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recA Mutations That Reduce the Constitutive Coprotease Activity of the RecA1202(Prt^c) Protein: Possible Involvement of Interfilament Association in Proteolytic and Recombination Activities

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Twenty-eight *recA* mutants, isolated after spontaneous mutagenesis generated by the combined action of RecA1202(Prt^c) and UmuDC proteins, were characterized and sequenced. The mutations are intragenic suppressors of the *recA1202* allele and were detected by the reduced coprotease activity of the gene product. Twenty distinct mutation sites were found, among which two mutations, *recA1620* (V-275→D) and *recA1631* (I-284→N), were mapped in the C-terminal portion of the interfilament contact region (IFCR) in the RecA crystal. An interaction of this region with the part of the IFCR in which the *recA1202* mutation (Q-184→K) is mapped could occur only intermolecularly. Thus, altered IFCR and the likely resulting change in interfilament association appear to be important aspects of the formation of a constitutively active RecA coprotease. This observation is consistent with the filament-bundle theory (R. M. Story, I. T. Weber, and T. A. Steitz, Nature (London) 335:318–325, 1992). Furthermore, we found that among the 20 suppressor mutations, 3 missense mutations that lead to recombination-defective (Rec$^-$) phenotypes also mapped in the IFCR, suggesting that the IFCR, with its putative function in interfilament association, is required for the recombinase activity of RecA. We propose that RecA-DNA complexes may form bundles analogous to the RecA bundles (lacking DNA) described by Story et al. and that these RecA-DNA bundles play a role in homologous recombination.

The *recA* gene product of *Escherichia coli* is a small yet versatile protein composed of 353 amino acids (41). Two major and well-studied roles of RecA are to promote homologous recombination (7) and to induce the SOS response (35, 51). In homologous recombination, RecA is required for both strand pairing and an ATP-dependent strand exchange reaction (18, 37, 40, 56). In the SOS response, RecA is activated to a coprotease state by cofactors such as single-stranded DNA (ssDNA) and ATP or dATP (8, 38, 39). This activated RecA then mediates the cleavage of the LexA repressor (22, 24) and allows the expression of SOS genes, which are those under the repression of LexA and include *lexA*, *umuDC*, and *recA* itself (1, 4, 25). The activated RecA also mediates the cleavage of UmuD into two fragments, the larger of which, the C-terminal UmuD′, is essential for the function of UmuD in SOS mutagenesis (5, 33, 43).

Although RecA is required for the cleavage of LexA, UmuD, and phage repressors (35, 51) in vivo and in vitro (under physiological conditions) and may act as a protease, the term coprotease (23) has been adopted to describe its proteolytic activity because the protein substrates of RecA can undergo autodigestion at alkaline pH in vitro (21, 44). We find it convenient, however, to retain the designation Prt (47) to describe the protease phenotype of RecA.

In wild-type *E. coli* cells, the RecA protein is not proteolytically active without inhibition of DNA replication or exogenous DNA-damaging treatments (35, 51). Some mutations in either *recA* or other genes result in the activation of the RecA protein in the absence of DNA-damaging agents. Mutations such as *dnaB*(Ts) and *dnaE*(Ts) can lead to changes in DNA metabolism and indirectly activate RecA and induce the SOS response constitutively (31, 42). This activation is likely due to an increase in the availability of ssDNA regions in the cell as a result of abnormal DNA replication (42). In addition, mutations in the *recA* gene, designated *recA*(Prt^c), can confer constitutive coprotease activity to RecA and turn on the SOS response at all times.

By using a method that involves plating mutagenized λ*recA*$^+$ (a λ phage carrying the *recA*$^+$ gene) on indicator strains with *recA* deleted and containing Mu d(Ap *lac*) fusions in SOS genes (*dinD* and *sulA*), Tessman and Peterson isolated several classes of novel *recA*(Prt^c) mutants (47), some of which are recombinase negative and are designated *recA*(Prt^c Rec$^-$) (48). Unlike mutants carrying the classical *recA441* (*tif-1*) allele, which confers the Prt^c phenotype only at high temperature (6, 14), these newly isolated *recA*(Prt^c) mutants display constitutive coprotease activity at any growth temperature, with some having considerably greater coprotease activity than *recA441* strains (47). Among these, *recA1202* cells showed the strongest coprotease activity (47).

Further studies on two of these *recA*(Prt^c) mutants with strong RecA coprotease activity, the *recA1202* and *recA1211* mutants, demonstrated that the strong RecA(Prt^c) phenotype for these strains is likely due to two factors: (i) the RecA1202 and RecA1211 proteins can use any one of the other natural nucleoside triphosphates besides ATP or dATP as a cofactor in activating the cleavage of LexA (52), and (ii) they can use tRNA or rRNA besides ssDNA as a cofactor in the cleavage reaction (55). These novel biochemical properties of RecA-(Prt^c) proteins provide an explanation for a mutagenic phe-

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nomenon observed with *recA1202*(Prt^c) cells; this phenomenon is termed proximal mutagenesis because the *recA1202* gene and nearby regions are preferentially mutated (26).

The proximal mutagenic activity was used in the present study to isolate mutations that reduce the constitutive coprotease activity of the *recA1202* allele. We characterized 28 such mutants. Each carried an additional *recA* mutation that can be viewed as an intragenic suppressor of the *recA1202* constitutive coprotease activity. These new double *recA* mutants, carried by λ phages, have been characterized for both recombination and coprotease phenotypes in a strain with its chromosomal *recA* gene deleted.

Story et al. (46) have solved the molecular structure of the RecA protein by X-ray crystallography to a 2.3-Å (0.23-nm) resolution. The crystallized RecA protein can be divided into three domains: a large central domain and two smaller flanking domains at the amino and carboxyl termini (from residue 1 to about residue 30 and from about residue 270 to residue 328, respectively), both of which protrude from the central domain (Fig. 1a). There are two types of interactions among RecA molecules in the crystal. First, monomers pack together to form a filament coil, with six monomers per turn of the coil. The coil of the filament is relatively open, with intermolecular associations only between adjacent monomers in the filament polymer. In addition, there are interfilament associations between monomers which allow filaments to form a filament bundle in the crystal. Thus, each monomer interacts with four other monomers, two within the filament and two from another filament. Each intrafilament (intermolecular) contact is extensive, involving at least 54 amino acids; in contrast, the interfilament contact is less extensive, involving only about 20 amino acids (46).

While Story et al. (46) pointed out that these interfilament contacts may be an artifact of crystallization, they suggested that they may be biologically relevant because mutations in and around these residues have major effects upon RecA functions. In particular they noted that many of the mutations that lead to constitutive coprotease activity are located in the interfilament contact region (IFCR). On the basis of this observation, they proposed that the formation of a RecA filament, from the dissociation of bundles of RecA polymer (a storage form lacking DNA), is an important step in forming an active coprotease. Thus, mutations mapped in the IFCR, including *recA1202* (Q-184→K), could reduce the interfilament contact and shift the equilibrium toward the formation of active RecA filaments, which in turn results in the generation of a constitutively active coprotease (46). The existence of RecA bundles (with or without DNA) has been documented by in vitro studies (3, 10, 11, 57).

To further understand the structure-function relationship of RecA, we integrated into the crystal structure of RecA the phenotypic and sequencing data for 28 suppressor mutants that we isolated. Although the crystal structure may not reflect the exact RecA conformation in vivo, the 2.3-Å structure (46) can serve as a model upon which the analysis of newly obtained data can be based. Our analysis of some of the 28 suppressor mutations provides additional evidence supporting the theory proposed by Story et al. (46). Furthermore, our extended analysis of the locations of Rec$^-$ mutations indicates that interfilament association may also play an important role in recombination. We propose a theory involving RecA-DNA multifilament bundles to explain why the IFCR is involved in recombination and how single *recA* mutations might result in Prt^c Rec$^-$ phenotypes.

MATERIALS AND METHODS

Bacterial strains and media. The host bacterial strain for λ*recA* mutant phages used in this work was *E. coli* K-12 strain EST2411 (Δ*recA306 sulA11 dinD1*::Mu d(Ap *lac*) *supE44* S13^s) (27), which is a derivative of AB1157. The *recA1202* control strain was IT1993 (EST2411/λ*recA1202* c**I** *ind*). The *recA*$^+$ strain was EST2422 (λ*recA*$^+$ c**I** *ind*) (27). The media, M9-CAA (a Casamino-Acids-supplemented M9), LB broth, and SFLB (a salt-free plating agar based on LB broth), have been previously described (26, 27). The antibiotics used were kanamycin (30 μg/ml) and rifampin (25 μg/ml). 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) was used at 60 μg/ml, and mitomycin (MitC) was used at 0.5 μg/ml.

Isolation and characterization of the suppressor mutants of the *recA1202* allele. $CaCl_2$-treated IT3111 (EST2411/λ*recA1202* c**I**857 *ind*) cells were mixed with DNA from the high-copy-number plasmid pSE117 (*umuD*$^+$*C*$^+$ Kan^r) (12), heat shocked at 42°C for 2 min, diluted sixfold with LB broth, and incubated at 30°C for 1 h before being spread on plates containing M9-CAA plus kanamycin and X-Gal. Strains with unmutated *recA1202* alleles produce dark blue (DB) colonies on X-Gal plates because the high coprotease activity derepresses the *dinD* gene and the fused *lacZ* gene (47). However, in combination with the pSE117 plasmid, the *recA1202* gene exhibits a very high frequency of mutation in the *recA* gene, which results in decreased RecA coprotease activity and pale blue (PB) colonies (26). Thus, after incubation at 32°C for 24 h, there were many transformants with stable PB or blue (B) colors that could be picked and purified. From these PB or B mutants, λ*recA* mutant phages were heat induced and then used to lysogenize EST2411. All phenotypic characterizations refer to these lysogenized strains. The temperature for all phenotypic characterizations was 32°C.

Sensitivity to UV was determined by spotting 10 μl of cells grown overnight in M9-CAA medium onto the surface of M9-CAA plates, which were then UV irradiated with a 15-W germicidal lamp with fluxes of 0, 20, 47, 75, and 103 J/m^2. Strains that were completely inactivated by 20 J/m^2 were designated S for sensitive, strains resistant to 103 J/m^2 were designated R for resistant, and strains inactivated by 47 or 75 J/m^2 were designated R/S. Determinations of the fraction of lethal lesions repaired (repair sector, *W*) by Weigle reactivation of UV-irradiated S13 and of the Rif^r frequencies were as described previously (26, 27).

Phage λ DNA purification. To induce the phage, lysogens of λ were grown in LB plus 0.01 M $MgCl_2$ to mid-log phase, heat induced at 45°C for 15 min, and incubated for another 2 h at 38°C. Chloroform was added to complete the lysis, and bacterial debris were removed by centrifugation. The λ DNA was extracted and purified from the lysate by a λ DNA minikit with the protocol provided by the manufacturer (Qiagen).

DNA sequence determination. The λ DNA containing the mutated *recA* gene was digested with *Eco*RI. A 1.8- and a 1.3-kb *Eco*RI fragment, which contained three-fourths (N terminal) and one-fourth (C terminal) of the *recA* gene, were purified by using low-melting-point agarose gel electrophoresis (29). The DNA fragments that contained parts of the *recA* gene were sequenced by inserting the purified fragments into M13 mp19. The orientation of the cloned *Eco*RI fragments in M13 mp19 was determined by a DNA hybridization test (13). Sequencing was performed by using a Sequenase kit (United States Biochemical). In order to sequence the entire *recA* gene without subcloning parts of it, three 19-mer synthetic DNA primers with the following sequences were used in addition to the universal primer: 5′-GCGGTGCGTCGTCAGGCTA-3′,

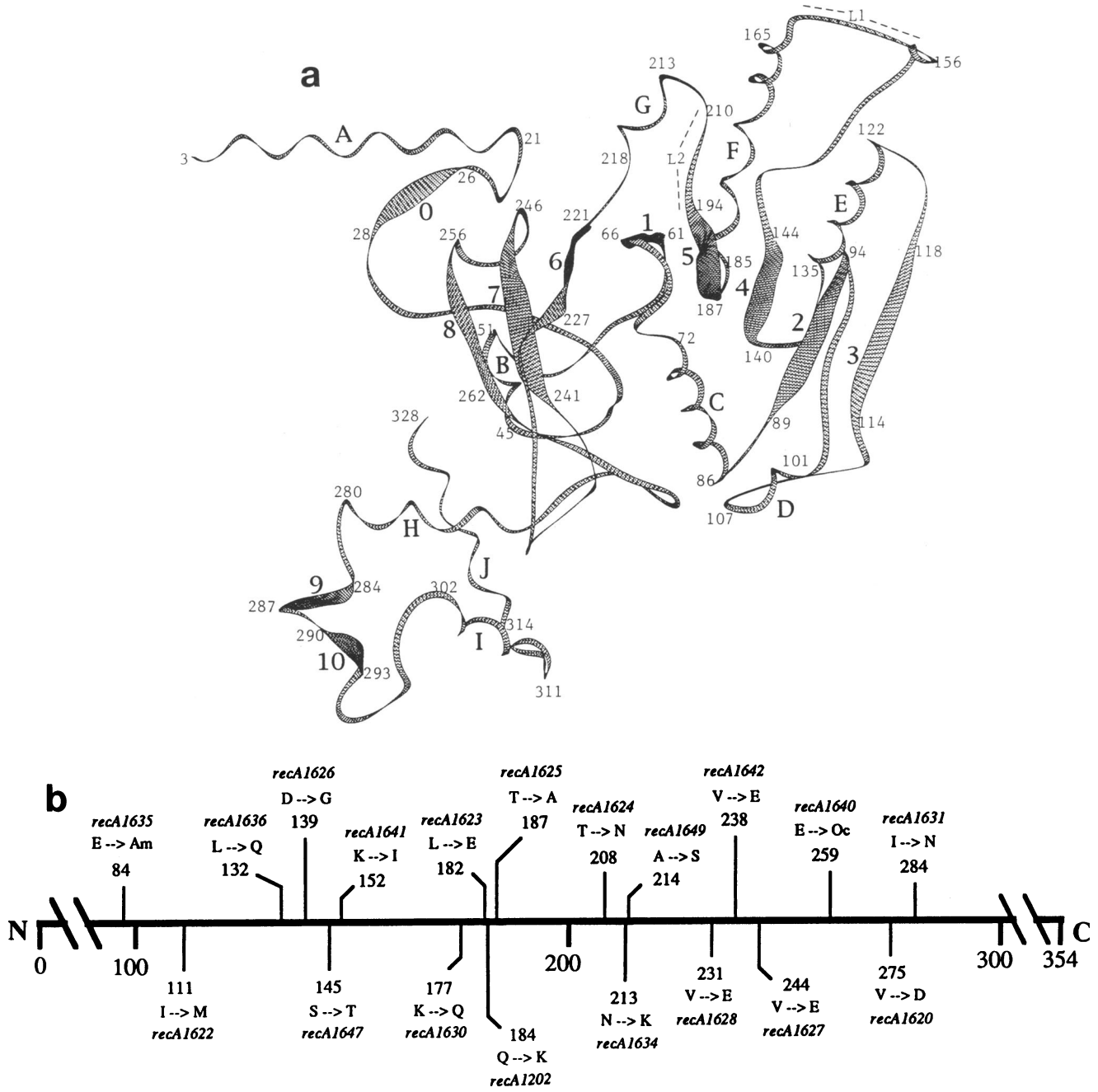

FIG. 1. RecA structure and sites of the suppressor mutations. (a) RecA monomer structure. The original coordinates (46) were obtained from the Brookhaven protein data base. The three-dimensional structure was drawn by using the computer program RIBBON (36). The labeling of secondary structure elements is according to Story et al. (46). The 10 α-helices are lettered A to J; the 11 β-strands are numbered 0 to 10. The numbers of the approximate first and last amino acid residues of each helix and strand are indicated, and the numbering is identical to that in panel b, which shows the mutational sites. The two disordered loops (L1 and L2) suggested as DNA-binding sites are labeled with dashed lines. The disordered N and C termini are not shown. (b) Map of the sites of the suppressor mutations in the RecA protein. Not shown is the *recA1643* mutation, which is a 10-bp deletion (Table 2) that alters the reading frame following codon 69 for proline.

5′-GCCGCAGCGCAGCGTGAAG-3′, and 5′-CTCCTGTCA TGCCGGGTAA-3′. The 5′ nucleotides of these primers correspond to nucleotides −98 and 293 in the nontranscribed strand and to nucleotide 44 in the transcribed strand of the *recA* gene.

Structural analysis. We downloaded the spatial coordinates of the *E. coli* RecA crystal as solved by Story et al. (46) from the Brookhaven protein data base. All subsequent structural analysis was performed with the Midas computer program on an Iris workstation. Possible effects of changes in the amino acid sequence of *E. coli* RecA on the tertiary structure were predicted by using the computer programs of Lee and Levitt (20).

Evolutionary comparisons. The amino acid sequences of the

RecA proteins from 32 species of bacteria, including *E. coli*, were downloaded from the National Center for Biotechnology Information data base via Internet. These species covered a wide evolutionary range within the eubacterial kingdom, including enterobacteria, gram-positive bacteria, bacteroides, spirochetes, mycoplasmas, cyanobacteria, and species from the α, β, and γ subgroups of the *Proteobacteria* (58). Sequences were aligned by the computer program CLUSTAL V (16) with the aid of the Genetic Data Environment computer program (kindly provided by Steve Smith, Millipore Corp.). Alignment ambiguities were limited to the C terminus (residue 315 or greater in the *E. coli* protein); the species showed a high degree of homology through the rest of the protein. Positions were scored for degree of conservation among the sequences. Completely conserved positions were those that are identical in all, or in all but one, species. Highly conserved positions were those with only conservative alterations among all species (e.g., valine, isoleucine, or leucine in all species) or among all but one. Moderately conserved positions were those that were identical or conservatively different in most species (>80%) but in which some nonconservative alterations were also present.

RESULTS

Isolation and characterization of *recA* mutants. The *recA1202* allele, in the absence of the *umuD*$^+$*C*$^+$ plasmid, produces a DB colony on X-Gal plates because it completely derepresses the SOS regulon, which includes the *dinD*::Mu d(*lac*) gene (17) in our strain. The high-copy-number *umuD*$^+$*C*$^+$ plasmid pSE117, in combination with the *recA1202* allele, causes an extraordinarily high frequency of proximal mutations in the *recA1202* gene itself (26). The basis of our isolation procedure was the fact that many of the mutations are easy to detect because they weaken the Prt^c phenotype, resulting in B or PB colonies that are easily distinguished from the DB parent colonies.

Immediately after transformation with pSE117 (*umuD*$^+$*C*$^+$ Kan^r), the cells were incubated for 1 h to allow expression of the Kan^r phenotype before being spread on M9–CAA–X-Gal–kanamycin plates. After 24 h of incubation at 32°C, Kan^r colonies with various degrees of blue color were observed. Among 660 transformed colonies, 66% were DB and 34% were B or PB. The DB colonies were similar to the plasmidless parent IT3111 in colony color except that many also contained B or PB sectors. The other B or PB colonies were presumed to have a change in color due to reduced constitutive coprotease activity. The 34% B or PB colonies looked homogeneous in colony color, suggesting that the mutations had occurred during the 1-h incubation period before plating. From these, 37 mutants were chosen to give a wide distribution of colony sizes and colors. These mutants were tested for their RecA functions by Weigle reactivation (an indication of SOS repair) of UV-irradiated phage S13, UV sensitivity, and crystal violet sensitivity (47, 48) as described in Materials and Methods. By these tests, 35 of the mutants could be distinguished from the *recA1202* control strain IT1993; it was inferred that these mutants were further mutated in their *recA1202* genes.

From the 35 potential *recA* mutants, 28 were selected by the ease with which the λ*recA* DNA could be isolated, and they were then analyzed for their DNA sequence and phenotype. The λ lysates from the other seven *recA* mutants gave consistently low titers, which may be an indication that the proximal mutagenesis phenomenon resulted in mutations in some important λ genes located near *recA1202* in the prophage. These mutants were not further characterized. The phage lysates from the 28 mutants were also used to lysogenize EST2411, in which characterization of the mutant RecA phenotype could be carried out free of the multicopy *umuDC* plasmid that might have complicated the studies. Sequencing of the DNA revealed that all 28 mutations represented 20 distinct sites within the *recA* gene.

The distinctive properties of the 20 different *recA* mutants allowed us to classify them into six groups, each containing a unique combination of Prt and Rec phenotypes (Table 1). Three tests, color on M9–CAA–X-Gal, repair sector (*W*) for the Weigle reactivation of UV-damaged S13 in unirradiated cells, and spontaneous mutation frequency to Rif^r, were used to measure the constitutive coprotease strength of RecA mutants in vivo. The correlation between the RecA coprotease activity and these three phenotypes has been established (47, 49). All 20 *recA* mutants appeared to have weaker-than-normal constitutive coprotease activity as indicated by the reduced values of *W* and the Rif^r frequencies, properties we expected from our mutant isolation strategy. Sensitivity to MitC and UV were used to estimate the recombinase activity of the mutants, since recombinase activity of RecA is a major determinant of resistance to UV and other DNA-damaging agents such as MitC (47, 54). The Rec$^-$ phenotype of all the UV- and MitC-sensitive mutants was further confirmed (data not shown) by the lack of recombination with DNA from an Hfr donor strain, as previously described (48).

In cases when constitutive coprotease activity of RecA was low, the ability of MitC to induce activity of RecA coprotease was also tested. Those mutants which were PB on M9–CAA–X-Gal and became B on plates with added MitC were then classified as Prt$^+$ if they also showed low-level constitutive Weigle reactivation ($W \leq 0.01$) and a low Rif^r frequency, both of which are characteristic of the *recA*$^+$ reference strain EST2422 (Table 1). This method could not be used to determine the inducible coprotease phenotype (Prt$^+$ or Prt$^-$) of Rec$^-$ strains because they are sensitive to MitC (designated S in Table 1). Therefore, the Prt$^\pm$ Rec$^-$ and Prt$^-$ Rec$^-$ phenotypes were determined by measuring Weigle reactivation of UV-inactivated phage S13 in cells induced with UV light at 16 J/m^2. The single mutant IT3200, classified as Prt$^\pm$, had the intermediate value of $W = 0.08$ when UV irradiated, while all the mutants classified as Prt$^-$ Rec$^-$ showed negligible values of W ($\leq$0.01). Because *W* was 0.04 for IT3170 in the absence of UV induction (Table 1), further study was unnecessary inasmuch as the Prt^c phenotype was apparent.

Distribution of the mutations in the *recA* gene. The DNA sequence changes and inferred amino acid substitutions were determined for all 28 mutants (Table 2). All mutants retained the original *recA1202* mutation, which is a Gln→Lys (GAG→AAG) change at amino acid residue 184. Thus, all alleles have double mutations in the *recA* gene, but for convenience only the second-site change is indicated. Among the 20 distinct second-site mutations, one was in the promoter region of *recA*, one was a 10-bp deletion, and two were nonsense mutations. The remaining 16 were single base pair missense mutations, which were distributed between residues 111 and 284. Of these mutations, 14 were in the region corresponding to the central domain of the RecA protein crystal, two were in the C-terminal domain, and none were in the N-terminal domain (Fig. 1). For each of the missense mutations, we analyzed the degree of evolutionary conservation of the residues shown in Table 2. The results indicate that 88% (14 of 16) of the suppressor mutations resulted in changes at either completely conserved (6 of 16) or highly conserved (8 of 16) residues of RecA (Table 2), implying that most of the

TABLE 1. Properties of the *recA* mutants

Strain	Allele[a]	Color on M9-CAA-X-Gal[b]:		Sensitivity to UV[c]	W^d (±0.01)	Mutant frequency[e] (Rif^S → Rif^r)
		Without MitC	With MitC[f]			
Prt^c Rec$^+$						
IT3174	*recA1624*	PB+	B	R	0.06	$(7.4 \pm 0.5) \times 10^{-8}$
IT3175	*recA1625*	B	DB	R	0.17	$(3.2 \pm 1.3) \times 10^{-7}$
IT3186	*recA1626* (2)	B	B	R	0.20	$(4.1 \pm 1.1) \times 10^{-7}$
IT3194	*recA1634*	PB+	B	R	0.02	$(1.9 \pm 0.7) \times 10^{-7}$
IT3167	*recA1647* (2)	PB+	B	R	0.06	$(8.2 \pm 1.1) \times 10^{-8}$
Prt^c Rec$^-$						
IT3170	*recA1620* (2)	B	S	S	0.04	$(2.5 \pm 1.2) \times 10^{-8}$
Prt$^+$ Rec$^+$						
IT3172	*recA1622*	PB	B	R	<0.01	$(3.6 \pm 0.7) \times 10^{-8}$
IT3190	*recA1641*	PB+	DB	R	<0.01	$(4.4 \pm 1.9) \times 10^{-8}$
IT3162	*recA1642* (3)	PB	B	R	0.01	$(5.2 \pm 0.8) \times 10^{-8}$
Prt$^+$ Rec$^\pm$						
IT3177	*recA1627* (2)	PB	B	R/S	<0.01	$<3.0 \times 10^{-8}$
IT3178	*recA1628*	PB	B	R/S	<0.01	$(1.6 \pm 0.4) \times 10^{-8}$
IT3180	*recA1630*	PB	B	R/S	<0.01	$(1.2 \pm 0.1) \times 10^{-8}$
IT3196	*recA1636*	PB	B	R/S	<0.01	$(2.0 \pm 0.6) \times 10^{-8}$
IT3169	*recA1649* (2)	PB+	B	R/S	<0.01	$(1.5 \pm 0.2) \times 10^{-8}$
Prt$^\pm$ Rec$^-$						
IT3200	*recA1640*	PB+	S	S	0.01	$<1.6 \times 10^{-8}$
Prt$^-$ Rec$^-$						
IT3173	*recA1623* (2)	PB+	S	S	<0.01	$<1.5 \times 10^{-8}$
IT3181	*recA1631*	PB+	S	S	<0.01	$<1.7 \times 10^{-8}$
IT3193	*recA1633*	PB	S	S	0.01	$(3.4 \pm 1.3) \times 10^{-8}$
IT3195	*recA1635*	PB	S	S	<0.01	$(1.7 \pm 0.9) \times 10^{-8}$
IT3163	*recA1643*	PB+	S	S	0.01	$<3.5 \times 10^{-8}$
Reference						
EST2411	Δ*recA306*	PB−	S	S	<0.01	NT[g]
EST2422	*recA*$^+$	PB	B	R	<0.01	$(1.1 \pm 0.4) \times 10^{-8}$
IT3111	*recA1202*	DB	DB	R	0.26	$(2.6 \pm 0.3) \times 10^{-6}$

[a] Multiple occurrences are indicated in parentheses. All mutations are missense except one ochre nonsense (*recA1640*), one amber nonsense (*recA1635*), and one deletion (*recA1643*).

[b] PB+, between PB and B; PB−, between PB and white.

[c] R, resistant; S, sensitive; R/S, intermediate sensitivity as described in Materials and Methods.

[d] The value of W is the repair sector for UV-irradiated phage S13. $W = 1 - \log S_a/\log S_b$, where S_a and S_b are the fractions of viruses surviving after and before reactivation, respectively. The viral survival after UV irradiation was between 1.5×10^{-6} and 3.9×10^{-7}. The *recA* mutant cells were not irradiated.

[e] Rif^r mutant frequencies were determined by growing cells in M9-CAA from small inocula (1,000 to 2,000 cells) to mid-log phase at 32°C. The cells were then spread on LB and LB-rifampin (25 μg/ml) plates and incubated at 32°C for 24 h. Each value is the average from three cultures ± standard error of the mean.

[f] MitC was at 0.5 μg/ml. S, sensitive to MitC and failed to grow.

[g] NT, not tested.

mutated residues play important roles in aspects of RecA structure and function.

Mutations mapped in the three-dimensional vicinity of Gln-184. Three suppressor mutations, *recA1630* (K-177→Q), *recA1623* (L-182→Q), and *recA1625* (T-187→A), alter residues that are very close to residue 184, the site of the *recA1202* mutation (Fig. 2), and they therefore may directly interact with it. In addition to compensating for the structural effect caused by *recA1202*, all three could also affect another area of the IFCR (see below). The importance of this region is also suggested by analysis of the *recA1623* allele. The suppressor mutation in this allele is a change from a polar to a nonpolar residue at a highly conserved residue that is in a nonexposed packing region immediately next to the original mutation (Fig. 2). Such a change would seem likely to destabilize the whole region and destroy its associated functions, a prediction that nicely fits with the Prt$^-$ Rec$^-$ phenotype of this allele. Of the remaining 13 suppressor mutations, only two appear to be reasonable candidates for changes that could have some direct influence on residue 184: *recA1636* (L-132→Q) and *recA1626* (D-139→G). These residues are relatively close to the original change at residue 184 in the three-dimensional structure. In particular, the mutation of *recA1626* is at a residue in the same hydrophobic packing region as *recA1623* and *recA1625*.

Mutations mapped in the putative binding site for the LexA repressor. Story et al. (46) proposed that a pocket (Fig. 3) formed by two adjacent RecA monomers in the crystal may be the binding site for the UmuD, LexA, and phage repressors. This proposal was based on mutation information and physical considerations (46). Residues 229 and 243, cited by Story et al. (46), have also been indicated as a contact region between RecA and LexA in a recent study on the structure of the LexA-RecA filament complex (60). Three of our suppressor mutations, *recA1627*, *recA1628*, and *recA1642*, were mapped to this region of the crystal (Table 2; Fig. 3). These three mutations resulted in a Prt$^+$ phenotype (Table 2). Thus, the resultant RecA mutant proteins are no longer constitutively active and have a coprotease activity like that of wild-type RecA: they become active only after DNA-damaging treatments. It is possible that the reduction of the RecA Prt^c activity in these mutants is due to defects in binding to the LexA repressor.

Mutations mapped in the putative intermolecular packing regions in a RecA filament. Four of the suppressor mutations were mapped to residues that may be involved in the contact regions between RecA monomers within a polymer filament (Table 2) (46). It is not surprising that mutations in these regions would reduce the coprotease activity, since it has been

TABLE 2. Amino acid substitutions of the *recA* mutants classified by the putative functions affected by the mutations

Allele[a]	Phenotype	Amino acid no.	Amino acid change	Evolutionary conservation[b]
IFCRs				
recA1620	Prt^c Rec^-	275	Val→Asp	HC
recA1623	Prt^- Rec^-	182	Leu→Gln	HC
recA1625	Prt^c Rec^+	187	Thr→Ala	HC
recA1630	Prt^+ $Rec^\pm$	177	Lys→Gln	CC
recA1631	Prt^- Rec^-	284	Ile→Asn	HC
Repressor-binding sites				
recA1627	Prt^+ $Rec^\pm$	244	Val→Glu	HC
recA1628	Prt^+ $Rec^\pm$	231	Val→Glu	HC
recA1642	Prt^+ Rec^+	238	Val→Glu	MC
Intermolecular packing regions				
recA1622	Prt^+ Rec^+	111	Ile→Met	HC
recA1626	Prt^c Rec^+	139	Asp→Gly	CC
recA1634	Prt^c Rec^+	213	Asn→Lys	MC
recA1636	Prt^+ $Rec^\pm$	132	Leu→Gln	CC
DNA-binding sites				
recA1624	Prt^c Rec^+	208	Thr→Asn	CC
recA1634[c]	Prt^c Rec^+	213	Asn→Lys	MC
recA1641	Prt^+ Rec^+	152	Lys→Ile	HC
recA1649	Prt^+ $Rec^\pm$	214	Ala→Ser	CC
ATP-binding site				
recA1647	Prt^c Rec^+	145	Ser→Thr	CC
Others				
recA1633	Prt^- Rec^-	−12[d]		
recA1635	Prt^- Rec^-	84	Gln→Am	
recA1640	$Prt^\pm$ Rec^-	259	Glu→Oc	

[a] Unlisted is the *recA1643* mutation, which is a 10-base deletion between T-446 and T-456 in the sequence 5′-CGCCCT-446GGCGCGTTCT-456GGCG-3′; this sequence contains a 4-base direct repeat and a 4-base inverted repeat, either of which might conceivably encourage formation of the deletion.
[b] CC, completely conserved; HC, highly conserved; MC, moderately conserved.
[c] The mutation site of *recA1634*, residue 213, is likely to be involved in both intermolecular packing and DNA binding.
[d] Refers to the base at −12 in the *recA* promoter.

indicated that the coprotease activity depends upon the formation of a filament in the presence of ATP and ssDNA (46, 60). None of the four mutations produced a Prt^- phenotype; two of them produced a Prt^c phenotype with reduced coprotease activity, and the other two mutants exhibited a Prt^+ phenotype (Tables 1 and 2).

Mutations in possible DNA- or ATP-binding sites of RecA. Story et al. suggest that three regions are particularly likely to be involved in DNA binding: regions in or near loop 1 (residues 157 to 164), loop 2 (residues 195 to 209), and helix G (residues 213 to 218) (Fig. 1a) (46). This suggestion was based on structural comparisons with other DNA-binding proteins and on DNA-binding properties of mutant proteins with known mutation sites (46). It should be emphasized that the structure of a RecA-DNA complex has not been solved, and these assignments should be considered tentative. Four mutations were mapped to or near these three regions (Table 2). Story and Steitz solved the crystal structure of a RecA-ADP complex (45). One suppressor mutation was found in sequences corresponding to the proposed ATP-binding site (Table 2) (45). If these regions are indeed involved in DNA and ATP binding, these mutations may reduce the Prt^c activity by affecting the binding activity of RecA1202 for DNA (or similarly RNA) or ATP (or similarly other nucleoside triphosphates).

Mutations in the IFCR. The remaining five suppressor mutations were mapped to the IFCR of the crystal. Of these, three were discussed above as likely having effects partly due to direct interaction with the mutant residue 184. The two others (*recA1620* and *recA1631*) are missense changes in the C-terminal domain of the RecA monomer, and the altered sites evidently could not interact intramolecularly with the IFCR near residue 184 (Fig. 1a) (46). Interestingly, these sites of *recA1620* (V-275→D) and *recA1631* (I-284→N) are in the C-terminal portion of the IFCR that is close to the original *recA1202* (Q-184→K) site in the adjacent filament (Fig. 2). Both of these mutations are nonpolar-to-polar changes in a hydrophobic core of the C-terminal domain. These drastic changes could lead to an altered spatial position of the IFCR and thus compensate for the alteration caused by the mutation at residue 184 in the IFCR. Such a situation is similar to that suggested by Story et al. (46) for the temperature-sensitive suppression of the Prt^c phenotype of the *recA1211* (E-38→K) allele by an I-298→V change (53). Thus, our finding is consistent with the filament-bundle theory, which suggests that the interference with the interfilament association is the cause of the constitutive coprotease activity of RecA(Prt^c) mutant proteins (46).

Effect of the suppressor mutations on recombination. The *recA1202* allele has a wild-type recombination phenotype (Rec^+). Of the 16 missense suppressors, 8 led to defective Rec phenotypes (Rec^- and $Rec^\pm$), and they were mapped in four different functional regions of RecA (Table 2). All eight Rec^- and $Rec^\pm$ mutations were mapped at residues that are either completely conserved or highly conserved among bacterial species; seven of the eight mutations produced a change from nonpolar to polar (Table 2). Thus, the relatively dramatic change in phenotype caused by these mutations can be explained by the fact that almost all cause drastic changes at critical residues. The fact that changes at DNA-binding and intermolecular packing regions can lead to a $Rec^\pm$ phenotype is further testimony that DNA binding and filament formation

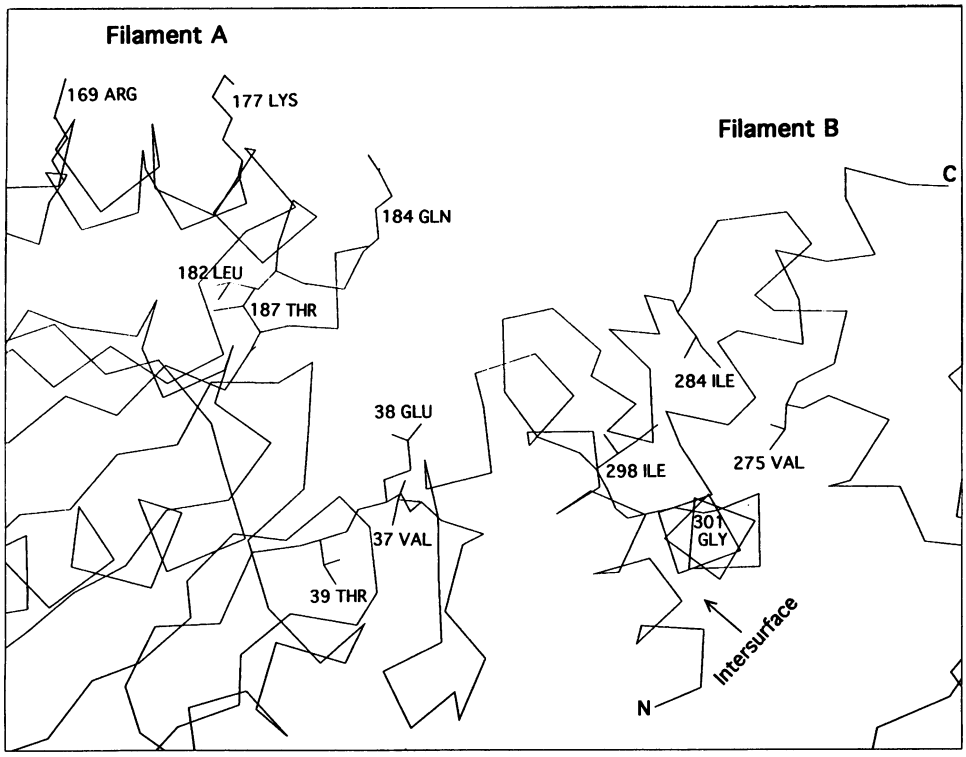

FIG. 2. Sites of mutations at the IFCR in the RecA crystal. The side chains shown are for the wild-type amino acids. The Prt^c Rec$^+$ mutation sites are *recA1202* (Q-184→K), *recA1211* (E-38→K), *recA1235* (T-39→I) (54), *recA1625* (Q-184→K, T-187→A) (Table 2), and the temperature-dependent Prt^c allele *recA441* (E-38→K, I-298→V) (53). Prt^c Rec$^+$ mutations also mapped in the IFCR but not shown are *recA1222* (S-25→F) and *recA1213* (A-179→V) (54). Prt^c Rec$^-$ mutation sites are *recA1620* (Q-184→K, V-275→D) (Table 2), *recA1601* (G-301→S), *recA1206* (G-301→D), and *recA1203* (R-169→C) (54). Prt$^-$ Rec$^-$ mutation sites are *recA1623* (Q-184→K, L-182→K) and *recA1631* (Q-184→K, I-284→N) (Table 2). The *recA1630* (Q-184→K, K-177→Q) allele showed a Prt$^+$ Rec$^\pm$ phenotype (Table 2). The *recA803* mutation at residue 37 (V-37→M) enhances the recombinase activity (32). The arrow indicates the location of the immediate contact points (intersurface) between two contacting filaments (A and B). The actual contacting amino acids, determined from our three-dimensional analysis with the computer program Midas, are residues 12, 15, 16, 19, 23, 33, 35, 36, 38, and 60 in the N-terminal domain, residue 183 in the central domain, and residues 290, 294, 296, 297, 298, 308, 311, 312, and 314 in the C-terminal domain.

are required for RecA to promote recombination activities (37, 40). Of the eight recombination-defective mutations, four were mapped in the IFCR, strongly suggesting that this region is involved in recombination (see below).

Involvement of IFCR in the recombination activities. While Story et al. (46) emphasized the involvement of the IFCR in the Prt^c phenotype, we explored the possibility of IFCR involvement in recombination. Among the 16 distinct missense mutations, 3 changes resulted in a Rec$^-$ phenotype (Table 1). Strikingly, all three mutations, *recA1623* (L-182→Q), *recA1631* (I-284→N), and *recA1620* (V-275→N), were mapped in the IFCR. In fact, among the five suppressor mutations mapped in the IFCR, four led to defective Rec phenotypes (Table 2). These observations imply that this region and its putative function in interfilament association play a role in promoting recombination activities (see Discussion).

DISCUSSION

Usefulness of the proximal mutagenic system. The proximal mutagenic system provides a simple way to produce and isolate mutations in the *recA1202* gene and in nearby genes as well. In the presence of a high-copy-number *umuDC* plasmid, the mutation frequency in the *recA1202* gene can be as high as about 5% per generation (26). The proximal mutagenesis is self-controlled because the agent of the mutagenesis (the *recA1202* allele) is preferentially targeted for mutation, and frequently such mutations suppress mutagenic activity (26), thereby precluding multiple mutations. In none of the 28 mutants we sequenced was there a multiple mutation in the *recA* gene.

General considerations for a structure-function analysis of the suppressor mutations. Our analysis of the *recA1202* suppressors was organized as an attempt to understand how the structure of RecA might contribute to the coprotease and recombination functions, particularly in the context of the filament-bundle theory proposed by Story et al. (46). First, the possible effects of the original *recA1202* mutation (Q-184→K) on the structure of RecA were examined by using a computer program designed by Lee and Levitt (20). The only predicted effects are in the immediate vicinity of position 184. This appears to be a reasonable prediction inasmuch as Q-184 is on the surface of the protein and the hydrophilic side chain is in solution and not buried; the *recA1202* mutation replaces a polar amino acid with one containing a basic side chain, which would not be likely to have much effect on the hydrophobicity of this region. Thus, the structural change caused by *recA1202* is probably restricted to a small region around residue 184 of the IFCR. This is consistent with a basic part of the theory proposed by Story et al. (46), which explains the phenotypic effects of the *recA1202* mutation: this change at residue 184

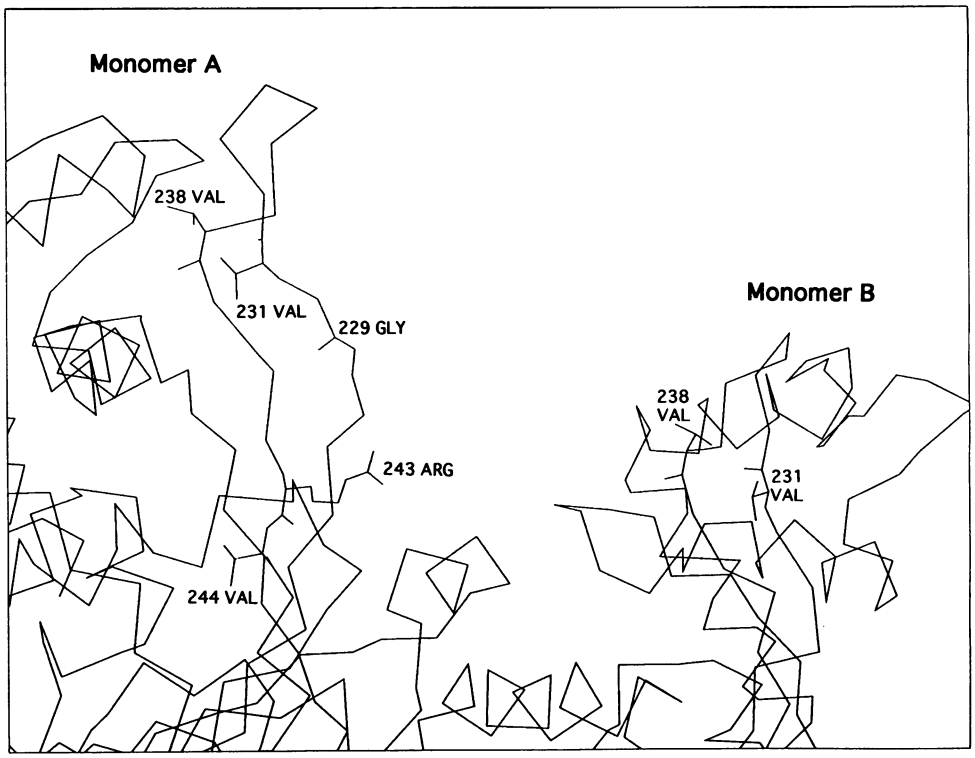

FIG. 3. Locations of mutations at the repressor-binding sites of RecA. The repressor-binding sites of RecA consist of regions of two adjacent monomers in a RecA filament (46), indicated as Monomer A and Monomer B. The mutation sites are at three residues: 231 (*recA1628*), 238 (*recA1642*), and 244 (*recA1627*). Two additional mutation sites, residue 229 (*recA91*) (34) and residue 243 (*recA1734*) (9), are also indicated; these mutations differentially affect cleavage of repressors, and the sites are close to those of the three suppressor mutations noted above. The side chains shown belong to the wild-type forms of the mutated amino acids.

alters only the local structure involved in contact between filaments.

We can imagine that the new mutations may suppress the Prt^c phenotype of the *recA1202* mutant either (i) directly by a change complementary to Q-184 that essentially restores the original structure or (ii) indirectly by altering a completely separate functional site that reduces the overall coprotease activity. We attempted to distinguish between these two possibilities by determining whether the secondary mutations would be likely to alter the structure in the same region as the original mutation (direct effect) or in some other regions (indirect effect) or both. It should be emphasized, however, that although a mutation may map in what appears to be a distinct functional region of the protein, it could also have effects on other functional regions as well, depending on how drastically it affects the structure and how close it is to other functional regions. Thus, its effect on the phenotype could be due to changes in other functional regions. Nevertheless, when several different mutations causing a similar phenotype all map in the same region, it is likely that the function of that region is directly responsible for the altered phenotype.

Interfilament association and the Prt^c activity. It is possible, as indicated in the filament-bundle theory (46), that without an inducing signal(s), $RecA^+$ monomers tend to form protein filaments which in turn have the tendency to form multifilament bundles. These protein bundles are a storage from of RecA and are not active in promoting proteolytic reactions (Fig. 4) (46). When a Prt^c mutation occurs in the IFCR sequences (Fig. 2), the following two events leading from a RecA(Prt^+) to a RecA(Prt^c) phenotype may both occur: (i) the mutation shifts the equilibrium from bundle formation to favor single filament formation, and (ii) the mutation causes a conformation change that allows the RecA filament to bind to unusual cofactors and thus promote the constitutive cleavage of repressors. Therefore, a second-site mutation at the IFCR in a RecA(Prt^c) protein that shifts the equilibrium back toward bundle formation can suppress the Prt^c phenotype (Fig. 4). The mutagenic effect could produce a phenotype that is either Prt^c (with reduced coprotease strength), Prt^+, or Prt^-, depending on how much it shifts the equilibrium.

IFCR mutations that also affect the recombinase activity. In addition to the four IFCR suppressor mutations described in Results, there are several previously known *recA* mutations that affect the recombinase activity and also map in the IFCR. *recA803* is a V→M change at residue 37 (32), which is in the N-terminal portion of the IFCR (Fig. 2) (46). The *recA803* mutant is Prt^+, but the mutant protein shows a higher-than-normal rate of strand pairing and strand exchange activity (32). Thus, not only can mutations in or around the IFCR decrease the recombinase activity, but some can also enhance the RecA recombination activities, an observation in agreement with our suggestion that the IFCR is involved in the recombinase activity of RecA.

A total of three single-site Prt^c Rec^- mutations were previously sequenced by Wang and Tessman: *recA1206* (G-301→D), *recA1601* (G-301→S), and *recA1203* (R-169→C) (54). All three single-site mutations, like the *recA1620*(Prt^c Rec^-) allele, mapped in or near the IFCR, where changes could cause defective association between filaments (Fig. 2) (46). Therefore, it appears that the IFCR and its possible

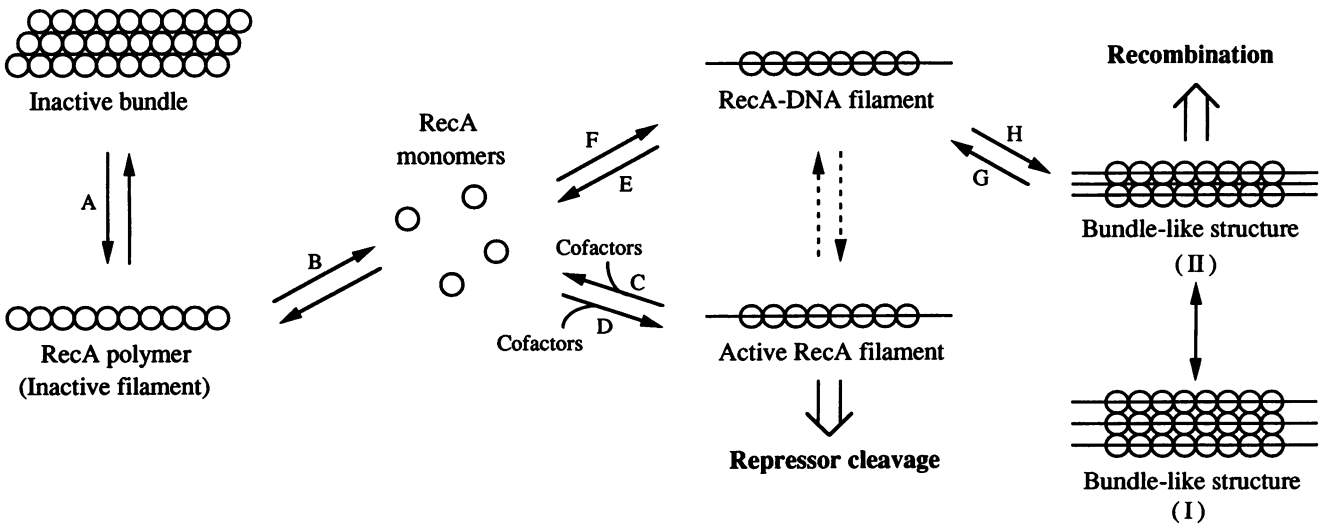

FIG. 4. RecA structural models to explain how single *recA* mutations can give rise to the Prt^c Rec$^+$, Prt$^+$ Rec$^-$, or Prt^c Rec$^-$ phenotype. Mutations that affect step A, B, or D can shift the equilibrium toward filament formation and result in Prt^c coprotease activity (46), mutations at step C, E, or G can shift the equilibrium toward monomer or filament formation and lead to a Rec$^-$ phenotype, a mutation that acts at both steps A and G can cause a Prt^c Rec$^-$ phenotype, and a mutation can act at step F or H to cause more efficient formation of the bundle-like structure and give rise to a mutant protein with enhanced recombinase activity. The DNA shown can be ssDNA or dsDNA.

function of interfilament association are important in regulating proteolytic activity and promoting recombination activity. It should be noted that the mutation site of *recA1203*, residue 169, is also close to a proposed DNA-binding site (46), and its change could also affect the DNA-binding activity.

Roles of interfilament association: bundles and bundle-like structure. Why is the IFCR important for the recombinase activity? How can single point mutations at the IFCR give rise to a Prt^c Rec$^-$ split-phenotype mutant protein? These observations can be explained if we assume the following: (i) Prt^c mutations affect the IFCR structure to favor the formation of the active filament (containing DNA) rather than the formation of the pure protein bundles; (ii) the pairing of homologous DNA strands and the subsequent strand exchange, which are required in part for the Rec$^+$ phenotype, are enhanced when the RecA-DNA combination forms a bundle-like structure; and (iii) the bundle-like structure is similar, but not identical, to the inactive form of bundle proposed by Story et al. (Fig. 4) (46) that is inactive for both coprotease and recombinase activities. Thus, if RecA bundle formation reduces coprotease activity but a similar bundle-like RecA-DNA structure promotes homologous recombination, mutations that hinder the formation of both bundle structures can produce a Prt^c Rec$^-$ phenotype. These mutations could favor the formation of individual active filaments, which presumably results in enhanced coprotease activity; in our model these mutations would also remove the RecA-DNA bundle-like structures required for recombination (Fig. 4), thereby producing the Prt^c Rec$^-$ split-phenotype effect. Mutations in the IFCR that block the formation of only one of the two bundle structures will give rise to a phenotype that is either Prt^c Rec$^+$ (defective only in bundle formation) or Prt$^+$ Rec$^-$ (defective in the formation of the RecA-DNA bundle-like structure).

The bundle-like structure proposed here may be a transient and dynamic aggregate of RecA-DNA filaments. In order to complete the pairing of homologous DNA strands, hydrogen bonds are likely to be formed between complementary DNA strands, which could require dynamic exchanges between two or more multifilament structures. The relatively weak contact between RecA filaments, as compared with that between monomers within a filament, may be an important factor in acquiring these dynamics. Specifically, we postulate a sandwich form of the bundle-like structure, consisting of RecA-DNA filament–DNA–RecA-DNA filament, which may continuously exchange with the other form of bundle-like structure composed of only RecA-DNA filaments (Fig. 4). The DNA wrapped in the RecA-DNA filament could be either ssDNA or double-stranded DNA (dsDNA).

Several observations are consistent with the notion that the RecA-DNA filament can associate not only with another RecA-DNA filament but also with a DNA (ssDNA or dsDNA) molecule at the IFCR. First, the C-terminal portion of the IFCR between residues 280 and 310 is rich in basic and aromatic residues and fits the sequence of the DNA-binding domain of some DNA-binding proteins (35, 37). In addition, the *recA441* allele, which consists of two missense mutations (53) that are both in the IFCR (46), codes for a mutant RecA protein that has altered DNA-binding kinetics (28, 30). The RecA1202 and RecA1211 Prt^c proteins, whose alterations are at different parts of the IFCR (Fig. 2) (46), also have enhanced DNA-binding activity (52, 55). Furthermore, in the three-dimensional crystal structure of the IFCR, most of the C-terminal residues described above, and the region around residue 184, are not buried in the intersurface between contacting filaments (Fig. 2). In fact, the two regions defined by residues around 184 and nonintersurface residues between 280 and 310 look like two sides of a small pocket in the three-dimensional structure, and the small pocket is big enough to provide a binding site for an ss- or dsDNA molecule (Fig. 2). Thus, the binding of a RecA-DNA filament to DNA may be enhanced, rather than excluded, by the association between two such filaments. This could explain, in part, the importance of the postulated RecA-DNA bundle-like structure in recombination.

The bundle-like structure may be formed after the pairing of complementary DNA strands, and its involvement in the

strand exchange reactions could still require a dynamic structure. Mutations that affect the dynamics and flexibility of this structure could result in a Rec$^-$ phenotype. Such mutations could actually stabilize the transient association between RecA filaments and reduce the coprotease activity as well. All three Rec$^-$ suppressors isolated in this study resulted from nonpolar-to-polar changes at residues involved in interfilament association (Table 2). These mutations could significantly disrupt the integrity of the IFCR and form additional hydrogen bonds, leading to the stabilization effect described above.

The RecA-DNA bundle-like structure that we postulate and its involvement in strand pairing and/or exchange activities is supported by several lines of evidence. In a study designed to understand how RecA promotes the alignment of homologous DNA strands, it has been observed that under strand-pairing conditions, RecA-ssDNA filaments and heterologous dsDNA formed coaggregates (50). This aggregation may provide a concentration effect that facilitates the search for homologous DNA sequences (15). This coaggregate structure is conceivably a form of the proposed bundle-like structure. Furthermore, in various electron microscopy studies of the structure of the RecA-DNA filament, bundle formation from RecA-DNA filaments has been repeatedly observed (10, 11). In the presence of Mg^{2+} and ATP-γS, RecA-DNA filaments regularly aggregate into bundles composed typically of three or six RecA-DNA filaments (10).

If formation of a bundle-like structure is important for promoting homologous recombination, how can one reconcile this with the data showing that RecA protein monomers truncated at the C terminus can still have recombinase activity, both in vivo (19) and in vitro (2)? The answer may lie in the electron microscopic study of Yu and Egelman (59). In their study of the conformational change of a truncated RecA protein, it was found that removal of 18 residues from the C terminus of the RecA protein results in a significant change in the structure of the RecA-DNA filament; a 15-Å (1.5-nm) outward (from the DNA axis) movement of an inner domain is observed, but RecA monomers still form a stable filament complex with DNA (59). Their result suggests that the RecA structure is flexible in forming a RecA-DNA filament. Our inability to isolate Prt$^-$ suppressor mutations in regions involved in DNA binding and filament formation (see below) is also consistent with this implied flexibility. It is conceivable, therefore, that a 50-amino-acid deletion at the C terminus (2) may result in a mutant RecA protein whose conformation is altered even more dramatically than that of the truncated RecA with 18 amino acids removed from its C terminus. The drastic conformation change caused by the 50-amino-acid deletion (2) could allow the formation of RecA-DNA filaments which in turn are capable of forming the bundle-like structure and promoting homologous recombination. Without such a notable change in conformation, most point mutations affecting the interfilament association will not allow efficient formation of the bundle-like structure, and, as a result, they will lead to defective recombinase activity. Thus, the evidence from the studies on the deletion mutants is not sufficient to rule out our theory that parts of the C-terminal domain containing the IFCR are important for recombination. In any case, it appears that conclusions from structure-function analysis based on deletions, especially multiresidue deletions, could be misleading.

Flexibility of RecA1202 structure in promoting repressor cleavage. It is intriguing that 11 mutations, consisting of all 5 mutations mapped in the DNA- or ATP-binding sites and all 7 mutations in repressor-binding and intermolecular packing regions, failed to reduce the coprotease activity to a level less than that of RecA$^+$ (Tables 1 and 2). This is significant because most of the amino acid substitutions caused by the 11 mutations are in themselves rather severe: 7 of the 11 substitutions are either nonpolar-to-polar or polar-to-nonpolar changes, and 1 of the other 4 substitutions is from a moderately polar to an extremely polar residue (Asn→Lys) (Table 2). This suggests flexibility in the three-dimensional structures required for DNA and repressor binding and subsequent repressor cleavage. If this is the case, most single-residue alterations in these regions are not likely to cause a dramatic change in the protein structure and function and resultant coprotease activity. An alternative explanation for this specific lack of Prt$^-$ mutations is that such mutations confer a selective disadvantage under our experimental procedures. We do not favor this alternative hypothesis because we were able to isolate many Prt$^-$ mutants; among 20 distinct mutants isolated, 6 showed a coprotease activity much weaker than that of the wild type (5 Prt$^-$ and 1 Prt$^\pm$ [Table 1]). This number is equal to the number of Prt^c mutants isolated and only slightly lower than the number of Prt$^+$ mutants isolated (Tables 1 and 2). Thus, while Prt$^-$ mutants can be easily isolated by our procedure, 11 mutations in the three regions described above did not produce a Prt$^-$ phenotype.

Furthermore, one of the nonsense mutations, *recA1640* (E-259→Oc), mapped at residue 259, and the resulting RecA is missing 94 C-terminal residues (Fig. 1b and Table 2). Surprisingly, this mutant protein could still be partially activated to a coprotease by UV irradiation (16 J/m^2) of the cell; it induced W to rise from 0.01 to 0.08, which can be compared with $W = 0.18$ for UV-activated RecA$^+$ protein (27). It seems, therefore, that the coprotease activity of RecA1202 is "buffered" by structural flexibility in maintaining the appropriate conformation to promote the cleavage of LexA repressor. Amino acid substitutions at a number of sites, including all the missense mutations at intermolecular packing, repressor-binding, and DNA-binding sites, and the 94-amino-acid truncation (caused by the *recA1640* mutation) at the C terminus do not eliminate the coprotease activity completely. It is unclear whether such a structure-function flexibility also exists in the RecA$^+$ protein. If so, it would be consistent with the evidence that RecA is not a true protease, but rather a coprotease (20, 23, 44), and can play a role in proteolytic reactions that is relatively easy to fulfill.

Inducibility of mutant proteins by MitC treatment. It is intriguing that many mutations mapped in the putative sites for repressor binding, intermolecular packing, and DNA binding lead to a coprotease that is inducible by MitC but not by constitutive cofactors such as nucleoside triphosphates and RNAs (Tables 1 and 2). One possibility is that cofactors for constitutive activation of RecA1202 (nucleoside triphosphates and RNAs) may activate RecA1202 to a conformation different from that activated by damaged DNA; mutations in different regions of RecA1202 may exaggerate this difference.

Conclusions. Analyses of our newly isolated suppressors of the *recA1202*(Prt^c) mutant provide additional evidence for the molecular mechanism proposed by Story et al. (46), by which *recA* mutations can lead to a Prt^c phenotype. Of 16 distinct missense suppressor mutations, 5 were mapped in the proposed IFCR, and 4 of them markedly changed the parental Prt^c Rec$^+$ phenotype of *recA1202* (Q-184→K) (Table 2). Two of these five suppressor mutations were mapped in the RecA C-terminal domain. This small domain, according to the 2.3-Å X-ray crystal structure, does not interact intramolecularly with the large central domain where the *recA1202* mutation resides. The two mutations were located in a part of the IFCR that apparently can interact intermolecularly with residue 184 in an

adjacent, contacting filament (Fig. 2). Thus, alterations in interfilament association could lead to a mutant RecA with a Prt^c phenotype and also to a reduction of the Prt^c activity of an existing Prt^c protein. A major part of the model proposed by Story et al. was based on the sequencing studies of the *recA441* double-mutant allele (E-38→K and I-298→V) (53), a temperature-dependent Prt^c mutant. The first mutation confers a Prt^c phenotype, and the second one is a temperature-sensitive suppressor (53); though far apart in the linear sequence, both mutations map in the proposed IFCR of the crystal (46). Our finding of more suppressor mutations mapped in the same regions strengthens the idea that alterations such as the suppressor mutations may favor the formation of inactive bundles and reduce the formation of individual filaments that produce the Prt^c phenotype.

We also found that three suppressor mutations give rise to the Rec$^-$ phenotype; surprisingly, all three map in the IFCR. In addition, all the sequenced Prt^c Rec$^-$ mutations, including one reported in this study, map in or near the IFCR. Therefore, it appears that the IFCR is involved in both proteolytic and recombination activities. To account for the observations, we propose that while shifting from inactive RecA bundles to the active RecA-DNA single filaments is essential for coprotease activity, formation of a RecA-DNA bundle-like structure is required for RecA-promoted recombination activities such as strand pairing and/or strand exchange. From our analysis of the possible composition of the bundle-like structure, we suggest that a form of the structure contains a DNA molecule between two contacting RecA-DNA filaments (Fig. 4). Determination of the exact conformation and functions of this hypothetical bundle-like structure awaits further studies, particularly on the Prt^c Rec$^-$ mutants.

In addition to mutations mapped in the IFCR, including three located in the vicinity of *recA1202*, there are suppressor mutations mapped in sites possibly involved in repressor binding, intermolecular packing, and binding to DNA and ATP. Most of these mutation sites do not seem to interact with residue 184 either intra- or intermolecularly. Thus, the conformations of these putative binding or packing sites have to be appropriately maintained to form a constitutively active coprotease. The fact that there were no Prt$^-$ mutations among 11 distinct mutations mapped in the above-mentioned four sites suggests that the RecA protein is structurally flexible in its ability to form a RecA-DNA filament upon activation and to bind to repressors to promote the subsequent cleavage.

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