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RecA Protein of *Escherichia coli* Has a Third Essential Role in SOS Mutator Activity

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The DNA damage-inducible SOS response of *Escherichia coli* includes an error-prone translesion DNA replication activity responsible for SOS mutagenesis. In certain *recA* mutant strains, in which the SOS response is expressed constitutively, SOS mutagenesis is manifested as a mutator activity. Like UV mutagenesis, SOS mutator activity requires the products of the *umuDC* operon and depends on RecA protein for at least two essential activities: facilitating cleavage of LexA repressor to derepress SOS genes and processing UmuD protein to produce a fragment (UmuD′) that is active in mutagenesis. To determine whether RecA has an additional role in SOS mutator activity, spontaneous mutability (tryptophan dependence to independence) was measured in a family of nine *lexA*-defective strains, each having a different *recA* allele, transformed or not with a plasmid that overproduces either UmuD′ alone or both UmuD′ and UmuC. The magnitude of SOS mutator activity in these strains, which require neither of the two known roles of RecA protein, was strongly dependent on the particular *recA* allele that was present. We conclude that UmuD′C does not determine the mutation rate independently of RecA and that RecA has a third essential role in SOS mutator activity.

SOS mutagenesis is due to an error-prone mode of DNA replication expressed in wild-type *Escherichia coli* after exposure to UV light or to chemical agents that block DNA replication and induce the SOS response. In certain mutant strains in which the SOS response is expressed constitutively, SOS mutagenesis is manifested as a mutator activity by which spontaneous mutation rates are elevated as much as 50-fold. In either case, SOS mutagenesis depends on the products of the *recA*, *lexA*, and *umuDC* genes. RecA and LexA proteins regulate the SOS response, and UmuDC proteins are believed to be essential for the actual mutagenic event (for reviews, see references 36 and 37).

RecA protein has two essential roles in SOS mutagenesis. One is its regulatory role. DNA damage generates a signal that results in an altered form of RecA protein (RecA*). RecA* causes the proteolytic cleavage of LexA, the repressor of some 20 genes composing the SOS regulon, including *recA* itself and the *umuDC* operon (for reviews, see references 19 and 28). Amplification of UmuDC is necessary but not sufficient for SOS mutagenesis, as shown by the absence or weak expression of SOS mutator activity in $recA^+$ strains carrying a *lexA*(Def) mutation that inactivates LexA. Such strains become strong mutators only if the $recA^+$ allele is replaced by *recA441* or *recA730*, which encode spontaneously activated RecA proteins, indicating that RecA* has an essential role in SOS mutagenesis other than its antirepressor function (1a, 11, 38). It has recently been shown that RecA* promotes the proteolytic cleavage of UmuD protein both in vitro (5) and in vivo (32) and that only the larger COOH-terminal fragment (UmuD′) is active in UV mutagenesis, whereas the unprocessed UmuD protein is not (27). SOS mutagenesis thus depends on RecA for cleaving both LexA and UmuD, probably by allosteric interactions that facilitate the intrinsic capacity of these proteins for autodigestion (18).

It has been proposed that RecA protein has a third essential role in SOS mutagenesis, perhaps participating directly in translesion DNA replication (4, 9, 10, 36). Evidence for an additional role of RecA in SOS mutagenesis has been offered by Nohmi et al. (27) and by Dutriex et al. (9), both groups working with strains that do not require either of the two known RecA activities. In these strains, LexA cleavage is rendered unnecessary by a *lexA*(Def) mutation that inactivates the repressor, and the need for processing of UmuD is eliminated by a plasmid carrying an appropriately engineered *umuDC* allele encoding the activated COOH-terminal fragment, UmuD′. UV mutagenesis was not detectable in such strains if they carried either a deletion of *recA* (27) or the mutant allele *recA1730* (9).

In this report, we describe experiments using a somewhat different approach to the same question. We measured SOS mutator activity, rather than UV mutability, in a family of congenic *lexA*(Def) strains, each carrying one of nine different *recA* alleles and transformed or not with plasmid pGW2123, which carries *umuD′C* and therefore overproduces both UmuD′ and UmuC, or with pGW2122 (*umuD′*), which overproduces only UmuD′ (27). We anticipated that the magnitude of SOS mutator activity would not differ in these strains unless RecA protein can influence the mutagenic process and unless at least some of the various RecA proteins differ in their ability to exert this influence. Our results led us to conclude that RecA has a third essential role in SOS mutagenesis.

MATERIALS AND METHODS

Strains. Table 1 shows the strains and plasmids used and describes their mode of origin or source. Strain construction utilized standard methods of P1 transduction (25) and/or transformation (15). The presence of plasmids carrying the unaltered chromosomal inserts (*umuD′C* or *umuD′*) was verified by isolating plasmid DNA (22) from each chosen transformant, comparing its migration on agarose gels with

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TABLE 1. Bacterial strains and plasmids

E. coli strain or plasmid	Relevant genotype[a] or description	Source or reference
Strains		
EW301	*recA730 lexA71*::Tn*5*(Def)	38; formerly SC30-SP
EW302	*recA730 lexA71*::Tn*5 umuD44*	This study, from EW301
EW411	*recA441 lexA51*(Def)	38; formerly SC30-TF-SP
EW412	*recA441 lexA51 umuD44*	This study, from EW411
EW181	*recA718 lexA71*::Tn*5*	40; formerly SC18-SP
EW182	*recA718 lexA71*::Tn*5 umuD44*	This study, from EW181
EW501	*recA750 lexA71*::Tn*5*	This study[b]
EW502	*recA750 lexA71*::Tn*5 umuD44*	This study, from EW501
EWRP1	*recA*$^+$ *lexA51*	This study, from SC30-RP1 (13)
EWRP2	*recA*$^+$ *lexA51 umuD44*	This study, from EWRP1
JS201	*recA720 lexA51*	This study[b]
JS202	*recA720 lexA51 umuD44*	This study, from JS201
JS271	*recA727 lexA71*::Tn*5*	This study[b]
JS272	*recA727 lexA71*::Tn*5 umuD44*	This study, from JS271
JS431	*recA430 lexA71*::Tn*5*	This study, from SC430 (40)
JS432	*recA430 lexA71*::Tn*5 umuD44*	This study, from JS431
EW061	*recA* Δ(*srlR-recA*)*306*::Tn*10 lexA51*	This study, from SC30-TF (39)
Plasmids		
pGW2122	Encodes the COOH-terminal portion of UmuD corresponding to UmuD′	27
pGW2123	Like pGW2122, but encodes both UmuD′ and UmuC	G. Walker

[a] All strains are also *uvrA155 trpE65 lon-11 sulA1* and were derived by P1 transductions from strain SC30 (39), except strains EW181, -182, -501, and -502, which were derived by P1 transductions from strain SC18 (39); SC30 and SC18 were isolated as segregants from a single colony and are assumed to be congenic. Although they are B/K-12 hybrids, they have most of the markers and properties of their B/r parent, strain WP2s, including the B restriction and modification system. All *umuD44* strains are also *fadR613*::Tn*10* and were constructed by selection for tetracycline resistance. The *umuD*$^+$ strains all have *srl*$^+$ linked to their *recA* alleles.

[b] *recA750* was selected as a temperature-resistant revertant of strain SC1812 (*recA718 polA12*), which does not grow on rich medium at 37°C or higher (E. Witkin, unpublished data); *recA720* and *recA727* were selected (J. B. Sweasy, Ph.D. thesis, Rutgers, the State University of New Jersey, Piscataway, 1989) as fully UV-resistant and partially UV-resistant revertants, respectively, of strain SC430 (*recA430*) (40).

that of the original plasmid, and testing its ability to complement the defects of *umuD* and/or *umuC* mutants in secondary transformations.

Culture media. Liquid medium was nutrient broth (Difco Laboratories, Detroit, Mich.) with 0.5% NaCl added (NB). Solid media were $MCHt_{.2a}$ (minimal medium E [35] supplemented with 0.4% glucose, 0.4% Casamino Acids [Difco], 0.2 μg of tryptophan per ml, and 100 μg of adenine per ml) and minimal medium E supplemented only with 0.4% glucose. Both media were solidified with 1.5% Bacto-Agar (Difco). Ampicillin (50 μg/ml; Sigma Chemical Co., St. Louis, Mo.) was routinely added to liquid and solid media used for growth of any strain carrying pGW2122 or pGW2123.

SOS mutator activity. Mutations from Trp$^-$ to Trp$^+$ were scored. Cultures were grown overnight in NB on a shaker at 30 or 37°C, diluted 10^{-1} into fresh NB, shaken at 30 or 37°C for 90 min, and then diluted 10^{-1} in E salts to yield suspensions of 2×10^7 to 4×10^7 cells per ml. Samples (0.1 ml) of this suspension were spread on the surface of E agar (to determine the number of fully expressed Trp$^+$ mutants present in the inoculum) and on $MCHt_{.2a}$ agar in triplicate or quadruplicate. Plates were incubated at 42°C. Trp$^+$ colonies were counted after 2 days.

Plasmid copy number. Copy number of pGW2122 was determined by the use of quantitative slot-blot hybridization (7). Radioactive probes for *E. coli* or plasmid (pBR322) sequences were prepared by nick translation of the appropriate DNAs. (pGW2122 and pGW2123 are derivatives of pBR322.) DNA for slot-blot hybridization was prepared from cultures incubated overnight at 42°C in NB plus ampicillin. DNA samples from various *recA* strains containing pGW2122 (*umuD′*) were hybridized to the two radioactive probes. Amounts of DNA used were within the ranges in which hybridization of the nick-translated DNAs to standards (*E. coli* DNA and pBR322 DNA) had been found to be linear. Chromosomal DNA concentration was estimated with the labeled *E. coli* probe, and the plasmid concentration was estimated with the labeled pBR322 probe. The copy number of plasmids per cell was calculated from the ratio of plasmid to chromosomal DNA, assuming that the chromosome is 3×10^6 base pairs and the plasmid is 4.7×10^3 base pairs in size.

RESULTS

Phenotypes conferred by nine *recA* alleles. Table 2 shows the nine *recA* alleles used in this study and compares some of their phenotypic effects. Four of these alleles encoded RecA proteins that can become constitutively activated for coprotease activity (RecA730, -441, -718, and -720), and four of the others required DNA damage to exhibit either normal (RecA$^+$ and RecA750) or partially deficient (RecA727 and -430) RecA* activity. Five of the nine alleles conferred normal UV resistance, and six conferred normal UV mutability, although they differed in SOS mutator activity. Only RecA730 and RecA441 conferred mutator activity in *lexA*$^+$ strains, indicating that uninduced amounts of these RecA proteins can cause cleavage of LexA and that both proteins can also cause processing of UmuD to UmuD′ in undamaged cells. Two of the other RecA proteins (-718 and -720) conferred strong mutator activity only in *lexA*(Def) strains, showing that amplified amounts of these proteins are able to process UmuD to UmuD′ but that uninduced amounts of RecA718 or RecA720 cannot cleave LexA in undamaged cells. The other RecA proteins (RecA$^+$, -750, -727, and -430)

TABLE 2. *recA* alleles used and some of their phenotypic effects

recA allele	Recombination	UV resistance	UV mutability	SOS response (*lexA*$^+$)	Relative spontaneous mutability[a]	
					lexA$^+$	*lexA*(Def)
+	+++	++++	++++	Inducible	1[b]	2.5
730	++++	++++	++++	Constitutive[c]	50	50
441	++++	++++	++++	Constitutive[c]	25	30[c]
718	+++	+++	++++	Inducible	1	30[c]
720	+++	++++	++++	Inducible	1	30[c]
750	+++	++++	++++	Inducible	1	5
727	+++	++	−	Partially inducible	1	1
430	+++	+	−	Partially inducible	1	1
Δ*306*	−	±	−	Noninducible	0.4	0.4

[a] Incubated at 42°C in the presence of adenine.
[b] Corresponds to a mutation rate of about 10^{-8} (Trp$^-$ to Trp$^+$).
[c] *recA730* strains are SOS constitutive at any temperature; *recA441* strains are SOS constitutive and are mutators only at elevated temperatures in the presence of adenine; when also *lexA*(Def), *recA718* and *recA720* strians are mutators at any temperature. Changes in protein sequence from RecA$^+$ (30) are Glu-38 to Lys in RecA441 (17); Glu-38 to Lys and Leu-126 to Val in RecA718 (23); Gly-204 to Ser in RecA430 (33); Gly-204 to Ser and Thr-39 to Ile in RecA720 (Sweasy, Ph.D. thesis); and Gly-204 to Ser and Glu-18 to Lys in RecA727 (Sweasy, Ph.D. thesis).

caused only weak mutator activity or none at all in *lexA*(Def) strains and are presumably deficient in the ability either to effect UmuD processing or to perform some other unknown RecA function required for SOS mutagenesis or both, at least in undamaged cells. RecA430 is defective in processing UmuD but can promote UV mutagenesis when UmuD' is supplied on a plasmid (27).

Overproduction of UmuD' and UmuD'C in *recA*-deleted strain. Table 3 shows that spontaneous mutability is not increased by pGW2122 (*umuD'*) or pGW2123 (*umuD'C*) in a *lexA*(Def) *recA*-deleted strain, although both plasmids confer mutator activity on a *lexA*(Def) *umuD44 recA*$^+$ strain. RecA protein was clearly required for detection of SOS mutator activity even when amplified amounts of UmuD' and UmuC were present. However, this result does not prove that RecA protein is required for a third role in SOS mutagenesis, because RecA may be required only for the survival of potential mutants (see Discussion).

Overproduction of UmuD' and UmuC in *umuD44* strains carrying eight different *recA* alleles. Table 4 compares the spontaneous mutability of eight sets of *lexA*(Def) strains, each set comprising four strains carrying a unique *recA* allele, and Fig. 1 displays these data graphically. When *umuD*$^+$ was on the chromosome and no plasmid was present, four of the strains (*recA730*, *-441*, *-718*, and *-720*) showed strong mutator activity and the other four did not. The *umuD44* derivatives of these strains were transformed with pGW2123 (*umuD'C*). (Without the plasmid, the *umuD44* allele reduces the number of Trp$^+$ colonies per plate in all the *lexA*(Def) strains to fewer than six per plate, except in the *recA730* strain, in which about 30 Trp$^+$ colonies per plate are produced [data not shown].) Strikingly, the overproduction of both UmuD' and UmuC restored about the same amount of mutator activity to the *umuD44* derivatives of the four strong mutator strains that they expressed without the plasmid but did not significantly increase this activity. The low spontaneous mutability of each of the other four *lexA*(Def) *umuD*$^+$ strains was somewhat increased by pGW2123 (*umuD'C*) in their *umuD44* derivatives, to an extent that varied with the *recA* allele, although in none of these strains did the mutator activity approach that of the four strong mutators. The presence of chromosomal *umuD*$^+$ instead of *umuD44* does not significantly alter the mutability of strains transformed with pGW2123 (*umuD'C*) (data not shown). Different RecA proteins evidently modulate the magnitude of SOS mutator activity distinctively even in strains not requiring RecA-mediated cleavage of LexA or of UmuD.

TABLE 3. Effect of pGW2122 (*umuD'*) and pGW2123 (*umuD'C*) on spontaneous mutability of *recA*$^+$ *lexA*(Def) and Δ*recA306* *lexA*(Def) strains

Strain	*recA* allele	No. of Trp$^+$ colonies per plate[a]
EWRP1	*recA*$^+$	17.6 ± 2.4
EWRP2(p*umuD'*)	*recA*$^+$	157.5 ± 17.6
EWRP2(p*umuD'C*)	*recA*$^+$	149.3 ± 9.8
EW061	Δ*recA306*	2.0 ± 1.4
EW061(p*umuD'*)	Δ*recA306*	2.8 ± 1.9
EW061(p*umuD'C*)	Δ*recA306*	2.5 ± 1.7

[a] Each number is the average of at least 12 plates and four separate experiments.

Selective overproduction of UmuD' in *umuD*$^+$ and *umuD44* strains carrying eight different *recA* alleles. Table 4 and Fig. 1 also show the spontaneous mutability of *umuD*$^+$ and *umuD44* strains carrying the same eight *recA* alleles but transformed with pGW2122 (*umuD'*) instead of pGW2123 (*umuD'C*). Within the group of four alleles that encode RecA*-constitutive proteins and therefore promote strong mutator activity without the plasmid, the effect of selective amplification of UmuD' varied greatly with the *recA* allele. When *umuD*$^+$ was on the chromosome, overproduction of UmuD' decreased the mutator activity of the *recA730* strain about 25% but did not significantly reduce the mutability of the *recA441* and *recA718* strains. In the *recA720* strain, however, overproduction of UmuD' eliminated nearly 80% of the spontaneous mutability of this stain. This drastic effect in the *recA720* strain was surprising, because its mutator activity was otherwise very similar to that of the *recA441* and *recA718* strains, either without the plasmid or when both UmuD' and UmuC were overproduced. In the *umuD*$^+$ *recA750*, *recA*$^+$, and *recA727* strains, overproduction of UmuD' alone caused about the same moderate increase in mutability as the overproduction of both Umu proteins. The low spontaneous mutability of the *umuD*$^+$ *recA430* strain, which was barely increased at all by pGW2123 (*umuD'C*),

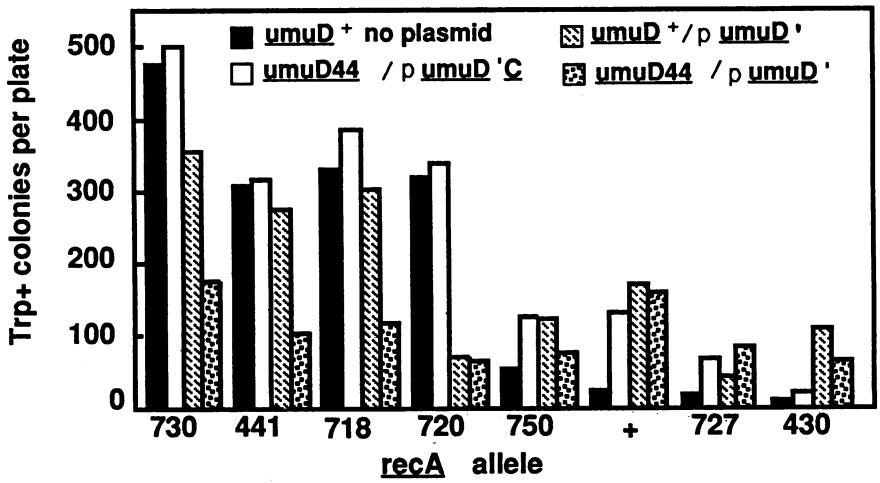

FIG. 1. Spontaneous mutability of *lexA*(Def) strains carrying eight different *recA* alleles. Data are the averages shown in Table 4.

was actually increased 10-fold by overproduction of UmuD' alone.

The effect of overproducing UmuD' in three of the four RecA*-constitutive strains (*recA730*, *-441*, and *-718*) depended on the chromosomal *umuD* allele. pGW2122 (*umuD'*) restored much less mutator activity to the *umuD44* derivatives of these strains than was expressed in their *umuD*$^+$ counterparts transformed with this plasmid. In the *recA720* strain, mutability was low when UmuD' was overproduced whether the chromosomal allele was *umuD44* or *umuD*$^+$, and the same was true of the four nonmutator strains. The presence of *umuD44* had a leveling effect, in that the overproduction of UmuD' failed to confer strong mutator activity on any of the eight *umuD44* strains and thus largely eliminated the sharp distinction between strains having constitutively activated RecA proteins and the others.

Plasmid copy number in strains carrying four different *recA* alleles. To rule out the unlikely possibility that different *recA* alleles might affect the copy number of the plasmid and that the observed differences in spontaneous mutability might result, we determined the copy number of pGW2122 (*umuD'*) in four of the strains showing the most widely disparate responses to the presence of the plasmid: EW302 (*recA730*), EWRP2 (*recA*$^+$), JS212 (*recA720*), and JS432 (*recA430*). The copy number varied between 94 and 130 per cell in overnight cultures grown in NB plus ampicillin and thus could not have determined the differences in mutability.

DISCUSSION

The spontaneous mutability of strains carrying eight different *recA* alleles is determined by the particular RecA protein that is present, even when the need for the two known functions of RecA in SOS mutagenesis is obviated by a *lexA*(Def) mutation and a plasmid that supplies UmuD' or UmuD'C (Fig. 1; Table 4). The strong SOS mutator activity expressed in four of the *umuD*$^+$ strains without any plasmid (those containing constitutively activated RecA730, -441, -718, and -720 proteins) was eliminated by the *umuD44* allele and was fully restored, but not significantly increased, by pGW2123 (*umuD'C*). In the strains whose RecA proteins are not constitutively activated, pGW2123 (*umuD'C*) did not confer strong mutator activity, although it increased spontaneous mutability to an extent that depended on the *recA* allele, causing a 10-fold increase in the *recA*$^+$ strain but a

TABLE 4. Effect of pGW2123 (*umuD'C*) and pGW2122 (*umuD'*) on spontaneous mutability of *lexA*(Def) strains carrying eight different *recA* alleles

Strain	Chromosomal genotype		No. of Trp$^+$ colonies per plate[a]
	recA	*umuD*	
EW301	*730*	+	479.2 ± 32.4
EW301(p*umuD'*)	*730*	+	362.7 ± 28.2
EW302(p*umuD'*)	*730*	*44*	174.6 ± 22.2
EW302(p*umuD'C*)	*730*	*44*	504.3 ± 31.1
EW411	*441*	+	312.2 ± 27.4
EW411(p*umuD'*)	*441*	+	280.1 ± 26.5
EW412(p*umuD'*)	*441*	*44*	105.9 ± 15.9
EW412(p*umuD'C*)	*441*	*44*	317.0 ± 31.1
EW181	*718*	+	328.5 ± 24.9
EW181(p*umuD'*)	*718*	+	305.1 ± 27.9
EW182(p*umuD'*)	*718*	*44*	115.7 ± 8.9
EW182(p*umuD'C*)	*718*	*44*	392.1 ± 33.3
JS201	*720*	+	317.0 ± 29.2
JS201(p*umuD'*)	*720*	+	68.2 ± 9.5
JS202(p*umuD'*)	*720*	*44*	67.9 ± 10.4
JS202(p*umuD'C*)	*720*	*44*	338.3 ± 34.6
EW501	*750*	+	52.0 ± 7.0
EW501(p*umuD'*)	*750*	+	118.4 ± 10.7
EW502(p*umuD'*)	*750*	*44*	75.2 ± 9.9
EW502(p*umuD'C*)	*750*	*44*	131.6 ± 6.9
EWRP1	+	+	17.6 ± 2.4
EWRP1(p*umuD'*)	+	+	167.4 ± 14.8
EWRP2(p*umuD'*)	+	*44*	159.1 ± 21.2
EWRP2(p*umuD'C*)	+	*44*	161.9 ± 13.7
JS271	*727*	+	17.0 ± 2.8
JS271(p*umuD'*)	*727*	+	39.3 ± 7.8
JS272(p*umuD'*)	*727*	*44*	79.1 ± 5.8
JS272(p*umuD'C*)	*727*	*44*	82.2 ± 6.2
JS431	*430*	+	10.4 ± 3.0
JS431(p*umuD'*)	*430*	+	109.8 ± 11.7
JS432(p*umuD'*)	*430*	*44*	68.4 ± 6.8
JS432(p*umuD'C*)	*430*	*44*	19.5 ± 2.8

[a] Average of four experiments and at least 16 plates; any Trp$^+$ mutants present in the plated inocula were subtracted.

barely 2-fold increase in the *recA430* strain. These results, encompassing a 25-fold difference in mutator activity between the *recA730* and *recA430* strains, lead to the conclusion that UmuD'C, even when overproduced, does not determine the spontaneous mutation rate independently of RecA and that SOS mutator activity requires a third RecA function in addition to cleavage of LexA and processing of UmuD.

The failure of pGW2122 (*umuD'*) or pGW2123 (*umuD'C*) to increase the spontaneous mutability of the *recA*-deleted strain (Table 3) is consistent with this conclusion but does not prove it, because another explanation is possible in this case. It has been suggested (4) that Umu-dependent SOS mutator activity is actually targeted at spontaneously occurring replication-blocking lesions such as apurinic sites, which are mutagenic via SOS processing (31). RecA protein would probably then be required for the survival of any potential mutant, and all potential mutants in a *recA*-deleted strain would die. A similar possibility was suggested as one way to explain the failure to detect UV mutagenesis in a *lexA*(Def) *recA*-deleted *E. coli* K-12 strain transformed with pGW2122 (27). UV mutagenesis is targeted at UV photoproducts that block DNA synthesis (26), and RecA is required for the ability to restart UV-inhibited replication (16, 40) as a prerequisite for survival. Thus, *recA*-deleted strains may not be informative about a possible third role of RecA in SOS mutagenesis.

Our data suggest that the ability to perform the third role is correlated with the ability of the various RecA proteins to become spontaneously activated. Four of the RecA proteins (RecA730, -441, -718, and -750) showed strong RecA* activity in undamaged cells, and only these four promoted strong mutator activity in the *umuD44* strains transformed with pGW2123 (*umuD'C*). The other four RecA proteins, which promoted much less mutability when UmuD' and UmuC were overproduced, either required DNA damage to become activated ($RecA^+$ and RecA750) or were partially deficient in RecA*-mediated activities even after DNA damage (RecA727 and -430).

The ability of the various RecA proteins to perform the third role in SOS mutator activity is probably also correlated with their ability to bind to small single-stranded regions of DNA in undamaged cells. In vitro, the activation of RecA to its coprotease form (RecA*) requires its interaction with polynucleotide and an adenine nucleotide (8). Purified RecA441, but not $RecA^+$, can be activated for repressor cleavage by binding to very small single-stranded oligonucleotides (as small as hexamers) and is therefore thought to become constitutively activated by binding the small regions of single-stranded DNA that exist in undamaged cells, whereas activation of $RecA^+$ protein requires longer stretches of single-stranded DNA, such as those resulting from DNA damage (24, 29). Although the DNA-binding activities of RecA730, -718, and -720 have not been determined, their ability to become constitutively activated suggests that they too, like RecA441, do so by binding the small single-stranded regions in undamaged cells. Lu and Echols (20) have shown that the decreasing ability of RecA441, $RecA^+$, and RecA430, in that order, to bind UV-irradiated double-stranded DNA (binding which they believe to be to the single-stranded DNA flanking helix-distorting bulky UV photoproducts) correlates with their decreasing efficiencies in LexA cleavage and SOS mutagenesis, and it clearly correlates as well with their decreasing ability to perform the third role of RecA in SOS mutator activity (Fig. 1). Whatever the additional role of RecA in SOS mutator activity may be, we propose that it can be performed only to the extent that a given RecA protein can bind small regions of single-stranded DNA, probably either at the replication fork (30a) or at mutational target sites, or both, to become activated in undamaged cells.

Six of the eight RecA proteins involved in this study showed normal UV mutability, although many of them differed quite markedly in their SOS mutator activity (Table 2). All six of these RecA proteins (RecA730, -441, -718, -720, -750, and $RecA^+$) are probably equally able to bind the longer stretches of single-stranded DNA generated by UV irradiation and are then equally able to cleave LexA, process UmuD, and perform the additional role(s) of RecA required for UV mutagenesis. We suggest that they differ from each other, however, in their ability to bind the smaller single-stranded DNA regions in unirradiated cells, and that among these six RecAs, $RecA^+$ and RecA750, even when amplified in *lexA*(Def) strains, are less efficient than the other four in this binding ability and thus show lower spontaneous mutability both with and without pGW2123 (*umuD'C*).

The effects of selective overproduction of UmuD' are more difficult to interpret than those of overproducing both Umu proteins. The influence of pGW2122 (*umuD'*) is strongly allele specific within the group of four strains having constitutively activated RecA proteins when $umuD^+$ is on the chromosome. Overproduction of UmuD' decreases mutability either moderately (RecA730), drastically (RecA720), or not significantly (RecA441, −718). Such allele specificity is consistent with protein-protein interaction and suggests that a particular kind of RecA interaction with the Umu proteins, in addition to RecA binding to single-stranded DNA, is required for SOS mutator activity. Another puzzling result is the powerful decrease in mutability caused by the chromosomal *umuD44* allele in the RecA*-constitutive strains when UmuD' is overproduced. In the presence of *umuD44*, UmuD' overproduction restores relatively little mutability to any of the eight strains, regardless of its *recA* allele.

One way to explain these observations stems from the assumption that a RecA*-UmuD'C complex bound at the site of mutation is a necessary intermediate in SOS mutagenesis. When UmuD' is overproduced but UmuC is not, the chromosomally encoded $UmuD^+$ may combine rapidly with UmuC (perhaps because they are coordinately translated from the same mRNA), leaving very little free UmuC to combine with plasmid-encoded UmuD'. In that case, overproduction of UmuD' will greatly reduce mutator activity in any strain whose RecA protein preferentially combines with UmuD' rather than with UmuDC (RecA720?), because nonfunctional RecA*-UmuD' complexes will saturate potential sites of mutation. In strains whose RecA proteins preferentially combine with UmuDC (RecA441 and -718?), functional RecA*-UmuD' C complexes will form more readily at such sites, and mutator activity will be high. Chromosomally encoded UmuD44 might also titrate out UmuC, and this could be why mutability is low in all eight *umuD44* strains transformed with UmuD', whatever their *recA* allele. This possibility is consistent with our observation (not shown) that the mutability of strains transformed with pGW2123 (*umuD'C*) is the same whether $umuD^+$ or *umuD44* is the chromosomal allele. Thus, UmuD44 exerts its strong antimutagenic effect when UmuD' alone is in excess but does not inhibit or poison SOS mutagenesis when UmuC protein is also overproduced. Although other explanations are certainly possible, it is difficult to account for these results without postulating that different RecA proteins interact

differentially with UmuD, UmuD44, UmuD', UmuC, or complexes of the various UmuD proteins with UmuC and that some of these interactions are more conducive to mutagenesis than others.

The low spontaneous mutability of the *recA750*, *recA*$^+$, *recA727*, and *recA430* strains was increased, albeit sometimes only slightly, by transformation with either plasmid (Fig. 1). This could mean that the ability of the corresponding RecA proteins to process UmuD is limiting without the plasmid, but given UmuD', these RecA proteins can then perform the third role required of RecA somewhat better, possibly because interaction with UmuD' or UmuD'C may cause allosteric effects that enhance the binding efficiency of these RecA proteins.

Our results are compatible with any of several possible third roles that have been proposed for RecA in SOS mutagenesis (9, 27). RecA* activity could be required to activate another protein proteolytically; to inactivate another repressor; or to interact with DNA polymerase III, which has been implicated in SOS mutagenesis (3, 14), perhaps to inhibit the proofreading activity of its epsilon subunit (21, 34) or to promote a template conformation conducive to translesion replication (4, 10). Interaction of RecA* with UmuD or a UmuDC complex is implied by the RecA*-mediated processing of UmuD and by the inability of *recA718* strains to restart DNA replication after UV irradiation unless functional UmuD'C proteins are present (40). Radioactively labeled UmuC in crude extracts of maxicells containing pGW2123 has recently been shown to interact with a RecA* affinity column (12). RecA* could guide the Umu proteins to the site of mutation, providing lesion recognition in targeted SOS mutagenesis. Once having delivered UmuD'C to the target, RecA could still be required for one or more of the other activities mentioned above, or it could have no further role, as suggested by the occurrence of some UV mutagenesis in delayed photoreversal experiments in a *recA*-deleted Umu$^-$ strain (2). RecA might even have to be released from the site as a prerequisite for translesion replication. In that case, the failure of RecA1730 to be released from the target site after delivering UmuD'C could account for the dominance exerted by this mutant protein in preventing the conferring of UV mutability by pGW2122 (*umuD'*) (9).

Our conclusions concerning the third role of RecA protein apply only to SOS mutator activity. However, it appears likely that Umu-dependent SOS mutator activity is largely targeted and that its mechanism, like that of SOS mutagenesis induced by DNA-damaging treatments, is translesion replication. An untargeted component of SOS mutator activity, usually eliminated by mismatch repair, was revealed in SOS-constitutive strains lacking mismatch repair activity (6), implying that the Umu-dependent residue of mutations not subject to mismatch correction is targeted. Bridges and Woodgate (4) have demonstrated that UV-induced mutations can be detected in Umu$^-$ strains after delayed photoreversal of UV damage, leading them to propose a two-stage model of targeted SOS mutagenesis. According to this model, Umu proteins are not required for the misincorporation step of translesion replication, which is influenced by RecA protein, but only for the resumption of replication after the misinsertion. This model also assumes that Umu dependence is indicative of targeted mutagenesis. Battista et al. (1) have observed a sequence similarity between UmuD' and T4 gene product 45 (which promotes processivity of T4 polymerase) and between UmuC and T4 gene product 44/62 (which functions as a single-stranded DNA ATPase). If these sequence similarities bespeak functional homologies, the comparable activities of UmuD'C might well foster successful translesion replication but would scarcely be needed for untargeted replication errors.

A convincing description of the third role (and possible additional roles) of RecA protein in SOS mutagenesis will probably require the development of an in vitro system of translesion DNA replication with purified proteins. Studies with the purified RecA mutant proteins used in this investigation and with their interactions with purified UmuD, UmuD', and UmuC proteins may eventually explain many of our results and should provide a test of the predicted relative binding affinities of the mutant RecA proteins for small regions of single-stranded DNA. Evidence for interaction of purified UmuC with both UmuD and UmuD' in vitro has been described previously (41), and in another study (J. Battista, personal communication), UmuC has been found to interact with UmuD'.

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