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Dominant negative *umuD* mutations decreasing RecA-mediated cleavage suggest roles for intact UmuD in modulation of SOS mutagenesis

(SOS response/UV mutagenesis/*umuDC*/posttranslational regulation)

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ABSTRACT **The products of the SOS-regulated *umuDC* operon are required for most UV and chemical mutagenesis in *Escherichia coli*. The UmuD protein shares homology with a family of proteins that includes LexA and several bacteriophage repressors. UmuD is posttranslationally activated for its role in mutagenesis by a RecA-mediated proteolytic cleavage that yields UmuD′. A set of missense mutants of *umuD* was isolated and shown to encode mutant UmuD proteins that are deficient in RecA-mediated cleavage *in vivo*. Most of these mutations are dominant to *umuD*+ with respect to UV mutagenesis yet do not interfere with SOS induction. Although both UmuD and UmuD′ form homodimers, we provide evidence that they preferentially form heterodimers. The relationship of UmuD to LexA, λ repressor, and other members of the family of proteins is discussed and possible roles of intact UmuD in modulating SOS mutagenesis are discussed.**

umuD and *umuC* mutants of *Escherichia coli* are virtually nonmutable with UV and many chemicals, suggesting that the UmuD and UmuC proteins are required for most mutagenesis by these agents (1–7). Evolutionarily diverged analogs of these proteins that can substitute for UmuD and UmuC function have been characterized and are encoded by naturally occurring plasmids: MucA and MucB by plasmid pMK101 (8, 9) and ImpA and ImpB by plasmid TP110 (ref. 10; P. Strike, personal communication). Recent evidence has indicated that the RecA protein (11–14) and the heat-shock-regulated chaperonins GroEL and GroES (15) are also required for SOS mutagenesis.

Both the *umuDC* operon and the plasmid-derived *mucAB* operon are repressed by the LexA protein (1, 2, 16) and are regulated as part of the SOS response of *E. coli* (3–5, 17, 18). SOS induction occurs when activated RecA (designated RecA*) mediates the proteolytic cleavage of the bond between Ala-84 and Gly-85 of LexA (19), apparently by facilitating an otherwise latent capacity of the LexA to autodigest (20). LexA shares homology with the repressors of bacteriophages λ, 434, P22, and φ80 (21, 22), and cleavage of these proteins appears to occur by an analogous mechanism.

Our observation that UmuD and MucA share homology with the carboxyl-terminal domains of LexA and the phage repressors led us to postulate that these proteins are posttranslationally activated for their roles in mutagenesis by a RecA-mediated proteolytic cleavage (8). RecA-mediated cleavage of UmuD at its bond between Cys-24 and Gly-25 has now been shown to occur *in vivo* (23) and *in vitro* (24). Genetic studies have shown that the purpose of this cleavage is to activate UmuD for its role in mutagenesis and that the carboxyl-terminal fragment of UmuD, UmuD′, is both necessary and sufficient for this role (11). Genetic observations suggested that the physical interaction of UmuD (or a UmuD derivative) with UmuC is necessary for UV mutagenesis (8) and recent biochemical studies have provided evidence that renatured UmuC can form a complex with a homodimer of UmuD′ (25). The biochemical roles of UmuD′ and UmuC in mutagenesis have not yet been determined although various models have been suggested (26–29). On the basis of amino acid similarity of UmuD and UmuC to the bacteriophage T4 *45*, *44*, and *62* gene products, we have suggested (30, 31) that the molecular actions of these proteins may be related to those of these DNA polymerase accessory proteins.

The genetic studies described in this paper help define the relationship between the mutagenesis proteins UmuD, MucA, and ImpA and the closely related group of repressors that includes LexA and λ repressor. In addition, we provide physical evidence that UmuD and UmuD′ form heteodimers *in vivo* and discuss the possible biological implications of our observations.

MATERIALS AND METHODS

pGW2020 (11) was treated with hydroxylamine under conditions that gave rise to approximately one adduct per plasmid. Mutagenized plasmids were transformed into GW3200 (*umuD44*) (11) and screened for failure to complement fully the deficiency of GW3200 in a UV-induced *argE3* reversion to an Arg+ phenotype (*argE3* → Arg+ reversion) (1). Dideoxy-nucleotide sequencing was carried out as described (11). After UV-irradiation of cells and incubation at 37°C for 1 hr, UmuD cleavage was assessed by centrifuging the cells, electrophoresing the proteins from 4×10^8 cells on a 14% polyacrylamide gel containing SDS, transferring the proteins to Immobilon-P, and blotting with affinity-purified antibodies raised against UmuD′. The antibody reacted equally well with UmuD and UmuD′ at the 1:10,000 dilution used in these studies. Cross-reacting material was visualized by using goat anti-rabbit antisera conjugated to horseradish peroxidase. The overproduction and purification of UmuD and UmuD′ will be described elsewhere. Glutaraldehyde cross-linking studies were carried out as described (32).

RESULTS

Isolation and Sequencing of *umuD* Mutants. After treatment with hydroxylamine, pGW2020, a multicopy plasmid carrying the *umuD*+ gene but not the *umuC*+ gene (11), was transformed into a *umuD44* strain. We then screened the transformants for derivatives that showed a reduced frequency of UV-induced *argE3* → Arg+ reversion (1). Of the 5000 transformants screened, 15 independent plasmids were isolated that appeared to carry *umuD* mutations. These mutant plasmids did not have any obvious deleterious effects on the growth of the strains carrying them. The location of the *umuD* mutation in each plasmid was determined by sequenc-

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ing the upstream and coding regions of *umuD* from the −90 position through the termination codon. Each mutant plasmid carried a single GC → AT transition mutation within the coding sequence of UmuD. Of the 15 mutations we sequenced, 11 were different (Table 1). Ten missense mutations and 1 nonsense mutation were obtained. Examination of the plasmid-encoded proteins synthesized in maxicells (33) revealed that all of the missense *umuD* plasmids encoded a single band that migrated on the gel to a position identical to, or close to, that of UmuD$^+$ (data not shown). These bands were of essentially the same intensity as the UmuD$^+$ band indicating that, at least in maxicell conditions, these mutations did not affect the stability of the UmuD derivatives.

Deficiencies of the *umuD* Mutants in UV Mutagenesis. All of the plasmids carrying *umuD* mutants were reduced in their ability to complement a *umuD44* strain in UV mutagenesis relative to a plasmid carrying the *umuD*$^+$ gene (Fig. 1*A*). The UmuD proteins that were most deficient in UV mutagenesis were those with alterations at the cleavage site (CY24, GS25, and GD25) and also GD129. Furthermore, we noted that, for several of the mutants (GS25, GR65, GD92, TM95, LF107, and GD129), the deficiencies of the *umuD* mutants in UV mutagenesis relative to a *umuD*$^+$ strain were not as great in cells that had been irradiated with 50 J/m^2 as they were in cells that had been irradiated with 20 J/m^2. This observation suggested that the higher UV dose can compensate for the impaired function of these mutant UmuD proteins. The *umuD* mutants exhibited a modest (2- to 4-fold) increase in sensitivity to killing by UV relative to the *umuD*$^+$ strain when irradiated at 50 J/m^2. Increased sensitivity was not detected at 20 J/m^2.

Deficiencies of the Mutant UmuD Proteins in RecA-Mediated Cleavage *in Vivo*. Given the demonstrated importance of RecA-mediated cleavage of UmuD in the mutagenic process (11), it seemed possible that the deficiencies of the mutant UmuD proteins in UV mutagenesis might be due to a reduced ability of these proteins to undergo RecA-mediated cleavage. We therefore used affinity-purified UmuD antisera to examine the extent of UV-induced cleavage of the mutant UmuD proteins *in vivo* in a manner similar to that described by Shinagawa *et al.* (23). By using this methodology, we were able to detect UmuD and UmuD′ generated from the multicopy *umuD*$^+$ plasmid pGW2020 but were not able to detect chromosomally encoded UmuD and UmuD′ unless the gels were substantially overloaded (data not shown). After a dose of 20 J/m^2 and an incubation of 1 hr, >90% of wild-type UmuD was cleaved to UmuD′ (Fig. 2*A*). In contrast, cleavage of the 10 missense UmuD proteins was either not detectable or just barely detectable, indicating that they are severely deficient in RecA-mediated cleavage. When the identical experiment was conducted with a higher UV dose of 50 J/m^2 (Fig. 2*B*), we were able to detect cleavage of all 10 missense proteins. In each case the UmuD′ derivative was ≈3 kDa smaller than the intact protein indicating that cleavage had occurred at, or very close to, the normal cleavage site between residues 24 and 25. Five of the mutant UmuD proteins (PS27, AT30, GD92, TM95, and LF107) were cleaved to a substantial extent and two (GD25 and GR65) were cleaved to a lesser extent. At this dose of UV, the other three mutants (CY24, GS25, and GD129) were also cleaved, but poorly, giving rise to barely detectable bands. Subsequent experiments (data not shown), in which larger amounts

Table 1. DNA sequence changes of *umuD* mutations causing reduced UV-mutability in a *umuD44* background

Plasmid	Base substitution	Mutant UmuD proteins
pGW2054	^{70}TGT → TAT	CY24
pGW2052	^{73}GGC → AGC	GS25
pGW2060	^{73}GGC → GAC	GD25
pGW2053	^{79}CCT → TCT	PS27
pGW2062	^{88}GCA → ACA	AT30
pGW2059	^{124}CAG → TAA	Ochre42
pGW2055	^{193}GGA → AGA	GR65
pGW2051	^{274}GGC → GAC	GD92
pGW2070	^{283}ACG → ATG	TM95
pGW2064	^{319}CTT → TTT	LF107
pGW2050	^{385}GGT → GAT	GD129

In the mutant proteins, the first letter represents the amino acid present at the wild-type UmuD and the second letter represents the amino acid that is present at that position in the mutant UmuD protein.

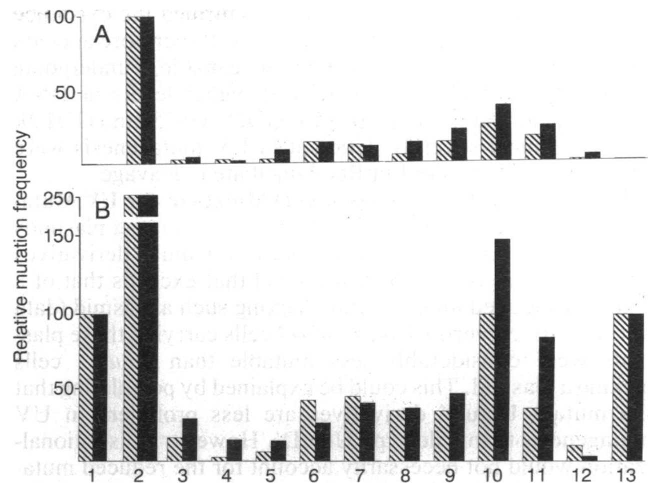

FIG. 1. Effect of plasmids carrying *umuD* mutations on UV-induced reversion (1) of *argE3* → Arg$^+$ in an AB1157 *umuD44* strain (GW3200) (11) (*A*) and an isogenic *umuD*$^+$ strain (AB1157) (11) (*B*). The UV doses used were 20 J/m^2 (hatched bars) and 50 J/m^2 (solid bars). The frequencies of induced Arg$^+$ revertants per survivor (× 10^7) at 20 and 50 J/m^2, respectively, were as follows: for GW3200, 6 and 13; for GW3200(pGW2020), 95 and 563; for AB1157, 37 and 152; and for AB1157(pGW2020), 92 and 450. The spontaneous Arg$^+$ reversion frequencies (× 10^7) for these four strains, respectively, were 1.1, 3.2, 0.8, 1.5. Introduction of the vector lacking the *umuD* gene did not affect the frequencies of either spontaneous or UV-induced *argE3* → Arg$^+$ reversion. Bars: 1, no plasmid; 2, UmuD$^+$; 3, CY24; 4, GD25; 5, GS25; 6, PS27; 7, AT30; 8, GR65; 9, GD92; 10, TM95; 11, LF107; 12, GD129; 13, Oc42.

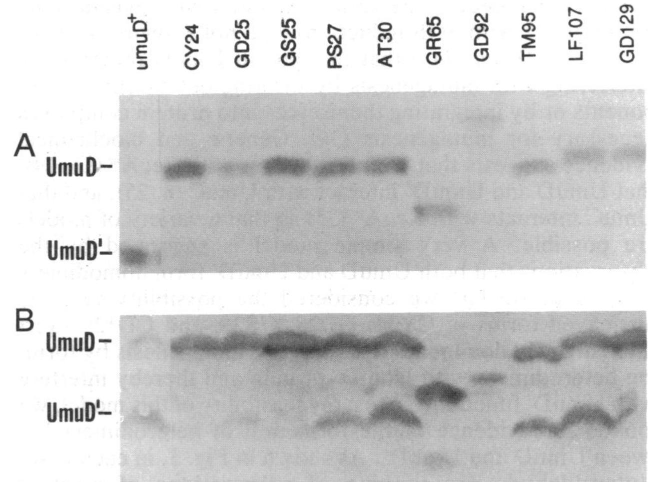

FIG. 2. UV-induced *in vivo* cleavage of various plasmid-encoded UmuD derivatives, as indicated. Cells were irradiated with 20 (*A*) or 50 (*B*) J/m^2 and incubated for 1 hr, and the extent of cleavage was assessed.

of protein were loaded on the gels, confirmed the existence of the UmuD′ product for these mutants. It therefore appears that all 10 missense UmuD proteins are capable of undergoing some degree of cleavage *in vivo* at higher levels of DNA damage. The four mutants (CY24, GD25, GS25, and GD129) that were most severely deficient in UV mutagenesis were most severely impaired in RecA-mediated cleavage.

Dominance of the Missense *umuD* Mutations for UV Mutagenesis. After a UV dose of 50 J/m^2, cells carrying plasmids encoding the most efficiently cleaved UmuD derivatives contain a cleavage product at a level that exceeds that of a similarly induced *umuD*$^+$ strain lacking such a plasmid (data not shown). Nevertheless, *umuD44* cells carrying these plasmids were considerably less mutable than *umuD*$^+$ cells lacking a plasmid. This could be explained by postulating that the mutant UmuD′ derivatives are less proficient in UV mutagenesis than wild-type UmuD′. However, this rationalization would not necessarily account for the reduced mutability seen in the *umuD44* derivative carrying the CY24 mutation. If, after induction, cleavage were to occur at the bond between residues 24 and 25 in this mutant, the UmuD′ derivative produced would be identical to wild-type UmuD′. This suggested the possibility that the uncleaved UmuD protein might actually interfere with UmuD′-dependent UV mutagenesis. If this were the case, one would expect *umuD* mutations that cause severe deficiencies in RecA-mediated cleavage to be dominant to *umuD*$^+$.

We therefore assessed the effect of the mutant *umuD* plasmids isolated in this study on the mutability of a *umuD*$^+$ strain, AB1157. As shown in Fig. 1*B*, the introduction of a *umuD*$^+$ plasmid into the *umuD*$^+$ strain increased the UV-induced mutation frequency by a factor of ≈2.5, suggesting that UmuD, directly or indirectly, influences a rate-limiting step in the process of UV mutagenesis. Introduction of a plasmid carrying the *umuD* ochre mutation had no effect on UV mutagenesis, indicating that this mutation is recessive. The TM95 mutation was also recessive as was the LF107 mutation at the higher UV dose. In contrast, the introduction of the plasmids carrying the other *umuD* mutations reduced the level of UV mutagenesis below that of a *umuD*$^+$ strain lacking a plasmid. Thus all of these *umuD* mutations are dominant, at least when present on a multicopy plasmid. The mutations having the strongest dominant negative effects were the three that affect the cleavage site (CY24, GD25, and GS25) and GD129. These are the same mutations that cause the most severe deficiencies in RecA-mediated cleavage.

Formation of Heterodimers Between UmuD and UmuD′. Since the presence of the various mutant *umuD* plasmids did not interfere with SOS induction (data not shown), it seems likely (see *Discussion*) that the mutant UmuD proteins are interfering with mutagenesis by titrating out essential components or by integrating themselves into protein complexes necessary for mutagenesis (34). Genetic and biochemical evidence suggests that UmuD interacts with RecA* (11, 24), that UmuD and UmuD′ interact with UmuC (8, 25), and that UmuC interacts with RecA* (35) so that a variety of models are possible. A very simple model is suggested by the observations that both UmuD and UmuD′ form homodimers (25). In particular, we considered the possibility that the uncleaved forms of CY24, GD25, GS25, and GD129 exert their strongly dominant effects on UV mutagenesis by forming heterodimers with UmuD′ protein and thereby interfere with UmuD′ function. To test the feasibility of this model, we looked for evidence of the formation of heterodimers between UmuD and UmuD′. As shown in Fig. 3, in each case, glutaraldehyde crosslinking of preparations of purified UmuD′ and UmuD allowed us to detect the formation of homodimers with no evidence of the generation of higher multimers. This dimerization is specific. When either UmuD or UmuD′ were incubated with equimolar amounts of LexA or λ repressor and then treated with glutaraldehyde, we were not able to detect the presence of any heterodimers although we did detect the presence of all of the expected homodimers (data not shown). In contrast, when equimolar amounts of UmuD′ and UmuD proteins were mixed, we could detect the time-dependent formation of heterodimers (Fig. 3, lanes 6–11). The failure to detect either UmuD or UmuD′ homodimers after 20 min suggests that the UmuD·UmuD′ heterodimer is a stronger complex than either of the homodimers. We are not yet sure why we are detecting two species of cross-linked heterodimers but suspect that cross-linking between different pairs of lysines results in complexes with slightly differing mobilities on SDS/polyacrylamide gels. This explanation is supported by the segregation of the UmuD monomer into two separable forms upon treatment with glutaraldehyde.

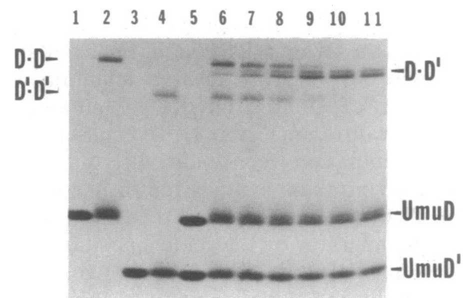

FIG. 3. Formation of heterodimers of UmuD and UmuD′. UmuD and UmuD′ were at 10 μM in 10 mM sodium phosphate, pH 6.8/0.1 mM EDTA/100 mM NaCl. Glutaraldehyde cross-linking was carried out by adding glutaraldehyde to 0.05%, incubating 3 min at room temperature, and stopping the reaction by adding 0.13 M Tris·HCl in the form of SDS gel sample buffer and freezing quickly. Samples were electrophoresed on a 13% polyacrylamide gel containing SDS and visualized by staining with Coomassie blue. Lanes: 1 or 2, UmuD with no treatment or with glutaraldehyde, respectively; 3 or 4, UmuD′ with no treatment or with glutaraldehyde, respectively; 6–11, UmuD and UmuD′ treated with glutaraldehyde after 1, 3, 5, 10, 20, or 30 min of incubation, respectively. D, UmuD; D′, UmuD′.

DISCUSSION

UmuD, MucA, ImpA, and the carboxyl-terminal domains of LexA and the repressors of bacteriophages λ, 434, P22, and φ80 share not only amino acid similarities but also functional similarities including (*i*) an ability to interact with RecA*, (*ii*) an ability to be proteolytically cleaved at a particular Ala-Gly or Cys-Gly bond in a RecA-mediated fashion, (*iii*) an ability to autodigest at pH 9–10, and (*iv*) an ability to form homodimers. In Fig. 4, we present an alignment of these proteins. In preparing this alignment, we have attempted to maximize amino acid identities shared between the members of this family. Our alignment of the sequences of the bacteriophage repressors and LexA differs in several respects from published alignments (21, 22) and, in particular, indicates conserved regions close to the carboxyl-terminal ends of the proteins. We have indicated the positions in λ repressor (36), LexA (37), and UmuD (ref. 11 and this study) where amino acid substitutions have been reported to give stable proteins that are deficient in RecA-mediated cleavage and also amino acids of λ repressor whose alteration has been shown to interfere with the formation of homodimers (38).

As shown in Fig. 5, the fact that UmuD carries out a biological role subsequent to its RecA-mediated cleavage makes the UmuD situation more complex than those of λ and LexA in which RecA-mediated cleavage leads to loss of biological activity.

The screening procedure employed in this study identified *umuD* mutants on the basis of their deficiency in UV muta-

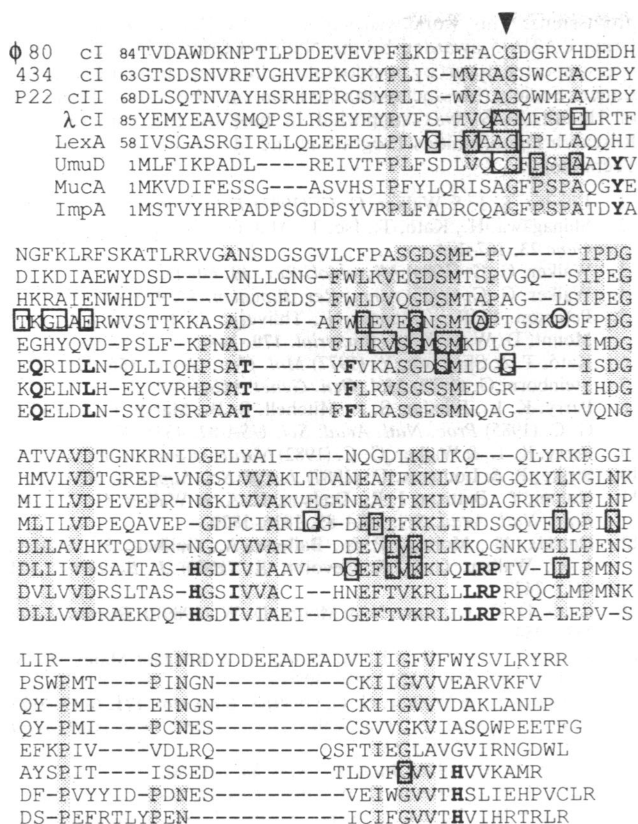

FIG. 4. Homology among the bacteriophage ϕ80, 434, and P22, λ repressors, LexA, and the mutagenesis proteins UmuD, MucA, and ImpA. Amino acids that are identical in four or more members of the set are shaded. Positions of λ (36), LexA (37), and UmuD (ref. 11 and this work) where amino acid substitutions have been shown to yield stable proteins that are defective in RecA-mediated cleavage are indicated by squares. Positions of λ repressor where an amino acid substitution has been shown to interfere with dimer formation are indicated by circles (38). Amino acids that are identical in the three mutagenesis proteins but are not shared with LexA or the bacteriophage repressors are indicated by bold lettering. The cleavage site is indicated by an arrowhead. The ImpA sequence is reproduced with the permission of P. Strike (University of Liverpool; personal communication).

genesis. In principle, the mutant UmuD proteins we identified could have been deficient (*i*) in the RecA-mediated cleavage event that activates UmuD, (*ii*) in the subsequent function(s) of UmuD in UV mutagenesis, or (*iii*) in both of these. As discussed by Gimble and Sauer (38), there are three simple ways in which mutations could affect the rate of a RecA-mediated cleavage reaction. First, if UmuD resembles λ repressor and LexA and undergoes the RecA-mediated cleavage reaction as a monomer, then a mutation that increased the population of UmuD dimers ($UmuD_2$) relative to monomers (UmuD) by changing the dimerization constant K_1 would decrease the rate of cleavage. Second, mutations could also decrease the rate of RecA-mediated cleavage by decreasing the affinity of the UmuD monomer for RecA* (changing K_B). Third, mutations could also decrease RecA-mediated cleavage by directly decreasing the rate of cleavage of the RecA*–UmuD complex (decreasing k_c). Such mutations could affect amino acids that play a direct functional role in the mechanism of the proteolytic cleavage reaction or some aspect of the architecture of UmuD that is necessary for the RecA-mediated cleavage reaction. Since the role of the resulting UmuD′ protein in the process of UV mutagenesis is not yet understood, it is not possible to identify particular steps subsequent to the cleavage reaction that might account for a reduced frequency of UV mutagenesis. However, plausible steps include the dimerization of UmuD′ proteins (ref. 25 and this study), association with UmuC (8, 25), and possible interaction with another protein such as RecA (14) or a DNA polymerase (30, 31).

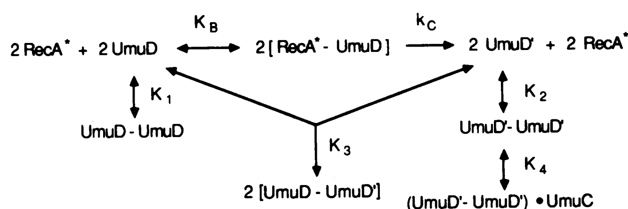

FIG. 5. Model of UmuD and RecA-mediated cleavage.

All of the mutant UmuD proteins analyzed in this study were severely deficient in their ability to undergo RecA-mediated cleavage *in vivo* but we were able to detect some cleavage of each protein at or very near the normal cleavage site between residues 24 and 25. However, we have found (unpublished data) that our *umuD* mutants SA60 and KA97 (11), which affect residues postulated to play a direct role in catalyzing the cleavage reaction (11, 39), are not cleaved to a detectable extent *in vivo*. In their characterization of RecA-mediated cleavage of 20 Ind⁻ LexA proteins *in vitro*, Lin and Little (40) found that several could be cleaved but at rates that were significantly lower than rates of the wild-type LexA protein. Similarly, Gimble and Sauer (36) reported that several of the Ind⁻ λ repressors they examined were capable of a diminished amount of RecA-mediated cleavage.

In their studies of λ repressor, Gimble and Sauer (36) found that most of the mutants that were defective in RecA-mediated cleavage were also defective in their ability to autodigest at alkaline pH. The exceptions were mutations affecting the λ repressor amino acids T122, G124, D125, and E127. This led Gimble and Sauer (36) to postulate that these mutations identified side chains that are involved in the binding of λ repressor to RecA*. As can be seen in Fig. 4, these residues of λ repressor are not conserved within this family of proteins suggesting that, if these amino acids do influence the interaction with RecA*, they do so in a λ-specific fashion. The existence of protein-specific interactions with RecA* could help to explain the observations that certain alleles of RecA differentially affect the ability of RecA to mediate the cleavage of certain members of this family of proteins (12, 13, 23, 24, 41) and that RecA proteins from other bacteria are able to mediate the cleavage of certain members of this family but not others (42). The region of UmuD (amino acids 33–41) that corresponds to the region of λ repressor affected by this latter class of λ *c*I mutations contains three amino acids that are conserved within the family of mutagenesis proteins (UmuD, MucA, and ImpA) but not with LexA and the various bacteriophage repressors (Fig. 4). This observation suggests the possibility that this region of UmuD might represent a domain that is required for some function or interaction that is particularly required for UmuD's role in UV and chemical mutagenesis, independent of whether it plays a role in RecA-mediated cleavage.

The mutant UmuD proteins with the most severe reductions in their ability to undergo RecA-mediated cleavage were those with alterations at the cleavage site (CY24, GD25, and GS25) and GD129, which has an alteration near the carboxyl-terminal end of the protein. In genetic studies of LexA and λ repressor (36, 37), no mutations affecting RecA-mediated cleavage have been reported that are so close to the carboxyl terminus. The fact that the GD129 mutation so severely impairs RecA-mediated cleavage of UmuD suggests that this carboxyl-terminal-most region of conservation may play some role in protein architecture that is extremely important for the RecA-mediated cleavage reaction.

Gimble and Sauer (38) have described two mutations causing amino acid substitutions in the carboxyl-terminal domain of λ repressor (AT152 and AT158) that interfere with the formation of homodimers (Fig. 4). These lie in a region in which there is relatively little conservation of amino acid sequence between the proteins that are members of this family, an observation that suggests that the various members of this family either might not form heterodimers with each other or at least might do so relatively inefficiently. Our failure to observe the formation of heterodimers between either UmuD or UmuD′ and LexA or λ repressor is consistent with this inference.

The results we have obtained in this study have led us to consider the hypothesis that intact UmuD is not simply an inactive form of UmuD′ but is rather a dominant inhibitor of UmuD′-dependent mutagenesis. The dominance of the umuD alleles that reduce RecA-mediated cleavage and the preferential generation of UmuD·UmuD′ heterodimers are observations consistent with this concept. UmuD is potentially well suited to a negative regulatory role. As the SOS response begins to shut off, an accumulation of UmuD could lead to the formation of UmuD·UmuD′ heterodimers and hence to an inhibition of UmuD′ activity. UmuD is cleaved much less efficiently than LexA *in vivo* (23) and *in vitro* (24). Thus one would expect some intact UmuD to accumulate before the increase in intact LexA would return expression of the *umuDC* operon to its basal level. Since the results of our *in vitro* experiments suggest that the formation of heterodimers is favored over homodimers, heterodimers should begin to form as soon as intact UmuD begins to accumulate.

The results of several previous studies, when considered collectively, suggest to us that there is indeed a mechanism for the deactivation of SOS-induced mutagenic capability that could be accounted for by the model we have proposed. By using two approaches, Witkin (44) and Defais *et al.* (45) showed that SOS mutagenesis decays with a half-life of ≈30 min in a Uvr⁻ background. However, Sassanfar and Roberts (43) have shown that, in a Uvr⁻ strain irradiated with an even lower dose of UV than that used by Defais *et al.* (45), LexA continues to be cleaved at a maximal rate for at least 60 min after the exposure to UV. Thus the decay of SOS mutagenesis cannot be accounted for by the disappearance of RecA*, the accumulation of LexA, and the subsequent repression of the SOS regulon. Furthermore, pulse–chase studies (J.R.B. and G.C.W., unpublished results) have indicated that the UmuD′ and the UmuC proteins are stable for at least 2 hr after translation, apparently ruling out proteolytic degradation of these proteins as a mechanism for inactivating SOS-induced mutagenic potential within the time frames discussed above.

The preferential formation of UmuD·UmuD′ heterodimers could have other regulatory consequences. For example, if the heterodimer is indeed inactive or weakly active in SOS mutagenesis, then substantial cleavage of UmuD would have to occur before the active $UmuD'_2$ homodimer would be produced in quantity. It is also possible that formation of UmuD·UmuD′ heterodimers could influence RecA-mediated cleavage of UmuD either positively or negatively.

It has been postulated that UmuD′ and UmuC proteins function by directly modifying a DNA polymerase in a fashion that permits that polymerase to bypass DNA adducts that would otherwise constitute a block to DNA replication (26–29). Given the elaborate control circuitry that has evolved to regulate the appearance of this activity, it seems reasonable that it might also have mechanisms for eliminating the potentially mutagenic effect caused by such a modified polymerase after its function is complete. Deactivating UmuD′ by heterodimer formation with intact UmuD may represent such a mechanism.

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