered, helical filament. The data currently available suggest that virtually all regions of the protein structure participate in this conversion. Binding of ATP sets off a cascade of conformational changes mediated primarily by Gln194, the allosteric switch. This appears to induce a coil to β-sheet structural transition in L2 which serves both to activate this region for DNA binding and to propagate the allosteric transition to the subunit interface near the region defined by the RecA signature sequence, residues 214–222. Changes in L2 may be detected by L1, perhaps via signaling through the "connector" region immediately C-terminal of the Walker B motif. This may contribute to the functional coupling of these two DNA binding domains. The arrival of ATP may also be communicated to other parts of the structure via the MAW motif in which there are residues that make direct connection from the ATP binding site to the subunit interface, *e.g.*, Glu63-Arg222. Now NTP-induced changes in the C-terminal region must be taken into account as well. Keeping in mind that this process activates RecA for two mechanistically unrelated functions—strand exchange and coprotease activity (see below)—much remains to be learned about the molecular events that coordinate these allosteric changes.

INTERACTIONS OF RECA WITH OTHER PROTEINS INVOLVED IN HOMOLOGOUS RECOMBINATION AND DAMAGE-INDUCIBLE DNA REPAIR

In addition to its inherent catalytic abilities, RecA function is dependent on its interactions with

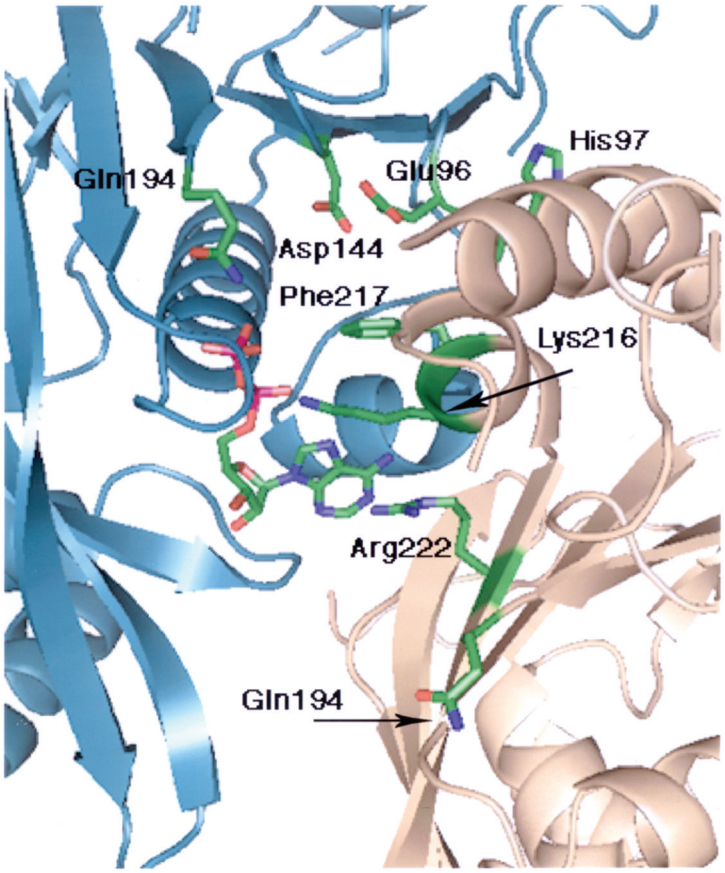

FIGURE 12. Alternate model of subunit interface and ATP-mediated transfer of allosteric information. As described by VanLoock *et al.* (2003), F217 and K216 of subunit 1 are proposed to interact with ATP in the neighboring subunit (1NO3). Coloring of side chains is same as Figure 10. See text for details. (Figure appears in color online at www.crbmb.com)

other proteins involved either directly in DNA repair or in the regulation of expression of DNA repair genes. Continued efforts have further defined domains in the RecA structure that participate in these interactions, and have also revealed previously undetected interactions that had been suggested by earlier genetic and biochemical studies.

Single-Stranded DNA Binding Protein (SSB)

SSB assists RecA-catalyzed strand exchange in at least two distinct manners. First, by melting out secondary structure in ssDNA, SSB promotes RecA polymerization (Figure 1). However, because SSB can compete with RecA for binding to ssDNA (Tsang *et al.*, 1985; Kowalczykowski and Krupp, 1987; Kowalczykowski *et al.*, 1987), SSB can inhibit RecA-mediated strand exchange if allowed to preincubate with ssDNA prior to the addition of RecA in *in vitro* reactions (Cox and Lehman, 1982). Secondly, SSB promotes strand exchange by binding the displaced ssDNA strand in the later stages of the reaction (Lavery and Kowalczykowski, 1992a). There is currently no direct evidence for protein-protein interactions between RecA and SSB, although Roca and Cox (1997) suggest that weak interactions between the two, especially toward the later stages of strand exchange, would provide increases in local concentrations of SSB, allowing it to bind the displaced strand and prevent the initiation of inappropriate secondary strand exchange events more easily. However, no such interaction has been established biochemically, and there are no *recA* mutants whose phenotype suggests such an interaction.

RecBCD Interacts Directly with RecA

The RecBCD enzyme is a highly processive helicase and nuclease that is essential for the initiation

of RecA-mediated repair of DSBs. Its nuclease function undergoes specific alterations when it encounters the sequence-specific recombination hotspot chi, thereby generating 3′-terminal ssDNA, which is the preferred substrate used by RecA, to initiate strand exchange (reviewed in Kowalczykowski, 2000). Work from the Kowalczykowski group has shown that in addition to its helicase and nuclease functions, RecBCD also facilitates loading of RecA onto the ssDNA that it produces. Their studies using mutant RecB proteins with single substitutions in the C-terminal region (RecB Asp1080Ala; *recB2109*-Thr807Ile) suggested that this part of the RecB protein may be involved in direct contacts with RecA (Anderson *et al.*, 1999; Arnold and Kowalczykowski, 2000). In fact, new work by Spies and Kowalczykowski (Spies *et al.*, 2003) demonstrates a direct interaction between this region of the RecB protein and RecA. The authors speculate that the RecA domain involved in this interaction is likely to be within the core region of the RecA structure. Currently no known *recA* mutants have been characterized regarding potential defects specific to RecA-RecBCD interactions.

RecF, RecO, and RecR Proteins

Early genetic studies revealed that the *recF*, *recO*, and *recR* gene products play subtle but significant roles in repair of DNA damage, that their activity is linked to RecA-mediated recombination, and that they are likely to function as a distinct complex of proteins (reviewed in Roca and Cox, 1997; Kowalczykowski, 2000). The current view is that the RecF, RecO, and RecR proteins play important roles in RecA-mediated repair of gapped DNA, lesions that most likely result when replication complexes stall at a DNA lesion (Webb *et al.*, 1997; Cox *et al.*, 2000; Kowalczykowski, 2000; Morimatsu and Kowalczykowski, 2003). Biochemical evidence suggested that RecFOR acts at an early step in the recombination pathway, and that its primary function is to relieve SSB-mediated inhibition of RecA through direct interactions between RecO and SSB. This allows more efficient clearing of SSB by cooperative polymerization of the RecA filament (Umezu *et al.*, 1993; Umezu and Kolodner, 1994). Although no direct interaction was observed between RecA and RecFOR in this work, recent studies suggest this possibility. Work in the Cox group has more clearly established the idea that RecFR and RecOR act as "alternative protein pairings with potentially complementary . . . functions" (Webb *et al.*, 1997). The RecOR pair is found to be particularly efficient in assisting RecA loading at a 5′-end of ssDNA, and the RecR protein remains associated with RecA filaments (Bork *et al.*, 2001). Using DNA substrates that mimic gapped dsDNA, the RecFR pair was found to prevent extension of the RecA filament from the single-stranded region into the double-stranded regions (Webb *et al.*, 1997). The data suggest that competitive association of RecR with either RecO or RecF plays an important role in regulating RecA filament assembly and disassembly, and the authors go on to describe the potential importance of these interactions for reinitiation of replication following fork collapse. Additional recent data from Morimatsu and Kowalczykowski (2003) support the idea that the RecFOR complex is a structure-specific mediator of RecA that targets recombinational repair to the ssDNA-dsDNA junctions in gapped DNA. The importance of RecF and RecR in replication restart had been demonstrated by Courcelle *et al*. (1997), and new work by Xu and Marians (2002, 2003) supports the idea that the RecF, RecO, and RecR proteins function by regulating RecA filament clearance from recombination intermediates so that they may be used to reinitiate replication. However, despite the apparent stable interaction of RecR with RecA filaments (Bork *et al.*, 2001) direct protein-protein interactions have not yet been demonstrated, and no *recA* mutants have been characterized that suggest this interaction. Several *recA* mutants have been shown to suppress the UV-sensitive phenotype of *recF* strains, *e.g.*, *recA441*-Glu38Lys/Ile298Val, *recA730*-Glu38Lys, *recA803*-Val37Met, *recA2020*-Thr121Ile and *recA801*-Gln257Pro (Thoms and Wackernagel, 1988; Wang *et al.*, 1993), but biochemical studies suggest that this results from increased cooperative polymerization of the RecA protein itself (Madiraju *et al.*, 1988) and/or an increased ability to displace SSB from ssDNA (Lavery and Kowalczykowski, 1992b).

Coprotease Activity

In addition to its role as a strand exchange enzyme, RecA catalyzes a mechanistically unrelated function termed coprotease activity. In this capacity it potentiates the autoproteolytic inactivation of several repressor proteins, *e.g.*, LexA, phage λ cI, and phage ϕ80 repressors (Little and Mount, 1982), as well as the activation of the UmuD protein (Sutton *et al.*, 2000; also see below). The LexA protein represses transcription of genes belonging to a DNA

damage-inducible family referred to as the SOS regulon. Ideas regarding an inducible DNA system in bacteria were advanced in the mid 1970s (reviewed in Radman, 1974, 1975), and current genomics efforts reveal that at least 48 genes are upregulated in a LexA/RecA-dependent manner in response to DNA damage (Courcelle *et al.*, 2001; Khil and Camerini-Otero, 2002).

RecA requires both a nucleotide and nucleic acid cofactor for this activity (Craig and Roberts, 1980). Although it appears that the nucleoprotein filament form of RecA is required for this activity, the oligomeric requirements have not yet been defined (see below). Initially it was thought that RecA had the catalytic ability to serve as a protease, but with the discovery by Little (1984) that the LexA and λ repressors undergo a specific autodigestion reaction, the model shifted to one in which RecA serves to bind the substrate proteins in such a way that they became competent for autocleavage (Little, 1993; Luo *et al.*, 2001).

The identity of a defined region within the RecA structure that serves as the binding site for coprotease substrates remains elusive. However, electron microscopic studies and several mutational analyses have revealed areas of the structure, and specific residues within these areas, that serve as likely docking sites. Before the first RecA structure was solved, genetic studies had identified two mutants that showed specificity for certain coprotease substrates. *RecA1734* (Arg243Leu) is active for cleavage of both the LexA and λ cI repressors but inhibited for cleavage of UmuD and the ϕ80 repressor (Dutreix *et al.*, 1989). Subsequently an Arg243His mutant was isolated (*recA433/435*) that showed a similar specificity (Ennis *et al.*, 1995). Additionally, *recA91* (Gly229Ser) permits cleavage of λ cI but not the ϕ80 repressor (Ogawa and Ogawa, 1986). Both Gly229 and Arg243 are positioned within a "notch" between adjacent subunits in the RecA filament, and Story *et al.* (1992) suggested that the coprotease substrate binding site lies in this region (Figure 13).

Genetic studies designed to pick up *recA* mutants with altered coprotease function were carried out by the Tessman group (Tessman and Peterson, 1985a, 1985b), and DNA sequence analysis of the constitutive alleles (coprt^c) revealed that mutations were clustered in 3 regions of protein sequence (Wang and Tessman, 1986). Further genetic and biochemical analysis of several of these mutants gave rise to the idea that coprt^c activity could result from structural changes in RecA that allowed more promiscuous use of different NTPs and nucleic acids as cofactors (Wang *et al.*, 1988a, 1988b). For example, whereas wild-type RecA cannot use NTPs other than ATP, dATP, ATPγS, and UTP effectively as cofactors for LexA cleavage, the *recA1202* (Gln184Lys) and *recA1211* (Glu38Lys) mutant proteins can also use CTP, dCTP, GTP, and dGTP, as well as rRNA, tRNA, and dsDNA, as nucleic acid cofactors more effectively than wild-type RecA.

In support of the idea that relaxed NTP and nucleic acid specificity can correlate with a coprt^c phenotype, Konola *et al.* (1995) found that the Pro67Trp protein uses all NTPs, dNTPs, and ddNTPs very efficiently for *in vitro* LexA cleavage, and relative to wild-type RecA can use shorter length oligonucleotides as cofactors for cleavage of the λ cI protein. Further analysis of different substitutions at Pro67 provided striking evidence that this residue may be involved in making specific contacts with coprotease substrates. It was found that Asp and Glu substitutions at Pro67 resulted in coprt^c activity, whereas Lys and Arg substitutions blocked coprt activity (Konola *et al.*, 1995). Later studies determined that while the Asp and Glu substitutions showed high levels of coprt^c activity that was further induced by DNA damage, these two mutants were inhibited for UmuD cleavage activity in both the absence and presence of DNA damage (Konola *et al.*, 1998). In contrast, the Pro67Arg mutant prevents LexA cleavage while allowing nearly 50% of the wild-type level of DNA damage-induced cleavage of UmuD. These results are consistent with the idea that Pro67 is located in a region of the RecA structure that makes direct contact with LexA and UmuD.

Electron microscopic analyses of RecA/DNA/LexA complexes revealed that LexA has two strong points of contact with the RecA protein filament (Yu and Egelman, 1993). One is within the helical filament groove (site A in Yu and Egelman, 1993), in support of the idea proposed by Story *et al.* (1992). The second area includes the L1 region (residues 157–165; site B in Yu and Egelman, 1993). Subsequent biochemical evidence supported the idea that L1 serves not only as part of the secondary DNA binding site, but as part of the coprotease substrate binding site as well. Studies from the Kowalczykowski group showed that the recombination and coprotease functions of RecA are mutually exclusive (Harmon *et al.*, 1996), and that LexA cleavage is competitive with binding of the secondary DNA (Rehrauer and Kowalczykowski, 1996). These results are entirely consistent with earlier studies showing that RecA-mediated cleavage of

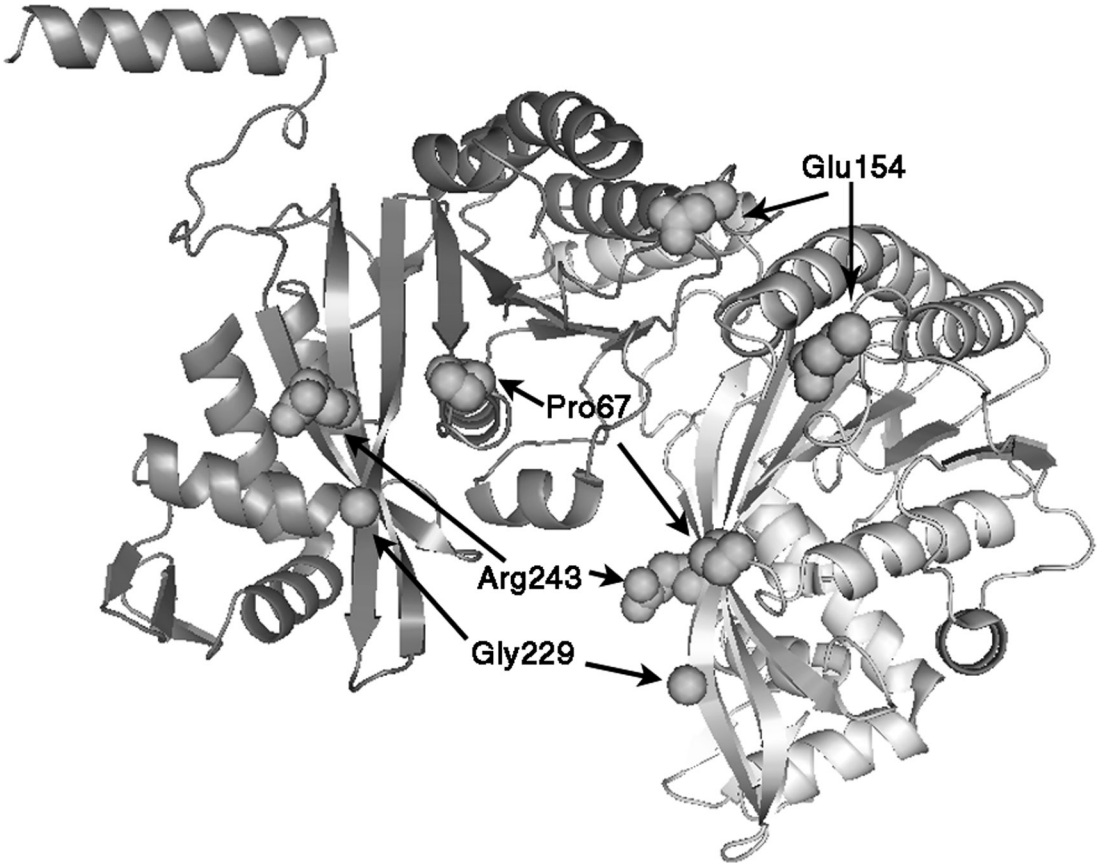

FIGURE 13. Model of dimeric RecA showing residues of potential sites of contact with coprotease substrates. The side chains of these residues all lie on the inside of the RecA lament and can be accommodated within a continuous surface that runs the length of the lament. The main chain of subunit 1 is colored beige and subunit 2 is teal. Residues 67, 154, 229, and 243 in each subunit are shown in green. This image was created using 2REB in Story and Steitz (1992). (Figure appears in color online at www.crbmb.com)

the λ cI or LexA proteins is inhibited by either excess ssDNA or dsDNA in a concentration-dependent manner (Weinstock and McEntee, 1981; Takahashi *et al.*, 1986; DiCapua *et al.*, 1992). A large number of mutations in and around the L1 region have been analyzed for specific effects on coprotease activity, as well as differential effects on LexA *versus* UmuD cleavage activity (Nastri and Knight, 1994; Nastri *et al.*, 1997). In two separate studies over 200 mutants were analyzed, and of the 67 single mutants greater than 50% were constitutively activated for cleavage of either LexA, UmuD, or both. Gly157 was identified as a hotspot for mutation-induced coprt^c activity as 10 of 14 and 12 of 14 single mutants gave rise to coprt^c activity toward LexA and UmuD, respectively. Two mutants in particular showed a clear preference for cleavage of UmuD, *recAN85* (Glu154Asp) and *recAN121* (Glu154Gln). Additionally, a significant number of mutants arose having one or more secondary substitutions that suppressed the coprt^c activity toward either LexA or UmuD seen in the primary mutant. Among these, three second-site mutants were found that completely suppress coprt^c activity toward LexA but had little or no effect on the coprt^c activity toward UmuD (see *recAN52*, *recAN113*, and *recAN118* in Table 1; Nastri *et al.*, 1997). The fact that a high frequency of second-site suppressor mutations arose within a small cluster of positions supports the idea that these residues interact as a functional domain and, together with the identification of mutants showing differential effects on LexA *versus* UmuD, that this domain is an important part of the coprotease substrate binding site.

To further define sites of interaction between RecA and coprotease substrates, Mustard and Little

(2000) carried out a targeted mutagenesis study in which Ala substitutions were placed at two positions close together in the RecA sequence. Of the 20 double Ala mutants analyzed, two were constitutively activated for cleavage of λ cI, but not LexA or UmuD (*recA* K256Q257, *recA* E259Q261). The RecA structures show that Lys256, Gln257, Glu259, and Gln261 are positioned within the "notch" region between subunits, similar to Gly229 and Arg243.

While it is assumed that coprotease activity requires formation of a RecA/DNA/ATP nucleoprotein filament similar to that needed for catalysis of strand exchange, this has not been specifically demonstrated. In fact, several pieces of evidence suggest that much smaller oligomers of RecA may be active for coprotease function. For example, wild-type RecA can use polydT oligonucleotides as short a 6 bases to achieve levels of LexA cleavage close to those seen with much longer lengths of ssDNA typically used in coprotease assays (Konola *et al.*, 1995). A 6 base oligonucleotide can maximally accommodate only two RecA subunits, although it was not determined in this study if these small RecA/oligonucleotide complexes can stack to form filament-like oligomers. Additionally, mutation of residues at the RecA subunit interface gives rise to a large number coprt^c mutants (M. C. Skiba and K. L. Knight, unpublished; see Table 1). For example, each of 16 substitutions at Lys216 has been shown to disrupt filament assembly by RecA in the absence of DNA and ATP, and 11 of these mutants are coprt^c. Of 12 substitutions at Arg222 that disrupt filament assembly, 9 are coprt^c. Detailed *in vitro* coprotease assays and binding studies have not been done with these mutants, and each mutant may be able to form stable nucleoprotein filaments that carry out coprotease function, but this information leaves open the possibility that a RecA nucleoprotein filament need not be the required oligomeric state for this activity. This is especially interesting given recent results regarding the oligomeric requirements for RecA in translesion bypass assays, where it appears that monomeric RecA may be all that is needed for this activity (see section below on RecA/pol V).

Clearly, there are several mechanisms by which RecA mutations result in coprt^c activity. In addition to the possibility of selective increases in binding affinity for specific substrates, other mechanisms include relaxed specificity for NTP and nucleic cofactors, which may result in the use *in vivo* of cofactors not accessible to wild-type RecA. Story *et al.* (1992) also proposed a model in which mutants that disrupt an aggregated storage form of RecA may give rise to coprt^c activity, but there is currently no evidence that such an inactive storage form exists. Several studies from the Kowalczykowski group support the idea that coprt^c activity is due to mutation-induced increases in the rate of association with ssDNA, which correlates with an increased ability to displace SSB from ssDNA. This has been shown for RecA441, RecA730, RecA803 (Lavery and Kowalczykowski, 1990, 1992b; Madiraju *et al.*, 1988, 1992), and recently for the Pro67Trp mutant (Mirshad and Kowalczykowski, 2003b). For these mutants one would anticipate that an increased ability to displace SSB from ssDNA would result in coprt^c toward all substrates. However, the fact that certain mutations at Pro67 show a clear selectivity for either LexA or UmuD is more suggestive of a selective interaction between RecA and the coprotease substrate. Mirshad and Kowalczykowski (2003b) also propose that coprt^c activity in mutants with significant recombination deficiencies may result simply from the accumulation of ssDNA that persists due to the inability of the mutant RecA protein to repair naturally occurring DNA damage. Their study of *recAN99* (Glu156Leu/Gly157Val) supports this claim.

Taking all published information into account, it appears that the coprotease substrate binding site is a continuous surface formed by the L1 region and the inside of the RecA filament that extends from the position of the Walker A motif to a region between subunits in which Gly229 and Arg243 are positioned (Figure 13). This surface can easily accommodate binding of a protein the size of LexA, UmuD, and other repressor substrates. Given the flexibility of the L1 region, binding to a repressor substrate may pull this region further toward the filament core than is apparent in current structures.

RecA/polV

The UmuD$'_2$C complex (pol V) is one of several known error-prone polymerases involved in restoring genomic structural integrity following DNA damage (reviewed in Goodman, 2002). The price for using low fidelity polymerases is the introduction of numerous mutations, and in bacteria this process was termed SOS mutagenesis or UV mutagenesis (Witkin, 1976; Walker, 1984). RecA's role in UV mutagenesis involves its coprotease activity for both the induction of the SOS gene family, to

which *umuC* and *umuD* belong, and for processing of UmuD to the form active in UV mutagenesis, UmuD′. But genetic evidence had suggested that RecA also collaborates in a direct way with the UmuC and UmuD′ proteins to promote UV mutagenesis, and this idea was further supported with the discovery of *recA* mutants that were active for recombination and coprotease functions but were deficient for UV mutagenesis: *recA1730* (Ser117Phe) in Dutreix *et al*. (1989), and *recA430* (Gly204Ser) and *recA727* (Glu18Lys/Gly204Ser) in Sweasy *et al*. (1990). A direct physical interaction between RecA and the UmuCD complex was also demonstrated *in vitro* (Freitag and McEntee, 1989; Frank *et al.*, 1993).

Elevated intracellular levels of the $UmuD'_2C$ complex had been shown to inhibit RecA-mediated activities *in vivo*, and Sommer *et al*. (1998) exploited this to isolate *recA* mutants that were resistant to $UmuD'_2C$-dependent inhibition, referring to these as UmuR mutants. Six different point mutants were isolated, with five of the substitutions occurring at positions within the subunit interface (Asp112Gly, Asn113Lys, Leu114Val, Ser117F, and Val247Met; see Figure 14). These mutants also had reduced levels of UV mutability. The idea that these mutations disrupted specific RecA/ $UmuD'_2C$ interaction supported an earlier hypothesis that $UmuD'_2C$ is positioned at the site of DNA damage by interacting with the end of a RecA filament (Sommer *et al.*, 1993, Boudsocq *et al.*, 1997). It is interesting to note that residues 112, 113, 114, and 117 all reside on the surface of the subunit interface that would be at the 3′ end of DNA, the end where pol V would act (Figure 14). Recently, Frank *et al*. (2000) provided direct evidence for this model by showing that $UmuD'_2C$ prefers to bind to the ends of RecA filaments. Thus, it now appears that specific protein-protein interactions between RecA and $UmuD'_2C$ serve to target pol V to the site of DNA damage.

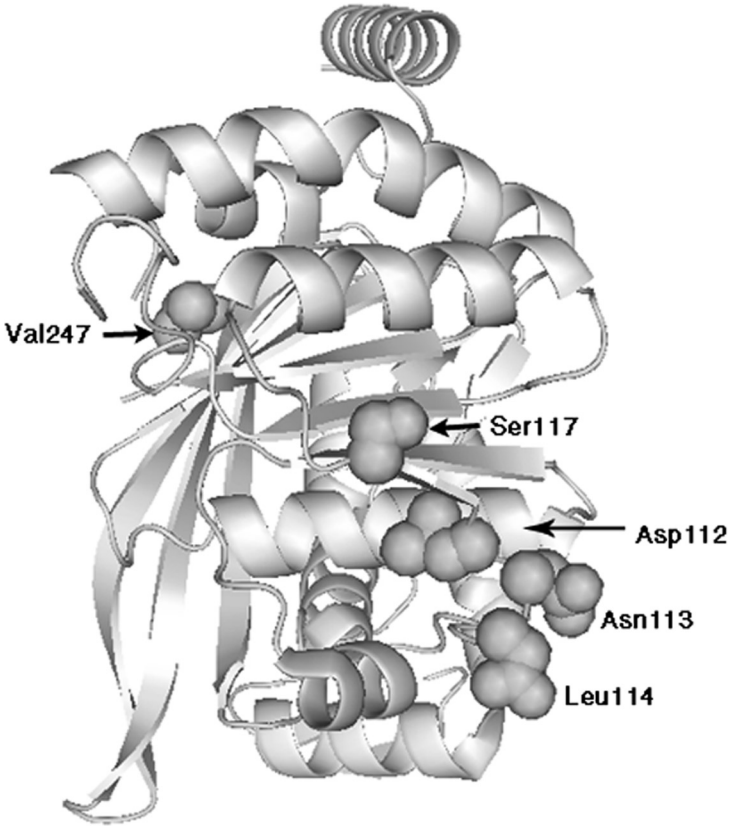

FIGURE 14. Potential sites of interaction between RecA and $UmuD'_2$ C (pol V). Mutations at each of these positions were selected as UmuR mutants. Four of the v e residues shown (Asp112, Asn113, Leu114, and Ser117) are on the surface of the subunit interface that would be at the 3′ end of bound DNA (Story *et al.*, 1992). This image was created using 2REB in Story *et al.* (1992). (Figure appears in color online at www.crbmb.com)

Recent findings by Pham *et al.* (2002) suggest that while a RecA filament may serve to initially target $UmuD'_2C$ to the site of damage *in vivo*, a nonfilamentous form of RecA is perfectly capable of promoting DNA replication across a damaged template. In fact, this study indicated that monomeric RecA is able to carry out this function. Thus, a productive protein-protein interaction appears not to require an oligomeric form of RecA and again raises the possibility that other activities of RecA, *e.g.*, coprotease, may not necessarily require an extended filament of RecA (see the "Coprotease Activity" section above).

RecX and DinI

The *recX* and *dinI* genes are part of the SOS regulon, and the proteins appear to be involved in attenuation of the SOS response following DNA repair by direct inhibition of RecA function. First described in *Pseudomonas aeruginosa* (Sano, 1993), the *recX* gene lies downstream of *recA* in *E. coli* and its expression is SOS regulated (Pages *et al.*, 2003). Studies in *P. aeruginosa*, *Mycobacterium smegmatis, Streptomyces lividans*, and *Xanthomonas oryzae* revealed that RecX is required to prevent deleterious effects caused by DNA damage-induced overexpression of *recA* (Sano, 1993; Papavinasasundaram *et al.*, 1998; Vierling *et al.*, 2000; Sukchawalit *et al.*, 2001), although in an *E. coli recX* deletion strain RecA overexpression is not harmful (Pages *et al.*, 2003) and in *Neisseria gonorrhoeae* RecX appears to be required for normal RecA function (Stohl and Seifert, 2001). RecX from both *Mycobacterium tuberculosis* and *E. coli* binds directly to RecA and inhibits its ATPase and strand exchange activities (Venkatesh *et al.*, 2002; Stohl *et al.*, 2003) as well as coprotease function (Stohl *et al.*, 2003). Recent structural studies using three-dimensional reconstruction of electron micrograph images now reveal that *E. coli* RecX binds to the inactive form of the RecA filament as opposed to LexA, which binds to the active form of RecA (VanLoock *et al.*, 2003a). This result is entirely consistent with genetic and biochemical results, showing that RecX serves to inhibit RecA activity.

The *E. coli dinI* gene was discovered by Yasuda *et al*. (1996) as a suppressor of a cold-sensitive mutant in *dinD*, one of several DNA damage-inducible genes isolated by Kenyon and Walker (1980). Overexpression of DinI blocks RecA-mediated cleavage of both LexA and UmuD (Yasuda *et al.*, 1998). Voloshin *et al*. (2001) have shown that DinI binds directly to the L2 region of RecA. RecA mutants carrying substitutions within L2, R196M, or K198M and do not bind DinI. Also, mutant DinI proteins carrying Ala substitutions at negatively charged residues in the C-terminal α-helix are inhibited for binding to wild-type RecA. Based on these biochemical data and the NMR structure of DinI (Ramirez *et al.*, 2000), a model was proposed in which the negatively charged side chains in this C-terminal α-helix mimic the negatively charged backbone of ssDNA and compete with DNA for binding to the L2 region of RecA (Ramirez *et al.*, 2000; Voloshin *et al.*, 2001). Further studies show that binding of DinI to RecA is enhanced upon RecA filament formation and suggest that DinI blocks RecA coprotease activity by competing with cleavable repressor substrates for binding to the deep helical groove in RecA filaments (Yoshimasu *et al.*, 2003).

CONCLUSION

The bacterial RecA protein functions in a number of mechanistically distinct processes, all of which contribute to the maintenance of genomic integrity. RecA is a classical allosterically regulated enzyme, in that ATP binding induces conformational changes that appear to propagate to virtually all regions of the protein structure. These structural changes are clearly inter-related and result in high affinity DNA binding, increased cooperative filament assembly, communication between primary and secondary DNA binding sites, and an increased association with other proteins such as coprotease substrates and the pol V complex. We now have a significant understanding of the molecular design of the protein and how various structural and functional domains coordinate their efforts to carry out these diverse activities. And all this has occurred in the absence of a detailed structure of the active form of the protein. However, there are still many questions that remain unanswered. For example, the structure of the DNA binding domains and the residues within them that make specific contact with DNA still remain largely undefined. Also, it appears now that the primary and secondary DNA binding sites are functionally and perhaps physically linked, and that the C-terminal domain also plays an important role in regulating the interaction between the RecA filament and the DNAs undergoing strand exchange. The possibility of such an intricate functional association is intriguing, and the molecular details remain to be worked out.

With the identification of Dmc1 and Rad51 as RecA homologs in yeast (Bishop *et al.*, 1992; Shinohara *et al.*, 1992; Story *et al.*, 1993), and the striking similarity of the filament structures formed by Rad51 and RecA (Story *et al.*, 1993; Ogawa *et al.*, 1993), the stage was set for more detailed investigations into homologous recombination and recombinational DNA repair in higher organisms. Work since that discovery has shown that there are RecA-like proteins in all free-living organisms and, while the overall process of Rad51/RecA recombination is similar across all species, there are significant differences in the fundamental mechanistic properties of the proteins involved as well as the regulation of their biochemical properties and catalytic abilities. Several excellent reviews of double-strand DNA break repair and homologous recombination in eukaryotes have appeared within the past three years, and the reader is referred to these and additional citations within these works (Symington, 2002; Thompson and Schild, 2002; van den Bosch *et al.*, 2002; Sonoda *et al.*, 2001; Modesti and Kanaar, 2001).

The eukaryotic proteins involved in DSB repair are members of the *RAD52* epistasis group, and it has been shown clearly that physical association among certain members is required for optimal function of the primary catalyst of DNA strand exchange, Rad51. In fact, continued examination of several well-known bacterial proteins, *e.g.*, RecA, RecFOR, and RecBCD, now provides evidence that specific protein-protein interactions play important roles in their function.

This review has focused on the molecular design of RecA and how various studies of mutant proteins have advanced our understanding of the molecular organization of this complex enzyme. Many excellent reviews have been written that provide further information on RecA, homologous recombination, and damage-inducible DNA repair in prokaryotes (Lusetti and Cox, 2002b; Goodman, 2002; Courcelle and Hanawalt, 2001; Cox, 2000; Kowalczykowski, 2000; Bianco and Weinstock, 1998; Roca and Cox, 1997; Eggleston and West, 1997; Clark, 1996).

Despite years of investigation, RecA remains a fascinating study in the use of sophisticated molecular design to accomplish a number of biologically related yet mechanistically distinct functions. It will undoubtedly continue as a paradigm for studies of allosterically regulated enzymes in general, as well as investigations into homologous recombination and recombinational DNA repair in other organisms.

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APPENDIX A

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16. (Konola *et al.*, 1994)
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18. (Skiba and Knight, 1994)
19. (Nastri and Knight, 1994)
20. (Nastri *et al.*, 1997)
21. (Bianco and Weinstock, 1998)
22. (Larminat *et al.*, 1992)

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25. (Kurumizaka *et al.*, 1996)
26. (Alexseyev *et al.*, 1996)
27. (Stole and Bryant, 1996)
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36. (Dutreix *et al.*, 1992)
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38. (Eldin *et al.*, 2000)
39. (Rehrauer and Kowalczykowski, 1993)
40. (Kelley and Knight, 1997)
41. (Ogawa and Ogawa, 1986)
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