Recombinogenic Activity of Chimeric *recA* Genes (*Pseudomonas aeruginosa/Escherichia coli*): A Search for RecA Protein Regions Responsible for This Activity

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ABSTRACT

In the background of weak, if any, constitutive SOS function, RecA from *Pseudomonas aeruginosa* (RecAPa) shows a higher frequency of recombination exchange (FRE) per DNA unit length as compared to RecA from *Escherichia coli* (RecAEc). To understand the molecular basis for this observation and to determine which regions of the RecAPa polypeptide are responsible for this unusual activity, we analyzed *recAX* chimeras between the *recAEc* and *recAPa* genes. We chose 31 previously described recombination- and repair-proficient *recAX* hybrids and determined their FRE calculated from linkage frequency data and constitutive SOS function expression as measured by using the *lacZ* gene under control of an SOS-regulated promoter. Relative to *recAEc*, the FRE of *recAPa* was 6.5 times greater; the relative alterations of FRE for *recAX* genes varied from ~0.6 to 9.0. No quantitative correlation between the FRE increase and constitutive SOS function was observed. Single ([L29M] or [I102D]), double ([G136N, V142I]), and multiple substitutions in related pairs of chimeric RecAX proteins significantly altered their relative FRE values. The residue content of three separate regions within the N-terminal and central but not the C-terminal protein domains within the RecA molecule also influenced the FRE values. Critical amino acids in these regions were located close to previously identified sequences that comprise the two surfaces for subunit interactions in the RecA polymer. We suggest that the intensity of the interactions between the subunits is a key factor in determining the FRE promoted by RecA *in vivo*.

THE RecA protein is an essential component of homologous SOS-independent and SOS-dependent recombination in bacteria. The two types of recombination processes differ in the frequency of recombination exchanges (FRE) per DNA unit length, which is about an order of magnitude higher under conditions of SOS response (Bresler and Lanzov 1978; Lloyd 1978; V. A. Lanzov and A. J. Clark, unpublished results). This dramatic increase results from derepression of the SOS regulon (controlled by the LexA repressor and the RecA allosteric inducer) and induction of a number of repair and recombination enzymes (Little and Mount 1982).

A distinguishing characteristic of RecA protein from *Pseudomonas aeruginosa* (RecAPa) is its hyperrecombinogenic activity in *Escherichia coli* cells in which the endogenous *E. coli recA* gene (*recAEc*) is replaced by its homolog from *P. aeruginosa*, *recAPa*. In fact, RecAPa increases FRE 6- to 8-fold in *E. coli* (Namsaraev *et al.* 1998). A similar increase can be also achieved with single amino acid substitutions in *recAEc* that show a 10- to 17-fold increase in constitutive expression of SOS functions due to a protease constitutive (Prt^c) phenotype. These mutations, however, do not cluster to a specific domain in the RecA molecule (Wang and Tessman 1986). On the other hand, RecAPa-mediated stimulation of FRE activity in *E. coli* most likely does not result from a constitutive SOS response, since a concomitant increase in β-galactosidase levels from an *sfiA::lacZ* fusion is not observed in these cells (Namsaraev *et al.* 1998). The uncoupling of the RecAPa hyperrecombinogenic (hyperrec) activity and the constitutive SOS has been explained by the tight binding of RecAPa to a single- and especially to a double-stranded DNA (ss- and dsDNA, respectively). Because the RecA::ATP::ssDNA presynaptic ternary complex can bind to either dsDNA or the LexA protein in a mutually exclusive manner (Yu and Egelman 1993; Rehrauer *et al.* 1996), the high affinity of RecAPa for dsDNA favors initiation of recombination instead of LexA repressor binding to induce the constitutive SOS response.

RecAEc is composed of 352 amino acids (Horii *et al.* 1980) that constitute three domains: an N-terminal domain from residues 1–36 ± 8, a central domain (residues 36–266 ± 4), and a C-terminal domain consisting of residues 266–352 (Story *et al.* 1992). The RecAPa protein, which is 71% identical and 86% similar to

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RecAEc (Sano and Kageyama 1987), is shorter than RecAEc by 6 amino acids (Figure 1) but probably has similar, if not identical, secondary structure. All 10 α-helices of the RecAPa protein have been reconstructed and analyzed as homologs of RecAEc (Petukhov *et al.* 1997).

A unique property of the RecA family of proteins is their ability to form nucleoprotein filaments composed of RecA, ATP, and ssDNA. This activates RecA for either recombination or the SOS response (Kowalczykowski *et al.* 1994; Roca and Cox 1997). The X-ray crystal structure analysis of RecA revealed that RecA monomers are packed together to form a continuous helical polymer (Story *et al.* 1992) with an axial hole that accommodates at least three strands of DNA (Cox 1995), including the ssDNA of one of the recombination partners and the dsDNA of the other. Each polymer subunit has two separate surfaces that form the contact interface (Figure 1). As is easily seen in Figure 1A, one surface (region I) consists of 14 residues located in positions 6–30 (region Ia) of the N-terminal and 13 residues located between positions 172 and 257 (region Ib) of the central domain, while the complementary surface includes 28 residues between positions 89 and 156 (region II) of the central domain (Story *et al.* 1992). Figure 1, B and C, show, respectively, a general and detailed spatial structure of the interface. One can see spatial positions of separate amino acid residues participating in the subunit-subunit interaction.

Fifty-four *recA* gene chimeras composed of *P. aeruginosa* and *E. coli recA* gene fragments (*recAX* genes) were either selected *in vivo* or constructed *in vitro* (Ogawa *et al.* 1992). In the present work, 31 recombination-proficient *recAX* genes from this collection were used in an attempt to determine which residues of RecAPa are critical for its hyper-rec activity. A comparison of both the recombinogenic and constitutive SOS characteristics of these *recAX* genes led us to conclude that, like RecAPa, some chimeric proteins contain an increased recombinogenic activity that does not correlate with their effect on constitutive SOS expression. A search for residues responsible for the increase of recombinogenic activity of these RecA chimeras (RecAX) suggests that intersubunit interactions specify the hyper-rec activity of both RecAX and RecAPa proteins.

MATERIALS AND METHODS

Bacterial strains and plasmids: The structure of *recAX* hybrids between *recA* genes from *E. coli* and *P. aeruginosa* was described earlier (Ogawa *et al.* 1992). Thirty-one original plasmids carrying the chimeric genes with Rec^+ phenotype were transformed both into the strain JC10289 (of AB1157 ancestry) with the relevant genotype Δ*recA thr-1 leu-6* Str^r (A. J. Clark's collection) to analyze FRE and into the strain GY7109 (of AB1157 ancestry) with the relevant genotype Δ*recA sfiA::lacZ* (R. Devoret's collection) to analyze the level of constitutive expression of *sfiA::lacZ* gene controlled by SOS regulon. In each transformation, several transformants were tested for Rec^+ and UV^r phenotypes; all tests were well reproducible, and two transformants were used in further analyses.

The strains KL227: HfrP4x *metB1 rel-1* Str^s and KL226: HfrC Str^s (*E. coli* Genetic Stock Center) were used as donors in conjugational crosses. The strains JC10289L, GY7109L, and KL226L (the same as JC10289, GY7109, and KL226, respectively, but *lexA3*) were made by P1 transduction of *lexA3* from RB800: *malE::Tn5 lexA3* (G. Walker's collection). *lexA3* conferred a UV^s phenotype and was 75% cotransducible with *malE::Tn5*. The presence of the *lexA3* mutation in JC10289L, GY7109L, and KL226L was confirmed by repeated P1 transduction of the *malE::Tn5 lexA3* from these strains to JC7623 (the same as AB1157, but *recBC*$^-$ *sbcB*$^-$) to observe again the Kan^r UV^s phenotype of these transductants in addition to the Rec^- phenotype that served as a specific characteristic of *lexA3 recBC*$^-$ *sbcB*$^-$ strains.

Plasmids pEC19 and pAK610 (Ogawa *et al.* 1992) contain, respectively, the *recAEc* and *recAPa* genes transcribed from the *lac* promoter. They were used for the expression of RecAEc and RecAPa proteins in determining both FRE and SOS characteristics. Plasmids carrying *recAEc/recAPa* chimeric genes (designated here as *recAX1*, *recAX2*, . . . , etc.) were constructed earlier from pEC19 and pAK610 (Ogawa *et al.* 1992).

Chemicals: *O*-Nitrophenyl-β-D-galactopyranoside (ONPG), ampicillin, and streptomycin were purchased from Sigma Chemical (St. Louis). All other reagents used were commercial products of the highest grade available.

FRE analysis: Quantitative estimations of FRE alterations (ΔFRE) promoted by different *recAX* genes relative to the FRE value promoted by the *recAEc* gene were done by use of the modified Haldane formula: $\mu = \frac{1}{2}[1 + \exp(2l/\lambda)]$, where μ is the coinheritance (linkage frequency) of selected and unselected transferred donor markers in Hfr × F^-crosses, l is the distance between these markers in minutes of *E. coli* map, and λ is an average distance between two neighboring recombination exchanges (Bresler and Lanzov 1978). If coinheritances of the same pair of markers are measured in two different crosses and promoted by two different *recA* genes (values μ_1 and μ_2, respectively), then the FRE alterations can be calculated via the formula $\Delta FRE = \ln(2\mu_1 - 1)/\ln(2\mu_2 - 1)$. The events of conjugational recombination proceeding in the 19-min interval *tsx-argE* of the 100-min *E. coli* genetic map (Rudd 1998) are well described by the Haldane formula in crosses between donors of HfrP4x or HfrC and recipients of AB1157 ancestry (Bresler *et al.* 1978; Lanzov *et al.* 1991). These strains were chosen for measurements of linkage between selected thr^+ and unselected leu^+ donor markers, located at 0 and 1.75 min of *E. coli* map, respectively. Because values of marker coinheritance, and consequently FRE, depend on different physiological factors including temperature, pH of the media, medium shift down, and growth phase of recipients (V. Lanzov, I. Bakhlanova and A. Clark, unpublished results), standard experimental conditions (including minimal medium for all stages of conjugation and exponential growth phase, pH 7.5, 37°) were used in all crosses.

Both Hfr and F^- strains were grown in minimal 56/2 medium (Adelberg and Burns 1960) supplemented with required growth factors. Selection was carried out on the same medium plus 1.5% agar and lacking threonine. Since strains of the AB1157 line are isoleucine- and valine-deficient at temperatures >37° (Tessman and Peterson 1985), growth and selective media contained these amino acids in all crosses. When Hfr donors were $metB^-$, growth and selective media contained methionine. Donors and recipients were mixed for conjugation in ratio 1:10; conjugation was allowed for 60 min; and the mating mixture was diluted 1:100 with 56/2 buffer and agitated with a vortex blender to stop mating. After conju-

gation, Thr^+ Str^r transconjugants were selected. Both Hfr KL227 and Hfr KL226 transfer markers in the following order: origin, *leu*$^+$, and *thr*$^+$. About 200–300 unpurified colonies from each selection were inoculated as patches in a regular array on selective media. After overnight incubation the transconjugants were replica plated onto the same selective media omitting threonine and leucine to score inheritance of unselected *leu*$^+$. As minimum, three crosses were performed with each pair of strains, the results were averaged, and standard deviations were determined.

Under the standard conditions described, the FRE value was found to be 4.43 ± 0.22 recombination exchanges per the 100-min length of DNA. This value is valid for the *recAEc*$^+$ recipients of AB1157 line in the *E. coli* map region between 90 and 10 min. All ΔFRE alterations found in this study were calculated relative to the FRE value for *recAEc*, 4.43 exchanges per 100 min.

β-Galactosidase assay: Spontaneous SOS gene expression was measured in strain GY7109 Δ*recA* carrying plasmids with different *recAX* chimeras. Cultures were grown overnight at 37° in mineral 56/2 medium containing all necessary growth factors and 25 μg/ml ampicillin. Overnight cultures were diluted 40-fold in the same medium and grown to an optical density of 0.1–0.2 at 600 nm. The β-galactosidase assay was essentially that described by Miller (1972). A portion (1 ml as a rule) of an exponentially growing cell suspension was diluted by the same volume of phosphate Z-buffer (pH 7) and the optical density at 600 nm (OD_{600}) was measured. The cell suspension was permeabilized by sodium dodecyl sulfate and chloroform; 0.4 ml ONPG (4 mg/ml) was added; the reaction, proceeding an appropriate time at 28°, was stopped by addition of 1 ml of 1 M Na_2CO_3; the mixture was centrifuged to remove debris; and the OD_{420} of the reaction mixture was determined. The protein activity was calculated in arbitrary units as recommended by Miller (1972), using the formula $\beta\text{-gal} = 1000(OD_{420}/OD_{600} \cdot t \cdot v)$, where OD_{420} is an optical density of the reaction mixture at 420 nm, OD_{600} is an optical density of cell suspension in Z-buffer at 600 nm, t is time of the reaction in minutes, and v is a portion (in milliliters) of the cell suspension in Z-buffer taken in the reaction. These units were proportional to the amount of *O*-nitrophenol increased per minute per bacterium. The data of two or three independent assays, each of which contained two or three probes taken at different optical density (OD_{600}) of growing culture, were averaged and standard deviations were determined. ΔSOS is a ratio of the β-gal level promoted by RecAX or RecAPa to that of RecAEc. Correlations between ΔFRE and ΔSOS data were calculated through Pierson correlation coefficients. Calculations of uncertainty in determinations of relative values of FRE and the β-galactosidase activity were done according to Robyt and White's (1990) manual. Standard deviation, s, from the mean (x_m) for a sample of data (x_i) of n observations was calculated by the following formula:

$$s = \sqrt{(x_i - x_m)^2/(n - 1)}.$$

Sequence analysis: The positions of chimera junctions in *recAX7*, *recAX8*, *recAX20*, *recAX21*, *recAX45*, and *recAX53* genes showing unusual FRE characteristics were confirmed by the Sanger method (Sambrook *et al.* 1989).

RESULTS

A lack of correlation observed between FRE values and constitutive SOS expression promoted by RecAX chimeras: Table 1 summarizes the changes in FRE values (named ΔFRE) for different structural types of chimeras described earlier (Ogawa *et al.* 1992) relative to the FRE value of *recAEc*. The ΔFRE values for the chimeras varied widely from ~0.6 (for X49 chimera) up to ~9.0 (for X53). The level of constitutive SOS expression mediated by the different chimeras is also presented relative to RecAEc, which was taken as 1.0. These ΔSOS values varied in range from ~1.3 for X54 to ~3.5 for X47 with a moderate constitutive SOS for several chimeras (X9, X35, X45–X47, and X49) and a weak SOS expression, if any, for most of them. For comparison, a high (17-fold) increase of constitutive SOS by a single *E. coli* mutant *recA730* (Tessman and Peterson 1985; Cazaux *et al.* 1993) results in a 7-fold increase of ΔFRE (V. Lanzov, I. Bakhlanova and A. Clark, unpublished results).

The quantitative correlation coefficient between ΔFRE and ΔSOS values for all 31 chimeras was found to be insignificant (0.144) and, furthermore, this coefficient was negative (−0.268) for the 15 chimeras (X8–X10, X21, X32–X35, X38, X45–X48, X53, and X54), which promoted a threefold and more increase of ΔFRE values. These estimations do not negate earlier observations of qualitative connection between the constitutive SOS expression and FRE increase, but rather they show the existence of some additional parameters that determine quantitative changes of ΔFRE values. In fact, two chimeras with similar ΔSOS values (for example, X7 and X48) showed different ΔFRE values and vice versa (compare X45 and X54). On the other hand, even small changes in the primary structures in related RecAX protein pairs (Figure 1, Table 1) such as RecAX7–X8 (three amino acid substitutions between positions 136 and 150), X20–X21 (one substitution in the region 29–31), X37–X38 (three substitutions in the interval 102–110), and X48–X49 (five substitutions among positions 251–266) resulted in, respectively, four-, three-, two-, and even sixfold ΔFRE alterations under conditions of insignificant differences in their ΔSOS values. Thus, the intrinsic recombinogenic ability of an individual RecA protein may be predetermined by this primary structure.

Inhibition of constitutive SOS expression by *lexA3* mutation does not affect the hyper-rec activity of RecAX proteins: If the hyper-rec activity of RecAX proteins even partially depended on constitutive SOS expression, the addition of a *lexA3* mutation that results in a complete loss of SOS expression to the *recAX* genetic background would proportionally inhibit its hyper-rec activity. This was not the case, because, like RecAXPa, five RecAX chimeras belonging to different structural types (Table 2) did not change the ΔFRE values under conditions of complete suppression of their constitutive SOS. This result was unexpected, at least for RecAX47, in which the ΔSOS value was significant at 3.5. A possible explanation of these findings is the existence of a threshold constitutive SOS expression that must be overcome

TABLE 1
Recombination properties of chimeric *recAX* genes and their proteins *in vivo*

Chimera[a]			Recombinogenic activity[c]		SOS activity	
Type	Name	Structure of RecAX chimeric protein[b] (1 — 100 — 200 — 300)	Number of clones analyzed	ΔFRE[d]	Number of probes analyzed	ΔSOS[d]
	Ec		1100	1.00[e]	12	1.00[e]
	Pa		600	4.42±0.54	12	1.80±0.10
I	X1	58	700	1.24±0.19	8	1.66±0.21
	X2	78	600	1.06±0.17	6	1.86±0.25
	X3	85	700	1.16±0.12	6	2.11±0.29
	X4	113	700	1.40±0.13	4	1.62±0.20
	X5	116	600	1.06±0.08	4	2.00±0.26
	X6	129	600	0.97±0.09	4	1.93±0.27
	X7	136	700	1.11±0.11	4	2.04±0.33
	X8	150	1400	4.91±0.49	4	2.15±0.27
	X9	159	900	4.64±0.50	4	2.39±0.34
	X10	167	700	2.92±0.26	4	1.76±0.21
	X16	256	600	1.45±0.16	4	1.41±0.18
	X17	266	600	0.86±0.09	4	1.50±0.20
	X18	294	600	0.92±0.06	4	1.50±0.21
II	X20	29	800	0.92±0.12	7	1.38±0.20
	X21	31	1400	2.94±0.17	7	1.65±0.28
	X32	189	600	6.17±0.89	5	2.11±0.30
	X33	228	600	5.97±0.68	6	2.03±0.29
	X34	235	600	6.52±0.56	6	2.18±0.30
	X35	251	600	6.20±0.64	6	2.39±0.35
III	X37	58 102	800	1.59±0.20	9	1.33±0.24
	X38	58 110	700	3.51±0.29	9	1.69±0.34
	X45	58 170	600	7.88±0.50	9	2.59±0.36
	X46	58 228	600	5.03±0.34	6	3.05±0.46
	X47	58 235	600	3.00±0.24	6	3.48±0.45
	X48	58 251	600	3.46±0.32	6	2.06±0.32
	X49	58 266	600	0.56±0.08	6	2.60±0.39
	X50	58 284	600	0.97±0.08	6	2.26±0.37
	X51	58 294	600	1.02±0.08	6	2.16±0.36
	X52	58 306	600	1.17±0.19	9	1.66±0.28
IV	X53	170 251	700	8.92±0.59	9	1.31±0.17
	X54	170 251 256	700	7.93±0.59	9	1.29±0.16

[a] Chimera types and names are those that have been used earlier (OGAWA *et al.* 1992).

[b] Solid and open bars show portions of RecAEc and RecAPa proteins, respectively. The position of a chimera junction is shown as that of the first different residue attributed to another protein in the sequence, read from left to right.

[c] The yield of Thr^+Str^r recombinants in all crosses was between 3.5 and 7.7% of donor cells, which were present at levels 10-fold less than that of recipients. The only exception was HfrP4x × JC10289 Δ*recAEc* / pRecA38, which resulted in a recombinant level of 0.5 ± 0.1%.

[d] ΔFRE, calculated from marker coinheritance data, and ΔSOS, measured from the spontaneous level of β-galactosidase from a *sfiA::lacZ* fusion, are FRE and SOS values of RecAX proteins relative to those of RecAEc.

[e] For RecAEc, the absolute value of FRE was 5.01 ± 0.43 (coinheritance of thr^+ and leu^+ markers was 0.931 ± 0.006), and the basal β-galactosidase level was 16.2 ± 1.6 arbitrary units (for details, see MATERIALS AND METHODS).

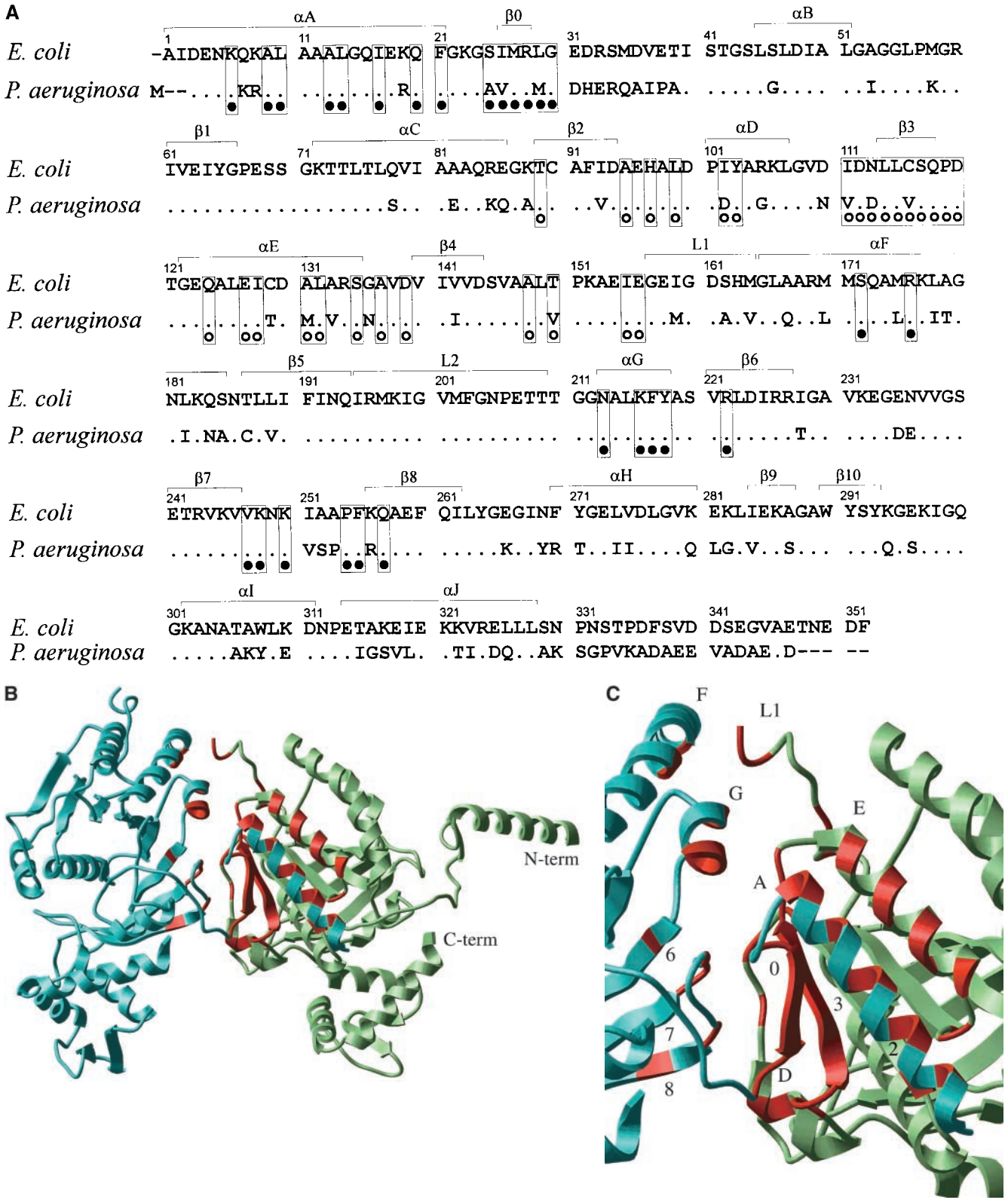

FIGURE 1.—Amino acid residues involved in the interface of subunit-subunit interactions in the RecA polymer structure according to STORY *et al.* (1992). (A) The amino acid sequence alignment of the RecAPa protein relative to RecAEc. The RecAPa amino acid residues identical to those of RecAEc are shown by dots. The residues involved in the interface of subunit-subunit interactions are framed. Solid circles below the sequences indicate residues participating in one face of the interface (they are located in region Ia between positions 6 and 30 and additionally in region Ib between positions 172 and 257). Open circles indicate the residues involved in the structure of the other face located in region II between positions 89 and 156. Designations of α-helices A, B, C, . . . , J; β-strands 0, 1, 2, . . . , 10; and loops L1 and L2 are those used by STORY *et al.* (1992). (B) The spatial structure of RecAEc dimer and the interface of subunit-subunit interaction (STORY *et al.* 1992). Two RecA protomers are shown in cyan and green, and the amino acid residues in the interface are colored red. (C) Details of the spatial structure of the interface shown in B. Letters (A, D, E, G, and F), numbers (0, 2, 3, 6, 7, and 8), and designation L1 show, respectively, the α-helices, the β-strands, and the loop, which form this interface.

TABLE 2

FRE and SOS activities of RecAPa and some RecAX chimeras in genetic background of either a wild-type or mutated *lexA* allele

Chimera			Recombinogenic activity		SOS activity	
Type	Name	*lexA*$^+$ or *lexA3*	No. of clones analyzed	ΔFRE	No. of probes analyzed	ΔSOS
I	X8	*lexA*$^+$	300	4.95 ± 0.45	3	2.36 ± 0.24
	X8	*lexA3*	800	4.09 ± 0.28	6	1.02 ± 0.12
II	X21	*lexA*$^+$	300	3.17 ± 0.25	3	1.83 ± 0.25
	X21	*lexA3*	800	3.00 ± 0.20	6	1.01 ± 0.12
	X32	*lexA*$^+$	300	5.92 ± 0.57	3	2.17 ± 0.22
	X32	*lexA3*	800	6.11 ± 0.57	6	1.01 ± 0.17
III	X47	*lexA*$^+$	300	3.00 ± 0.24	3	3.47 ± 0.37
	X47	*lexA3*	800	3.00 ± 0.20	6	1.09 ± 0.13
IV	X53	*lexA*$^+$	300	8.22 ± 0.57	3	1.43 ± 0.16
	X53	*lexA3*	800	7.12 ± 0.46	6	1.08 ± 0.11
RecAPa		*lexA*$^+$	700	6.52 ± 0.47	3	1.80 ± 0.22
RecAPa		*lexA3*	700	6.58 ± 0.53	6	1.03 ± 0.12

All designations and calculations are similar to those used in Table 1. Donor strain KL226 was used in the ΔFRE determination. The yield of Thr$^+$Str^r recombinants in all crosses was between 2.7 and 5.3% of donor cells.

to trigger FRE alterations. Since none of the proteins analyzed in Table 2 (including the ancestral RecAPa) overcome this threshold, the latter can be estimated as ΔSOS > 3.5.

The recombinogenic activity of the RecAX chimeras depends upon the amino acid content of the three protein regions: The comparison of RecAX chimera structures and ΔFRE alterations (Table 1) showed that all essential FRE determinants reside in the first 255 residues because the C-terminal amino acid residues (256–352) of either RecAPa or RecAEc had no significant influence. In fact, the ΔFRE of RecAX16, consisting of RecAEc over the first 255 residues and the RecAPa over 256 to the C-terminal end, differed insignificantly from the ΔFRE of RecAEc. Another example is the similarity (at least within the limit of error) of the FRE values for X53 and X54, which differ only at region 256–352.

On the basis of their contribution to the ΔFRE values, the RecA chimeras can be divided into three regions: A (residues 1–57), B (58–169), and C (170–255; Table 3). Relative to RecAEc, a three-, eight-, and ninefold increase in recombinogenic activity was seen in chimeras containing a significant portion of either region A (X21), B (X45), or C (X53), respectively, from RecAPa. On the other hand, the chimera with both region B and C from RecAPa showed a basal recombinogenic activity similar to that of RecAEc (compare X1 and X16–X18). However, a small expansion of RecAEc sequence from region A over region B as well as substitution of a small part of the RecAPa sequence in either region B (X8–X9 relative to X1–X7, Figure 1) or region C (X46–X48 *vs.* X1) by RecAEc restored, though incompletely, the high recombinogenic activity of X45 and X53, which was lost in the X1 structure. At last, the chimeric protein with all three (A, B, and C) regions from RecAPa showed a relatively high recombinogenic activity (compare X1 and X35).

The analysis presented above reveals the role of three separate regions of the RecAPa protein in mediating of the hyper-rec activity of RecAX chimeras. The analysis also indicates that some residues in these regions must be better compatible to generate the hyper-rec activity of a RecAX protein.

Several amino acid substitutions are critical for the

TABLE 3

Relative change of recombinogenic activity of RecAX proteins that differ by the amino acid content in three arbitrary regions of the N-terminal and central domains

Chimeric RecA proteins	RecAEc or RecAPa content of RecAX regions covering residue positions			ΔFRE[a]
	1–57 (A)	58–169 (B)	170–256 (C)	
X16, X17, X18	Ec	Ec	Ec	1.1
X21	Pa/Ec	Ec	Ec	2.9
X45	Ec	Pa	Ec	7.9
X53	Ec	Ec	Pa	8.9
X1	Ec	Pa	Pa	1.2
X8, X9	Ec	Ec/Pa	Pa	4.8
X46, X47, X48	Ec	Pa	Pa/Ec	5.0–3.0
X35	Pa	Pa	Pa	6.2

[a] These data were averaged from those presented in Table 1. For RecAEc and RecAPa, ΔFRE values are 1.0 and 6.5, respectively.

RecAX protein recombinogenic activity: Table 1 shows the roles of individual residues or combinations of residues in altering ΔFRE values. RecAX20 differs from X21 by a single residue substitution of residue 29 from leucine to methionine, [L29M] (Figure 1). This substitution resulted in a nearly threefold increase of FRE. Note that this position is located on one of the two surfaces involved in RecAEc subunit-subunit interactions.

RecAX10 and X9, which differ by three substitutions [I159M], [S162A], and [M164V], showed a 1.5-fold difference of ΔFRE. [T150V], the only difference between RecAX9 and X8, appeared to be neutral. The double substitution [G136N] and [V142I], the difference between RecAX8 and X7, showed a nearly 5-fold decrease of ΔFRE. These data indicate that either these residues themselves or the regions where these positions were present could be critical for the RecA protein recombinogenic activity. The substitution [I102D], the only difference between RecAX37 and X38, resulted in a 2-fold increase in recombinogenic activity.

Multiple substitutions in region 170–189 (RecAX45 *vs.* X46), a single substitution [I228T] (RecAX46 *vs.* X47), and the combination of [I251V, A252S, A253P, K256R] substitutions (RecAX48 *vs.* X49) consecutively decreased the RecAX recombinogenic activity from 7.88 to 0.56, making this activity even less than that of an ordinary RecAEc. On the other hand, the double substitution [E235D, N236E] (RecAX47 *vs.* X48) had no noticeable influence on ΔFRE value. These residues are all present in the C region of the RecAX chimeras and all the ΔFRE alterations were observed with the RecAEc sequence in region A and the RecAPa sequence in region B. In principle, these data indicate a functional interconnection between regions A, B, and C in the RecAX proteins. Both the A and C regions contain amino acids that comprise two separate parts, Ia and Ib, of one surface of the RecA protein interface for subunit-subunit interactions whereas the B region contains amino acids from the other surface, II (Figure 1). This suggests that intensity of subunit-subunit interactions during RecA protein polymerization could be one of the factors responsible for recombinogenic activity of the RecA presynaptic complex.

DISCUSSION

A wild-type RecAPa protein initiates recombination exchanges about sixfold more frequently than a wild-type RecAEc protein. This hyper-rec activity is not associated with a noticeable constitutive SOS expression (Namsaraev *et al.* 1998). To verify this observation, 31 recombination-proficient *recAX* chimeras were chosen for a comparative analysis of their constitutive recombinogenic and SOS activities. A quantitative comparison of these parameters showed all combinations of ΔFRE and ΔSOS values in different groups of chimeras, including such contradictory combinations as a high ΔFRE and low ΔSOS (X53 and X54), high ΔFRE and moderate ΔSOS (X33 and X45), and low ΔFRE and moderate ΔSOS (X49), etc. In no instance did a ΔSOS value achieve the level of RecA730 protein, a classical example of a direct correlation between FRE and SOS activities.

To determine if the observed FRE increase was at least partially coupled to the constitutive SOS increase, we measured the ΔFRE values of several chimeras belonging to different structural types in the presence of the *lexA3* mutation, which makes the LexA repressor uninducible. All five chimeras chosen for this analysis, as well as their ancestor RecAPa, showed similar ΔFRE values in both the *lexA*$^+$ and *lexA3* genetic background (Table 2). These findings imply that the moderate level of the constitutive SOS expression promoted by these chimeric RecAX proteins is not sufficient to increase the RecAX recombinogenic activity. It suggests also that a certain threshold in the ΔSOS value determines the

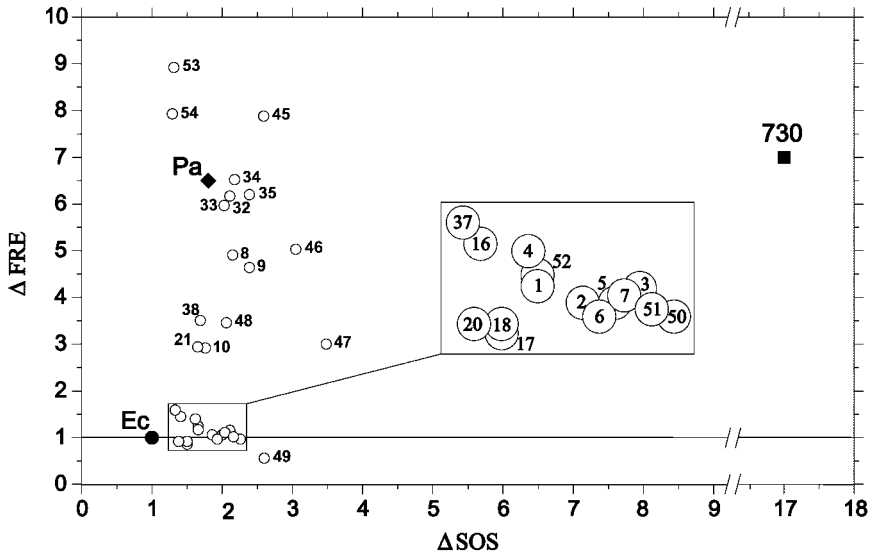

Figure 2.—Relationships between ΔFRE and ΔSOS values found for different RecA proteins. Numbers 1, 2, 3, . . . , 54 designate positions of chimeric RecAX proteins; Ec, Pa, and 730 indicate positions of RecAEc, RecAPa, and RecA730, respectively.

commencement of SOS stimulation of the RecA hyperrec activity. Such a threshold was overcome by RecA730 but not by either RecAPa or the protein chimeras analyzed. The described situation is illustrated in Figure 2. According to presented data, the threshold discussed can be estimated as $\Delta SOS > 3.5$. In other words, a SOS-dependent mechanism of hyperrecombination is at work under conditions of significant derepression of the SOS regulon. Another SOS-independent mechanism is based on RecA protein's internal ability to be more aggressive in initiation of recombination. Both mechanisms can be mutually additive.

The FRE and SOS characteristics of RecAX-related proteins support the idea that intrinsic biochemical properties of the RecA protein determine its ability to initiate recombination. In fact, single residue substitutions [L29M] and [I102D] resulted in a 3- and 2-fold increase of FRE, respectively; a double substitution [G136N, V142I] resulted in a 5-fold decrease of FRE; and several consecutive substitutions of 9, 1, 2, and 4 residues in the region between positions 170 and 265 (see chimeras X45 to X49, Table 1) resulted, respectively, in 1.6-, 1.7-, 0.9-, and 5.4-fold decreases of FRE that give by multiplication a 14-fold FRE decrease. It is noteworthy that these single, double, and multiple substitutions were not coupled to significant, if any, change of the RecAX constitutive SOS activity.

The critical substitutions mentioned above are located either in the N-terminal or in the central domain of RecA (but not in the C-terminal domain) where Ia, Ib, and II regions of the interface between subunits in the RecA polymer are also localized (Figure 1). Furthermore, some of the critical substitutions such as those in positions 29 and 102 directly participate in this interface. These observations suggest that the intensity of subunit-subunit interactions in the RecA filament structure can be one of the factors responsible for hyper- or hyporecombinogenic activity of the RecA protein.

The structure of subunit-subunit interface is crucial to the RecA filamentous structure and, consequently, for all of RecA genetic and biochemical activities. The loss of only one interprotomer contact (for example, by deletion of seven amino acid residues from the N terminus of RecA that includes the K6 residue from the interface, Figure 1A) appears to be sufficient to inactivate the RecA protein (Zaitsev and Kowalczykowski 1998). The [K6A] substitution changes several wild-type RecA activities, showing (1) a significantly reduced affinity for ssDNA, (2) a twofold inhibition of ssDNA-dependent ATPase activity, and (3) a marked delay in the appearance of final products in the strand transfer reaction (Eldin *et al.* 2000).

In contrast to wild-type RecA and the RecA[K6A] mutant protein, another interface mutant, RecA[R28A], shows a higher affinity for ssDNA, only slight decrease in the ssDNA-dependent ATPase, and unusual timing, duration, and level of intermediate and final product formation in the DNA strand exchange reaction. The latter is characterized by a quick accumulation of joint molecules as well as significant delay and decrease in accumulation of final products (Eldin *et al.* 2000). Additionally, the RecA[R28A] protein displays a time-dependent formation of so-called network products, the large DNA-protein complexes resulting from multiple initiation events. These latter characteristics are similar to those described earlier for such hyper-rec proteins as RecA441 and RecAPa (Lavery and Kowalczykowski 1990; Namsaraev *et al.* 1998) and, in principle, predict the hyper-rec phenotype of RecA[R28A].

The third example of interface substitutions is RecA[L29M]. This substitution has dramatic consequences for the RecA properties that are thought to be responsible for the protein recombinogenic activity. These include (1) an increased affinity for double-stranded DNA, (2) a more active displacement of SSB protein from ssDNA, (3) a decreased end-dependent RecA protein dissociation from presynaptic complex, and (4) a greater accumulation of intermediate products relative to the final products in the strand exchange reaction (D. Chervyakova, A. Kagansky, M. Petukhov and V. Lanzov, unpublished results).

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