The UmuD′ protein filament and its potential role in damage induced mutagenesis

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Introduction
During cell division, all living organisms attempt to replicate their genomes faithfully. Indeed, under optimal conditions DNA replication is extremely accurate. In Escherichia coli it has been estimated that only one error occurs for every $10^{10}$ nucleotides replicated [1]. However, because life persists in a somewhat hostile environment where exposure to a variety of natural and synthetic agents can damage DNA, repair mechanisms are required. As a consequence most organisms have equipped themselves with an array of enzymes to remove the damage before DNA replication proceeds (for a recent review see [2]). Situations can nevertheless arise where lesions cannot be repaired by conventional repair enzymes. The consequences of these unrepaired lesions can be disastrous; at least in E. coli they can block DNA replication and ultimately lead to cell death. Bacteria respond to DNA damage with the classic ‘SOS response’: inducing a set of regulated genes, among these is the $umu$ operon that codes for UmuD and UmuC mutagenesis proteins [2,3]. These proteins, together with RecA, are thought to act as specialized processivity factors that enable the DNA polymerase III holoenzyme to replicate through unrepaired DNA, but with a concomitant decrease in replication fidelity [4–6]. Thus, the price for continued cell survival is that occasional mutations are introduced into the genome.

Because this pathway can lead to errors in the genome, the activity of the mutagenesis proteins is regulated at several levels. In addition to negative transcriptional regulation provided by the LexA repressor, the UmuD protein remains functionally inactive until it is post-translationally processed to UmuD′ [7–9]. UmuD and its functional homologs share sequence homology to the C-terminal portions of the LexA protein and certain bacteriophage repressors like $\lambda$ cl [10]. These UmuD-like proteins are now known to undergo mechanistically similar RecA-mediated cleavage reactions upon SOS induction [11–14]. This cleavage reaction is a RecA-dependent intramolecular reaction under physiological conditions, but can be accomplished under alkaline conditions without RecA. Furthermore, studies with LexA mutants have shown that this is indeed a true enzymatic reaction that can occur intermolecularly as well as intramolecularly [15].

Another level of regulation is the formation of inactive heterodimer UmuD–UmuD′ complexes. Once DNA polymerase completes replication of the damaged chromosome,
RecA is inactivated and LexA and UmuD are no longer cleaved. Residual UmuD′ is then sequestered by the formation of the UmuD–UmuD′ heterodimer, which is a stronger complex than either the UmuD or the UmuD′ homodimer [10]. Degradation of this inactive heterodimer by the CtpX protease completes the cycle and removes the threat of spurious mutagenesis [16].

While UmuD shares similar cleavage properties with the transcriptional repressors, it does not possess the ability to bind directly to DNA. Instead, the protein is targeted to DNA via an interaction with a RecA nucleoprotein filament [17–19]. UmuD′ also interacts with UmuC to form a mutagenically active UmuD′–UmuC complex [5,20]. It is believed that through the interaction with RecA the UmuD′–UmuC complex is correctly positioned for the encounter with DNA polymerase III holoenzyme that ultimately allows translesion DNA replication [19].

We have previously described the crystal structure of UmuD′ emphasizing the implications of the structure for the process of proteolytic activation from UmuD [21]. The structure in this crystal consists of filaments in which UmuD′ protomeric units interact through two kinds of dimer interface. The unusually intertwined and intimate nature of one of these interfaces suggests the possibility that a similar UmuD′ filament might be involved in SOS mutagenesis. In this report we analyze the dimer interfaces in two additional UmuD′ crystal structures, as well as in the initial structure. We provide biochemical evidence that identifies one interface with the molecular dimer of UmuD′ and the other with filament-like interactions that occur in solution. We also describe observations that associate the filament-like interactions with the in vivo activity of UmuD′.

**Results**

**Crystal structure**

The initially reported structure of UmuD′ [21] was obtained using a selenomethionyl derivative of a mutant variant (Met138→Thr). We have now determined the structure of another polymorph of this protein at 2.6 Å resolution (type Ib) and that of the wild-type protein at 3.0 Å resolution (type Ic). The most significant differences in protomer structure are in the extent of C-terminal ordering, where an additional two residues are seen in the two new crystal forms, and in the electron density at position 138. A non-crystallographic diad axis relates the two UmuD′ protomers in such a manner as to generate a filament of molecules associated along the fourfold screw axis of the crystal. There is a diad axis relating protomer A to protomer B (Fig. 1b,c), which is perpendicular to and intersecting with the 4, axis. This in turn generates a set of such diad axes and also a second intervening set, rotated by 45° from the first. The filament then has the symmetry of a 4,22 axis (Fig. 1b,c). The second type of diad relates the AB dimer to an A′B′ dimer (Fig. 1b,c).

Based on experiments described below, we call dimers from the first set molecular dimers and those from the second set filament dimers.

Both interfaces of the crystallographic filament are appreciably flexible. The most disparate of the structures are those newly reported here for lattices Ib and Ic (see Table 1). When globular cores (residues 46–135) of the A protomers are superimposed, a transformation of 1.55° in rotation and −0.31 Å in translation is required to superimpose the B protomer in the molecular dimer of Ib onto that of Ic; the corresponding transformation needed to superimpose the B′ protomers in the filament dimer has parameters of 1.17° and 0.66 Å. Thus, whereas the protomeric structures are all very similar, ranging from 0.26–0.40 Å in root mean square (rms) deviation in core Ca positions, the corresponding deviations are as large as 0.61 Å over molecular dimers and 1.50 Å over filament dimers. As we describe below, filament formation and flexibility in this structure may also be important for the biological activity of UmuD′.

**Molecular dimer interactions**

The molecular dimer interface is primarily hydrophobic although there is also a salt bridge between residues Glu93 and Lys55 of the dimer mate on both sides of the interface. Approximately 550 Å² of accessible surface area from each protomer is buried in the formation of a molecular dimer. To test the hypothesis that the molecular dimer is indeed the predominant dimer found in solution, we used standard PCR technology to generate a mutant variant of UmuD′ (called ΔN-UmuD′ or UmuD′ 302) lacking the N-terminal extension. In our model, ΔN-UmuD′ is not expected to form the filament dimer, but would still be expected to form the molecular dimer (Fig. 2a,b). This N-terminal UmuD′ deletion mutant exhibited purification characteristics similar to the wild-type protein, suggesting that it is correctly folded. Both gel filtration and native acrylamide gel electrophoresis suggest that it is indeed a dimer in solution (data not shown). Physically cross-linking the subunits with glutaraldehyde and analyzing the products using denaturing SDS-polyacrylamide gel electrophoresis (PAGE) showed that, like wild type UmuD and UmuD′, ΔN-UmuD′ formed homodimers [10] (Fig. 3a).

As the molecular dimer interface is primarily hydrophobic, we hypothesized that molecular dimer formation might be disrupted by mild detergent conditions. We also expected sensitivity to high pH as UmuD autodigestion, which is thought to require dimer dissociation [22], only
happens under alkaline conditions. The molecular dimer did not dissociate at neutral pH, but at higher pH (as shown in Fig. 3a) UmuD, UmuD′ and ΔN-UmuD′ dissociate to monomers in detergent when sufficiently dilute. In all cases the sensitivity of the dimer interface to detergent, pH and dilution is essentially identical over a wide range of conditions. These results support our identification of the molecular interface in the crystal (Fig. 2a) with the molecular dimer found in solution.

Filament dimer interactions
The filament dimer is formed by interactions at the N and C termini of the protein (Fig. 2b). Residues 132–138 of the C terminus (protomer A) form hydrogen bonds across the interface with the related residues, 132–138 (protomer B′), extending two three-stranded β sheets into a six-stranded intersubunit β sheet. At the N termini, there are two α helices which cross over each other with the Asn41 Oδ of protomer A making a hydrogen bond with the mainchain Leu40 N of protomer B′. There are also several hydrophobic contacts in this area with Leu40 of protomer A being close to Leu43, Leu44, Ile45 and Ile73 of protomer B′, and vice versa (Fig. 2b). Leu44 (protomer A) is also close to Val135 (protomer B′), linking the α helix of the N terminus with the β7 strand of the C terminus, and forming a hydrophobic core central to this interface. About 850 Å² of solvent accessible surface is buried by each protomer when two protomers form a filament dimer.

Although N-terminal sequencing showed that the protein used for crystallization starts at residue Gly25, the first residue seen in our maps is Asp32. We believe the first seven residues are disordered in these crystals. Moreover, much of the N-terminal segment that could be modeled is poorly ordered, particularly in residues 34–37 where little sidechain density is seen. The one exception is residue Tyr33, which is well defined. The N terminus of one protomer (e.g. A) extends beyond the ‘filament dimer’ interactions (with protomer B′) to the next protomer along in the filament structure (A′), placing the Tyr33 ring on a patch of hydrophobic residues (Fig. 1b,c). These additional interactions add another 200 Å² of buried surface area per protomer, making the total buried accessible surface area of the filament about 2100 Å², almost twice that of the molecular dimer interface (1100 Å²).

Despite the extent of contact at the filament interface, it seems clear from the similarity of the properties observed in solution for ΔN-UmuD′, UmuD′ itself and the uncleaved UmuD (see above) that this interface is not the one generally associated with the dimer observed in solution. There is one striking difference in the in vitro behavior of these
Figure 1

The UmuD’ protomer and its organization within the filament. (a) Stereo view showing the secondary structure of the UmuD’ protomer; β strands are colored in green and the α and 3₁₀ helices are colored in red. (Figure generated using the program MOLSCRIPT [55].) (b) Picture of four dimers in a filament. For orientation purposes, the Asp32 (red) and His82 (blue) sidechains are shown. The A protomers are colored in shades of purple; the B protomers are colored in shades of pink. The N- and C-terminal ends are labeled only for the protomers at the ends of the filament. (Figure generated using the program GRASP [56].) (c) Stereo view of the Ca trace of four dimers in a filament; each dimer is colored differently to show how the N and C termini intertwine to form the filament. (Figure generated using the program MOLSCRIPT [55].)
three proteins, however, and that is the appearance of higher-order oligomers when the processed N-terminal segment is present, but not otherwise. In the case of wild type UmuD′, but not with ΔN-UmuD′ nor UmuD, we observe a concentration-dependent ladder of glutaraldehyde cross-linked species in a sensitive chemiluminescent assay (Fig. 3b). The oligomeric species have sizes consistent with tetramers, hexamers, and larger associations of UmuD′ subunits. Oligomers are seen through the pH range 7 to 10; they are less stable than the molecular dimers and they are very sensitive to the presence of detergent (Fig. 3). These properties are consistent with the essential involvement of the N-terminal extension in the crystallographic filament (Fig. 1b,c) and the importance of hydrophobic interactions in this interface (Fig. 2b). Thus, filament-like structures of UmuD′ may also form in solution. Moreover, as the concentration of UmuD′ used in here (0.2 μM dimer) is appreciably lower than that in an SOS-induced cell (1900 UmuD′ subunits [23] in 6.7×10^{-13} g water [24] which gives ~2 μM dimer), such filaments may also form under physiological conditions.

Biological consequences of filament formation

The first suggestion that structures like the UmuD′ filament found in the crystal may have biological significance arose from the observation that mutations at position 138 (Met138→Thr or Met138→Val) result in a gain of function. These mutant variants exhibit 2–3 fold higher levels of UV-induced mutagenesis than the wildtype protein (Fig. 4a). This residue is in the region of the extended filament interface. As can be seen in Figure 4b, the methionine sidechain curves away from this filament interface, whereas the threonine sidechain drops into a pocket with the Thr138 Oγ making a potential hydrogen bond with the backbone NH of residue 133 on the symmetry related B′ protomer. One hypothesis to explain this enhanced mutagenesis is that these mutant variants form a tighter UmuD′ filament dimer.

Analysis of additional UmuD′ mutants that were specifically constructed based on the structure of the UmuD′ protein provided further support for the biological significance of the filament interaction. The ΔN-UmuD′ mutant, which was shown to be proficient in forming molecular dimers but deficient in forming filament-like structures (Fig. 3a), was found to be completely defective in its ability to support SOS-dependent spontaneous or methyl methanesulfonic acid (MMS) induced mutagenesis (Table 2). Likewise, deletion of the C-terminal residues 136–139 caused a complete loss in the protein’s ability to facilitate the mutagenic process.

We have also identified a missense UmuD′ mutation, His82→Tyr, that has a greatly reduced capacity to promote SOS spontaneous mutagenesis but is proficient for MMS mutagenesis. This residue on one protomer (A′) is close to the N terminus of its symmetry related neighbor (A) (Fig. 5). The position of this histidine is in the only electrostatically positive region on the protein surface which is made up of residues His82, Lys98 and Arg102 and is in great

![Figure 2](image)

UmuD′ dimer interactions. (a) Overall view of molecular dimer interactions, shown as a ribbon representation. The blue portion of the ribbon, residues 32–45 of UmuD′, is the region deleted to make the ΔN-UmuD′ mutant protein. This shows that deletion of the N-terminal tail should not affect the formation of the molecular dimer. Hydrophobic sidechains are in green, acidic sidechains are in red, basic sidechains are in dark blue, and all other sidechains are in cyan. The dotted line represents a hydrogen bond between the Gln93 and Lys55 residues. Note the stacking of the two Phe94 rings; the other residues shown are Asp32, Val54, His82, lle87, and Phe128. (b) Overall view of the filament dimer interactions, as a ribbon representation; the color scheme is as described in (a). A hydrophobic core is formed by sidechains from residues in the N- and C-terminal regions of the protein (Leu3, Leu44, lle73, Val113 shown). There is β sheet hydrogen bonding across the interface involving residues 132–138 of β7 of each protomer. There are also hydrogen bonds across the α helices of the N-terminal regions (e.g., the Asn41 O6 forms a hydrogen bond with the backbone Leu40 N, Leu40 sidechain not shown). Deletion of residues 25–45 in the ΔN-UmuD′ mutant removes much of the hydrophobic core that was found with the wild-type UmuD′ structure. Although the C-terminal β strands can still form an extended β sheet in this mutant, many of the hydrophobic residues that were once buried would now be exposed. (Figures generated using the program GRASP [56].)
contrast to the rest of the protein which is quite negatively charged (−7), with 10% of its residues being aspartic acid. This histidine residue, as well as the arginine residue at position 102, is conserved in seven of the eight mutagenesis proteins shown in Figure 6. With these new structures, we can also see that the C terminus (of protomer B′′) is within 10 Å of His82, and only 4.1 Å from Lys98 (Fig. 5). So both extensions are in the same region, which may be important in filament formation and the mutagenic response.

Mutations that have been hypothesized to affect the ability of the λ cl protein to interact with RecA have been mapped to a region of the protein that would correspond to the extended N-terminal residues of UmuD′ (Fig. 6). This observation raises the intriguing possibility that UmuD′ residues that are important for filament interactions are also important for interaction with RecA. To test the hypothesis that the extended N terminus of UmuD′ may contact RecA, we analyzed the ability of the wild-type and ΔN-UmuD′ proteins to interact with a RecA nucleoprotein filament [19] (Fig. 7). Wild type UmuD′ clearly has affinity for the activated RecA filament, whereas the affinity of the ΔN-UmuD′ protein, lacking residues 25–45 of UmuD′, is much reduced. This suggests that the deleted residues are indeed important for a UmuD′–RecA–DNA interaction. It is clear, however, that other regions of UmuD′ must also contact RecA, as ΔN-UmuD′ did retain a limited ability to interact with the RecA nucleoprotein.
The reduced capacity of ΔN-UmuD′ to interact with RecA may explain why cells expressing ΔN-UmuD′ together with UmuC are rendered phenotypically non-mutable (Table 2).

Discussion

As is typical for soluble proteins, the globular core of UmuD′ (residues 46–135) and its associated molecular dimer are compact. In contrast, the conformations of the N- and C-terminal extensions (residues 32–45 and 136–139) clearly depend on the intimately intertwined character of the filament dimer interface. Such an interface is highly unusual as a mode of lattice packing. This observation led us to consider the possibility that filament-like structures may have biological relevance and to make further experimental tests of this hypothesis. Our demonstration that filament-like associations of UmuD′ dimers, but not of UmuD dimers, exist in solution at physiologically relevant concentrations itself suggests a possible involvement in the mutagenic response. Alternative explanations do need to be considered; such filaments may exist in the cell but have another yet undefined function. It is also possible that somehow the crystallographic filaments and filament-like associations in solution are artifacts of our crystallization and solution conditions. On the other hand, our observations of mutant variants of UmuD clearly do implicate the filament-forming residues in the N- and C-terminal extensions in the biological response. If the filament does play a direct role in translesion repair, how might this happen?

We know that UmuD′ interacts with activated RecA nucleofilaments [19] in the absence of UmuC and that this interaction is greatly attenuated for ΔN-UmuD′. We also know that UmuD′-UmuC complexes form in the absence of RecA and can interact with single-stranded DNA [25]; all three components are needed for mutagenic repair. An SOS-induced cell is likely to contain approximately 200

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**Figure 4**

Effects of mutations at Met138. (a) Quantitative mutagenesis assays with Met138→Thr and Met138→Val. A bacterial suspension of a His+ strain was exposed to UV-light and dilutions plated on minimal agar plates supplemented with 1 μg ml–1 histidine. His+ revertants were scored after four days at 37 °C and the induced mutation frequency calculated. (b) Position of residue 138 in wild type UmuD′ (Met138, in yellow) and in the Met138→Thr mutant (T138, in red). The methionine residue bends away from the filament interface whereas the threonine residue drops into a pocket and forms a potential hydrogen bond with the mainchain nitrogen of residue 133 of the other protomer in the filament dimer interface. Residues within ~5 Å of residue 138 are shown in green. The two structures diverge in the C-terminal residue; the darker color belonging to the Met138→Thr (T138) structure and the lighter color corresponding to the wild-type structure. (Figure generated using the program GRASP [56].)

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**Figure 5**

Close-up view of the region around His82 of protomer A′. Both the N and C termini (from different protomers, A and B′) come together in this region to form the filament structure. The His82, Lys98 and Arg102 sidechains are in dark blue. The N terminus of protomer A is denoted by Asp32 (shown in red), and the C terminus of protomer B′ is denoted by the mainchain of Arg139 (shown in yellow). Little density is seen for the sidechain of Arg139, although there is good density for the mainchain atoms in this region. Other residues, Tyr33 and Thr38 (from different protomers), are shown in cyan. (Figure generated using the program GRASP [56].)
The amino acid sequence alignment of 11 proteins of known homology to the E. coli UmuD protein. The numbering is according to the UmuD sequence, except to indicate the first residue of each protein. The first eight proteins are mutagenesis proteins and the last three proteins are well-known E. coli and phage repressors (LexA, cI and 434 cI). The repressor proteins start in the linker region between the N-terminal (DNA-binding region) and the C-terminal (homologous region to UmuD) portions of the proteins. There is a space between every ten amino acids of UmuD to help denote the numbering. The secondary structure elements of the UmuD’ model are designated above the UmuD sequence. The two residues cleaved apart in the self-cleavage reaction are underlined and in bold. Other amino acids in bold are conserved in at least seven of the eight mutagenesis proteins depicted in the figure. There are 12 absolutely conserved residues in this set of 11 proteins: Gly25, Ser60, Met61, Val74, Ala88, Glu93, Thr95, Lys97, Leu99, Leu107, and Gly129. Gly25 is at the cleavage site, with Ser60 and Lys97 being located in the catalytic site. The asterisk above residue Met138 denotes the mutation site for the protein that was crystallized (Met138 → Thr).
to be in this area. Asp32, Val34, His82, and presumably several of the sidechains of the disordered residues, are on a ridge of the filament and thus solvent exposed. It is known from previous studies that mutations in this part of the N terminus affect the ability of the protein to participate in mutagenesis [32]. For these reasons, we believe that this region of the UmuD′ filament is quite likely to be important for either RecA or UmuC interactions.

Several mutations in the RecA protein suggest that UmuD and UmuD′ interact in different ways with RecA. The RecA1734 and RecA433 proteins are deficient in their ability to act as the coprotease for UmuD, but are proficient for promoting cleavage of homologous proteins [33,34]. However, cells with the recA1734 or the recA433 mutation are proficient for mutagenesis if UmuD′ is supplied on a plasmid, suggesting that they retain the ability to interact with UmuD′, but not UmuD [34,35]. Conversely, the recA1730 gene product affects mutagenesis but not the cleavage reaction, thus appearing to interact with UmuD and not UmuD′ [18,19,34,36]. A recent study [37] shows that RecA stabilizes UmuD′ specifically (over UmuD), and RecA mutants that form more stable filaments further enhance the stability of UmuD′ in vivo. This suggests that UmuD′ (potentially in the filament structure) may make very different interactions with the activated RecA nucleoprotein filament than does the UmuD (monomeric) protein.

**Biological implications**

DNA damage in bacteria can induce the ‘SOS response’. This response involves the expression of a number of regulated proteins, including UmuD, UmuC and RecA. Together these proteins act to enable DNA polymerase III to replicate through damaged DNA. The structure of wild-type UmuD provides considerable insight into its regulated role in DNA mutagenesis. There is evidence suggesting that the filament seen in the crystal structure is functionally relevant and as such provides another example of how physiologically important quaternary interactions are maintained in the crystal lattice [28,38,39]. Consistent with the relevance of this filament structure, we find that mutations in both the N- and C-terminal regions of the protein affect the mutagenesis reaction, but do not affect formation of the dimer found in solution (this study and [32]). The mutagenic character of UmuD necessitates
that the activity of the protein is tightly regulated. One level of regulation is the protein’s activation by self-cleavage to form U muD’. This cleavage step is probably necessary to allow the protein to adopt the filament structure as we do not observe higher order oligomeric U muD structures. The N-terminal extension seen for U muD’ may be folded back onto the molecular dimer, perhaps in part occupying positions equivalent to those on symmetry mates in the U muD’ filament, thereby precluding inappropriate filament formation. This exchange may be yet another case of domain swapping [40].

The ability of U muD’ to interact with the RecA–DNA filament provides a mechanism whereby U muD’ can position its partner, U muC, appropriately for interactions with DNA polymerase III and thereby allow DNA lesion bypass. The filament structure found in U muD’ crystals gives us an idea as to how this positioning may take place in the cell. The fact that we were able to detect high order oligomeric U muD’ interactions in solution at physiologically relevant concentrations suggests that similar structures may exist in vivo. The inability of the U muD’ mutants to form filaments correlates with their inability to promote damage induced mutagenesis. The fact that U muD and a mutant of U muD’ with an N-terminal deletion (ΔN–U muD’) do not appear to form these higher order oligomeric structures in solution, suggests that this filament structure is specific to U muD’. Recently it has been shown that the U muD’–U muC complex permits bypass of DNA lesions in Xenopus oocytes, demonstrating that this process is not limited to bacterial systems [41].

Materials and methods

UmuD’ was purified and crystallized as described elsewhere [42]. The protein crystallizes in space group P4₁2₁2₁ with two UmuD’ polypeptide chains per asymmetric unit. The structure was initially determined [21] by the multiwavelength anomalous diffraction (MAD) method [43] from the selenomethionyl protein [44]. Rigid-body refinement and then Powell minimization using X-FLOR [45] were used to determine the structures of the other crystal forms, also in space group P4₁2₁2₁ [47]. This procedure introduced an Nde I restriction enzyme site at the N-terminus of the protein. This codon is located such that the second amino acid of the mutant protein corresponds to glutamine 46 of UmuD. Although all residues up through ile45 were deleted in the gene, we have found from N-terminal sequencing that the N-terminal methionine residue at position 45 is retained in this mutant protein. DNA sequence analysis confirmed that no errors were introduced during the PCR. UmuD’ 302 was subsequently cloned into two expression vectors: pEC62, a derivative of pET22b (Novagen) that places the gene under the control of an inducible T7 promoter and pJM72, a derivative of pRW66 [47]. In the pJM72 construct, ΔN–U muD’ (UmuD’ 302) is expressed from the natural LexA-regulated umuDC promoter and allows us to assay the functional activity of the protein in vivo. ΔN–U muD’ was purified from IPTG induced BL21 (DE3)/pEC62 using the same protocol developed for the wild-type UmuD’ protein [19]. Like wild-type UmuD’, ΔN–UmuD’ is eluted from a gel filtration column at a position consistent with it being dimeric. UmuD’ mutants that resulted in the deletion of residues from the C terminus of UmuD’ were similarly constructed by standard PCR technology. All of these mutations were sequenced to confirm that only the desired change had occurred and were then cloned into a low copy number derivative of pRW66 and assayed for functional activity.

Protein cross-linking in CHAPS detergent

Cross-linking of UmuD, wild-type UmuD’ and ΔN–UmuD’ was assayed at different detergent concentrations. The ability of UmuD, UmuD’ and ΔN–UmuD’ to form dimers and higher structures was analyzed at different CHAPS (3-(3-cholamidopropyl)dimethylammonio)1-propanesulfonate) concentrations and at different pH conditions (from 7.5–10.0). The 25 μl reaction included a pre-incubation step at room temperature for 30 min with a protein concentration of 5 μg ml⁻¹ (0.2 μM dimer) in 20 mM Tris-HCl pH 10.0 (for the gel shown in Fig. 3a), 50 mM NaCl, 1 mM dithiothreitol (DTT), and 0, 2, or 15 mM CHAPS, respectively. Proteins were chemically cross-linked at room temperature for 30 min in 0.01% glutaraldehyde and the cross-linking reaction was stopped by the addition of SDS sample buffer. Proteins were then separated by SDS-PAGE and were transferred to an Immobilon-P membrane where complexes were visualized using the chemiluminescent immunoassay. The positions of a set of protein standards are indicated with the molecular weights and arrows. The reaction conditions were similar for the gel shown in Figure 3b, but no CHAPS detergent was added and the pH of the reaction was 7.0 (i.e. still 20 mM Tris-HCl, 50 mM NaCl, 1 mM DTT). The volume of the reaction depended on the dilution factor, with the undiluted sample being 2.5 μl, 4 × dilution equalled 10 μl, etc. The cross-linking reaction was performed as described above.

Quantitative mutagenesis assays with Met138Δ and Met138ΔVal

While the wild-type and mutant UmuD’ genes were cloned behind a T7 promoter in a pALTER plasmid, a low level of protein is produced in strains lacking the T7 polymerase (RW, unpublished results). This basal level of expression is sufficient to assay the ability of the wild type and the mutant UmuD’ proteins to restore UV-induced mutagenesis functions to a normally non-mutable umuD77 strain [49]. Briefly, the E. coli K-12 strain TK614, lacking a plasmid or harboring one of the pALTER-UmuD’ plasmids (wild type, Met138Δ or Met138ΔVal) was grown in Luria broth medium to a cell density of 10⁶ ml⁻¹. Cells were then harvested and resuspended in SM buffer (50 mM Tris HCl, 100 mM NaCl, 8 mM MgSO₄, 0.01% gelatin). The bacterial suspension was exposed to UV-light and dilutions plated on minimal agar plates supplemented with...
1 μg ml⁻¹ histidine. His₃ revertants were scored after four days at 37°C and the induced mutation frequency calculated [50].

Quantitative mutagenesis assays with the ΔN- and ΔC-UmuD' mutants
To determine if the ΔN-UmuD' and ΔC-UmuD' proteins were mutagenically active, we used a qualitative reversion assay [47]. Mutant plasmids were introduced into the ΔumuDC E. coli strain RW126, together with the compatible UmuC expressing plasmid pRW124 [47]. The ability of the constructs to promote both spontaneous and EMS induced mutagenesis was assayed by following the reversion of the hisG40(c) allele. Plasmid pRW62, with a wild-type umuD' gene, was transformed into strain RW126 to obtain the wild type numbers. Under these conditions, ΔN-UmuD' (plasmid pM72) failed to promote either spontaneous or EMS induced mutagenesis. This phenotype is most likely explained by our observation that ΔN-UmuD' has a reduced capacity to interact with the RecA nucleoprotein filament and therefore fails to be suitably targeted to DNA. The ΔC-UmuD' proteins (plasmid pM73 for the ΔC-UmuD' and plasmid pM74 for the ΔN + ΔC mutant) were tested in essentially the same manner as the ΔN-UmuD' protein and we expect that the ΔC-UmuD' proteins also have a reduced capacity to interact with the RecA nucleoprotein filament.

UmuD' binding to a RecA nucleoprotein filament
The ability of UmuD' and ΔN-UmuD' (UmuD'302) to interact with a RecA nucleoprotein filament was assayed. RecA and the UmuD' or ΔN-UmuD' proteins were incubated with 1 mM ATP·S²⁻, with or without 60 ng of single-stranded αX174 DNA. Protein-DNA complexes were chemically cross-linked in 0.1% glutaraldehyde. Complexes were separated by electrophoresis in a 1.0% agarose gel. The ability of UmuD' and ΔN-UmuD' to interact with the RecA nucleoprotein filament was examined by transferring the DNA-protein complex to an Immobilon-P membrane that was incubated with a 1:10 000 dilution of affinity purified polyclonal UmuD' antisera. The position of the UmuD' and ΔN-UmuD' proteins was subsequently visualized using a chemiluminescent immunobassay.

Crytalization
The protein was crystallized by the hanging drop method in 100mM cacodylate buffer, pH 5.8, 600 mM Li₂SO₄, 20 mM MgCl₂, 5 mM DTT, 2 g l⁻¹ free ou-methionine at 20°C, with a final protein concentration of 12–15 mg ml⁻¹. Crystals formed in the course of 1–2 weeks and were typically 30 × 30 × 150 microns in space group P4₁2₁2. The crystals diffracted to ~3 Å at room temperature on a Rigaku R200 X-ray generator and to beyond 2.6 Å frozen at 100 K in paratone at the synchrotron.

Data collection and refinement
The Met138→Thr data were collected at X4A (NSLS) with an oscillation range of 3.0° (overlap of 0.9°) and exposure times of 240–480 s. The wild type data were collected at 4°C at beamline F1 of the Cornell High Energy Synchrotron Source (CHESS). The data were processed with DENZO [51] and scaled using a modified CCP4 [52] suite of programs. After rigid-body refinement in X-PLOR with the previously determined model, 2Fc–Fc maps were calculated in X-PLOR and displayed with the interactive graphics program O [53]. The model was refined using Powell minimization and manual rebuilding using the programs X-PLOR and O. Non-crystallographic symmetry (NCS) restraints were used for all rounds of refinement, except on residues involved in crystal contacts (residues 32–37, 102 and 138–139). The average B factor for the type Ic crystal was 150 microns in space group P4₁2₁2. The crystals diffracted to ~3 Å at room temperature on a Rigaku R200 X-ray generator and to beyond 2.6 Å frozen at 100 K in paratone at the synchrotron.

Δ and ΔC-UmuD' proteins were tested in essentially the same manner as the ΔN-UmuD' protein. Brian Meffle for help with the mutagenesis assays. Mary Ann Gawinowicz for N-terminal sequencing and David King (HHMI, UC Berkeley) for mass spectrometry analysis. This work was supported in part by a grant from the National Institutes of Health. Beamline X4A at the National Synchrotron Light Source, a DOE facility, is supported by the Howard Hughes Medical Institute. We also thank CHESS for synchrotron beam time.

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