Acta Biochimica Polonica

Vol. 45 No. 1/1998

163-172

QUARTERLY

Structural insights into the regulation of SOS mutagenesis*°

Martín Gonzalez, Ekaterina G. Frank, John P. McDonald, Arthur S. Levine and Roger Woodgate✉

Section on DNA Replication, Repair and Mutagenesis, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-2725 U.S.A.

Key words: *Escherichia coli, Salmonella typhimurium*, UmuD, UmuD', ClpX, ClpP, Lon, proteolysis

The *Escherichia coli* Umu proteins are best characterized by their role in damage inducible mutagenesis. Recently, we discovered that the intracellular levels of the UmuD and UmuC proteins are kept to a minimum by the Lon serine protease. Studies with the *Salmonella typhimurium* UmuD protein (which is 73% homologous with its *E. coli* counterpart) revealed that it too is degraded by Lon, suggesting that both UmuD proteins share conserved structural motifs. In contrast, *E. coli* UmuD' is removed from the cell by the ClpXP serine protease, but only when it is in a heterodimer complex with UmuD. We have generated deletion mutants of UmuD' and have co-expressed the mutant proteins with UmuD1 (a non-cleavable UmuD protein). By assaying the sensitivity of the mutant UmuD'-UmuD1 complex to ClpXP, we have been able to map regions of UmuD' that appear essential for efficient UmuD'-UmuD heterodimer formation.

Previous experiments have suggested that the *in vivo* posttranslational processing of UmuD to UmuD' is inefficient. We have, however, discovered that limited cleavage occurs in an undamaged cell, but that these small amounts of UmuD' are rapidly degraded by ClpXP, thus giving rise to the appearance of inefficient cleavage. The ClpXP protease therefore plays dual roles in regulating SOS mutagenesis: it keeps the basal levels of UmuD' to a minimum in undamaged cells but it also acts in damaged cells to reduce the elevated levels of mutagenically active UmuD' protein, thereby returning the cell to a resting non-mutable state.

The degradation of proteins has long been known to be vital to the health of both growing and nutritionally starved cells. Protein turnover places necessary controls on the levels of regulatory proteins whose concentrations must be rapidly adaptable to the changing needs and conditions of the cell. One example of the need for tight regulatory control in

*Presented at the Conference on "Mechanisms of DNA Repair and Mutagenesis" Commemorating the 100th Anniversary of the Discovery of Polonium and Radium, October, 1997, Warsaw, Poland.

°This work was performed while the author (M.G.) held a National Research Council SDRRM Research Associateship.

✉Corresponding author: Building 6, Room 1A13, NICHD, NIH, 9000 Rockville Pike, Bethesda, MD 20892-2725, U.S.A., tel: (301) 496 6175; fax: (301) 594 1135; e-mail: woodgate@helix.nih.gov

Escherichia coli is the SOS response, and in particular UmuD/D'C-mediated SOS mutagenesis. The UmuD and UmuC proteins are required, along with RecA and DNA polymerase III, to facilitate translesion DNA synthesis. This translesion DNA synthesis results in an increase in cell survival following DNA damage, but with a concomitant decrease in replication fidelity (for recent reviews see [1, 2]). Both UmuD and UmuC are labile *in vivo* and are degraded by the Lon protease [3]. Following SOS induction, UmuD and UmuC are expressed from the *umu* operon at about 2400 and about 200 copies per cell respectively [4]. A subset of the mutagenically inactive UmuD population then undergoes RecA-mediated cleavage to yield the mutagenically active UmuD' protein [5–7]. Under conditions of limited cleavage UmuD' preferentially interacts with the more abundant UmuD protein and this complex is subsequently targeted for degradation by the ClpXP protease [3]. However, substantial cleavage of UmuD to UmuD', as would be the case following significant DNA damage, favors the formation of the relatively stable UmuD' homodimer, which in turn is able to interact with and stabilize the otherwise labile UmuC protein [8]. The mechanism of UmuC stabilization is unknown, but it seems likely that by binding UmuD' the Lon degradation signal(s) on UmuC are somehow masked. It is evident that in order for translesion synthesis to proceed under SOS-inducing conditions several sequential protein-protein interactions must transpire to impart the necessary level of stability (or instability) for proper functioning of the mutagenesis proteins.

Clearly, regulated proteolysis is critical in maintaining tight control of the mutagenic response. In this report, we further investigate the roles of the Lon and ClpXP proteases. As part of these studies, we have characterized the stability of the *Salmonella typhimurium* UmuD protein and suggest that it too is a substrate of the Lon protease. We also provide evidence to suggest that limited UmuD cleavage occurs under non-SOS conditions but that the resultant UmuD' protein is rapidly degraded by ClpXP. Finally, we attempt to identify regions of UmuD' necessary for heterodimerization with UmuD, as well as identify potential sites involved in the targeting of UmuD', in a heterodimer context, for degradation by the ClpXP protease.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* K-12 strains used in this study are listed in Table 1. The low copy-number-plasmid encoding UmuD (pRW362) has been described previously [3]. Plasmid pRW364 which expresses the UmuD1 protein was constructed by cloning an *Eco*RI-*Bgl*II fragment from pGW2053 [9] into the *Eco*RI-*Bam*HI digested low-copy-number vector, pGB2 [10]. Low-copy-number plasmids expressing carboxyl-terminal deletions of UmuD' and medium-copy-number plasmids expressing *E. coli* or *S. typhimurium* UmuD were constructed by J.P. McDonald and will be described in detail elsewhere. The pKSD plasmids express the non-cleavable UmuD1 protein *in cis* with either the wild-type UmuD' protein or the UmuD' deletion mutants described in this study. These plasmids were constructed by inserting a 433 bp *Pst*I-*Pvu*II fragment encoding either wild-type UmuD'(pJM105) or a one (pJM79), two (pJM78), three (pJM77), or four (pJM73) amino acid deletion of the extreme carboxyl-terminus of UmuD' into the *Pst*I-*Pml*I digested vector, pRW364. Plasmid pKSD7, which co-expresses UmuD1 and the 20 amino acid amino-terminal deletion of UmuD' (UmuD'302) *in cis*, was similarly generated by inserting the *Pst*I-*Pvu*II fragment of pJM72 [11] into the *Pst*I-*Pml*I digested pRW364. More detailed descriptions of all plasmid constructs used in this study are available upon request.

In vivo *stability of wild-type and mutant Umu proteins.* The stability of the Umu pro-

Table 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Relevant genotype or characteristics	Source or reference
Strains		
EC10	*recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT*	[3]
EC18	*recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT* *lon146*::Tn*10*	[3]
EC22	*recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT* *clpP*::Kan	[3]
EC28	*recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT* *clpX*::Kan	[3]
Plasmids		
pJM125	Amp^r, medium-copy-number, pBR322 based plasmid that expresses wild-type *E. coli* UmuD	J.P. McDonald
pJM126	Amp^r, medium-copy-number, pBR322 based plasmid that expresses wild-type *S. typhimurium* UmuD	J.P. McDonald
pRW362	Spc^r, low-copy-number, pGB2 based plasmid expressing wild-type UmuD	[3]
pRW364	Spc^r, low-copy-number, pGB2 based plasmid expressing UmuD1	This study
pKSD1	Spc^r, low-copy-number, pGB2 based plasmid co-expressing UmuD1 and UmuD′	This study
pKSD2	Spc^r, low-copy-number, pGB2 based plasmid co-expressing UmuD1 and UmuD′308 (a one amino acid carboxyl-terminal deletion of UmuD′)	This study
pKSD3	Spc^r, low-copy-number, pGB2 based plasmid co-expressing UmuD1 and UmuD′307 (a two amino acid carboxyl-terminal deletion of UmuD′)	This study
pKSD4	Spc^r, low-copy-number, pGB2 based plasmid co-expressing UmuD1 and UmuD′306 (a three amino acid carboxyl-terminal deletion of UmuD′)	This study
pKSD5	Spc^r, low-copy-number, pGB2 based plasmid co-expressing UmuD1 and UmuD′303 (a four amino acid carboxyl-terminal deletion of UmuD′)	This study
pKSD7	Spc^r, low-copy-number, pGB2 based plasmid co-expressing UmuD1 and UmuD′302 (a twenty amino acid amino terminal deletion of UmuD′)	This study

teins in various genetic backgrounds was determined as previously described [3, 8]. Cells were grown in Luria broth at 37°C to early exponential phase. At time zero, 100 μg/ml chloramphenicol was added to the medium and 1.5 ml aliquots were removed at various time points thereafter. Cells were harvested by centrifugation and the resulting cell pellet was resuspended in electrophoresis sample buffer (50 mM Tris/HCl [pH 6.8], 10% glycerol, 2.0% sodium dodecyl sulfate [SDS], 0.1% bromophenol blue, 10 mM dithiothreitol). Aliquots representing equal cell numbers were electrophoresed on SDS/17% PAGE gels. Proteins were then transferred to an Immobilon P membrane (Millipore) and subsequently probed with a 1:8000 dilution of affinity purified polyclonal antibodies raised against UmuD/UmuD′. The transferred proteins were visualized using the CPSD-Western light chemiluminescent assay (Tropix, Bedford, MA, U.S.A.). Membranes were exposed to Kodak Bio-Max MR film for periods of 4–20 min.

RESULTS

Degradation of *Salmonella typhimurium* UmuD by the Lon protease

SOS mutagenesis is known to require stringent regulation of the *E. coli* Umu proteins at various stages of the mutagenic response. We recently demonstrated that the *E. coli* Umu proteins, UmuD and UmuC, are also regulated at the post-translational level. These pro-

teins were found to be extremely labile and their instability was mediated by the ATP-dependent serine protease, Lon [3]. In an attempt to further understand the role of Lon, and proteolysis in general, we chose to characterize the stability of the *S. typhimurium* UmuD protein. The *S. typhimurium* UmuD protein shares about 73% amino acid identity with the *E. coli* UmuD protein [12,13] and has been shown to substitute functionally for *E. coli* UmuD [14,15]. The premise behind characterizing *S. typhimurium* UmuD stability is quite simple. Should *S. typhimurium* UmuD protein remain stable in the presence of Lon, we could focus on areas of non-homology as potential degradation signals in the *E. coli* UmuD protein. Conversely, if *S. typhimurium* UmuD is degraded in the presence of Lon, it would suggest that the Lon degradation signal is in a region conserved between the two UmuD proteins.

To address these hypotheses, we determined the stability of each of the separately plasmid-encoded Umu proteins in isogenic *E. coli* Δ*umuDC lexA51*(Def) strains harboring either a *lon*$^+$ or *lon*$^-$ allele (Fig. 1). As is evident, *S. typhimurium* UmuD was highly labile in the *lon*$^+$ background with only a small percentage of the initial protein remaining after 10 minutes. In contrast, *S. typhimurium* UmuD protein levels remained consistently high throughout the assay period in the *lon*$^-$ strain. These results mimic that seen with *E. coli* UmuD (Fig. 1), and strongly suggest that the *S. typhimurium* UmuD protein is also a substrate of the Lon protease. Presumably, such proteolysis results from both proteins sharing common Lon degradation signals.

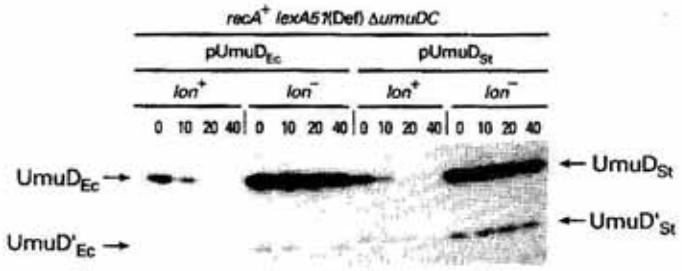

Figure 1. Stability of the *E. coli* and *S. typhimurium* UmuD proteins in isogenic *lon*$^+$ and *lon*$^-$ strains.

Plasmids expressing *E. coli* UmuD (pJM125) and *S. typhimurium* (pJM126) were introduced into the *recA*$^+$ *lexA51*(Def) Δ(*umuDC*)596::*ermGT lon*$^+$ strain, EC10, and the *recA*$^+$*lexA51*(Def) Δ(*umuDC*) 596::*ermGT lon*$^-$ strain, EC18. The relative stability of the UmuD species was measured after protein synthesis was inhibited by the addition of chloramphenicol (100 μg/ml) at time zero. Additional aliquots were taken at the indicated times.

Role of ClpXP in minimizing UmuD′ levels under non SOS-inducing conditions

As noted earlier, the lengths that *E. coli* goes to in order to keep the level and activity of the Umu proteins to a minimum are numerous and quite complex. Part of this regulation is the posttranslational processing of UmuD to the mutagenically active UmuD′ protein. Based upon previous *in vivo* studies, it was believed that this cleavage reaction was very inefficient and only occurred under conditions of severe DNA damage [4]. Such conclusions were, however, based upon the limited appearance of UmuD′ in undamaged cells or in cells exposed to modest DNA damage. Based upon our recent observations which indicate that UmuD′ is rapidly degraded *in vivo* by the ClpXP protease when in a heterodimer complex with UmuD, we hypothesized that such a targeting mechanism allowed the cell to return to a resting non-mutable state after DNA damage had been repaired [3]. There is, however, no reason to believe that ClpXP would only work *after* DNA damage; it is equally plausible that ClpXP also works *before* DNA damage. Indeed, when we assayed the stability of plasmid-encoded UmuD in *clp*$^+$ and *clpP* backgrounds, we were able to detect limited (but significant) quantities of UmuD′ even in an undamaged cell (Fig. 2). Similar to the ex-

periments where we co-express UmuD and UmuD′ *in cis* from a recombinant plasmid (see [3] and Figs. 3 and 4 below), the limited amount of UmuD′ generated by gratuitous cleavage was stable in a *clpP* background and appears to form a stable heterodimer with UmuD (Fig. 2). This gratuitous cleavage is not observed in the wild-type *clp*$^+$ strain, not because of a lack of cleavage, but because UmuD′ is rapidly degraded by ClpXP. Thus, it would appear that cleavage of UmuD to UmuD′, while still relatively poor, is not as inefficient as we had originally suggested. Rapid proteolysis of UmuD′ *in vivo* simply gives the false impression that cleavage is very inefficient. The fact that UmuD′ is actually observed at all in a *clp*$^+$ cell [4] presumably means that at some point the ClpXP-UmuD/UmuD′ degratory pathway becomes saturated, thereby allowing for the accumulation of significant levels of UmuD′.

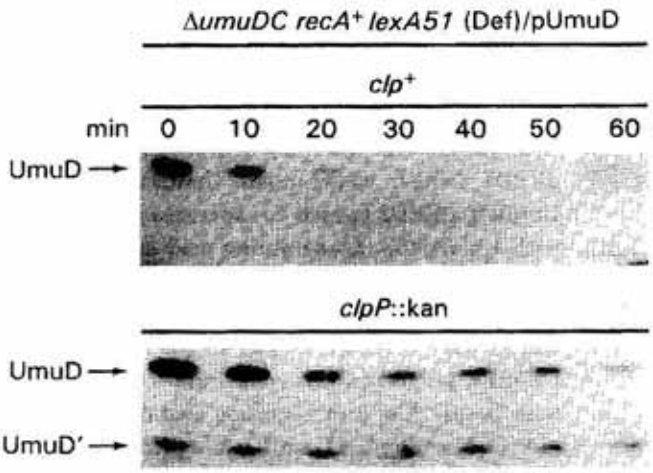

Figure 2. Proteolytic processing of UmuD to UmuD′ under non-SOS conditions.

Visualization of UmuD′ production was accomplished by introducing the UmuD expressing plasmid, pRW362, into the *recA*$^+$ *lexA51*(Def) Δ(*umuDC*)596::*ermGT clp*$^+$ strain, EC10, and the *recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT clpP*::kan strain, EC22. Chloramphenicol (100 μg/ml) was added at time zero to inhibit protein synthesis and aliquots were taken at the indicated times.

Role of the carboxyl-terminus of UmuD′ in heterodimerization

The carboxyl-terminus of the Mu transposase, MuA [16], and that of certain virulent derivatives of the Mu repressor [17, 18] are thought to be necessary for their degradation by the ClpXP protease. Other substrates of ClpXP such as the λO protein [19] and the P1 Phd protein [20] appear to have weak sequence similarity to that of MuA and the Mu repressor derivatives. Although the carboxyl-terminus of UmuD′ displays weak amino acid homology to the other ClpXP substrates, we were interested in assessing the role, if any, that the carboxyl-terminus of UmuD′ might play in ClpXP recognition and/or degradation.

The fact that UmuD′ is only targeted for proteolysis by ClpXP when it is in a heterodimer with UmuD rather than a homodimer provides us with a bio-assay by which we can follow heterodimer formation *in vivo*. If mutant UmuD′ proteins are able to interact with UmuD, the mutants, while potentially stable as homodimers, would be expected to be degraded upon forming a heterodimer with UmuD in *clp*$^+$ strains, but not in *clpXP* strains. As a consequence, we transferred low-copy-number plasmids that co-express *in cis* the UmuD1 (a non-cleavable UmuD derivative) and UmuD′ (or the individual carboxyl-terminal truncations of UmuD′) proteins into various *lexA*(Def) Δ*umuDC* strains. As expected, the wild-type heterodimer UmuD1/UmuD′ displayed characteristics similar to that described previously [3]. In the wild-type background, UmuD1 and UmuD′ are quite labile proteins (Fig. 3). Although both proteins of the heterodimer complex are stabilized in *clpX*::kan background, we have demonstarted that UmuD′ and not UmuD1 is the substrate for the ClpXP protease (E.G. Frank, unpublished results). As we have discussed previously [3], UmuD1 in a UmuD1/UmuD′ heterodimer may simply be insensitive to proteoly-

sis by the Lon protease in a *clpX*::kan background. In contrast, neither the homodimeric UmuD1 nor UmuD′ proteins are substrates of the ClpXP protease (unpublished observation). Based upon the stability of the various heterodimeric complexes in *clp*$^+$ and *clpX* backgrounds, it appears that limited amino acid truncations of the UmuD′ carboxyl-terminus (the largest truncation being 4 amino acids) apparently retain the ability to interact with UmuD1 and, as a consequence, remain substrates of the ClpXP protease (Fig. 3). The overall stability of a single amino acid truncation, UmuD′308, is comparable to wild-type UmuD′ (Fig. 3), whereas carboxyl-terminal truncations between 2 to 4 amino acids of UmuD′ are, however, generally more labile than wild-type UmuD′. It is most probable that these truncations are recognized by other cellular proteases since they all display some instability even in a *clpX*::kan background.

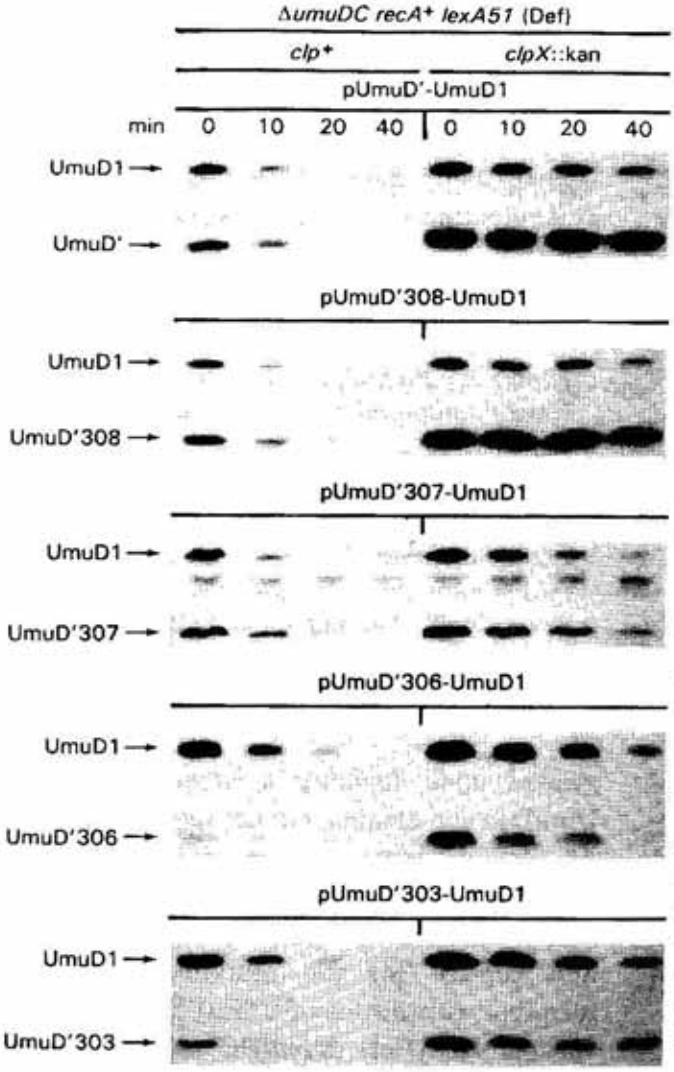

Figure 3. Stability of UmuD1 and carboxyl-terminal deletions of UmuD′ in isogenic *clp*$^+$ and *clpX* strains

The stability of UmuD1 and the various UmuD′ deletion mutants was analyzed by introducing plasmids pKSD1 (which co-expresses UmuD1 *in cis* with UmuD′), pKSD2 (which co-expresses UmuD1 *in cis* with UmuD′308, a one amino acid deletion of the carboxyl terminal of UmuD′), pKSD3 (which co-expresses UmuD1 *in cis* with UmuD′307, a two amino acid deletion of the carboxyl terminal of UmuD′), pKSD4 (which co-expresses UmuD1 *in cis* with UmuD′306, a three amino acid deletion of the carboxyl terminal of UmuD′), and pKSD5 (which co-expresses UmuD1 *in cis* with UmuD′303, a four amino acid deletion of the carboxyl terminal of UmuD′) into the *recA*$^+$ *lexA51*(Def) Δ*(umuDC)596*::*ermGT clp*$^+$ strain, EC10, and the *recA*$^+$ *lexA51*(Def) Δ*(umuDC)596*::*ermGT clpX*::kan strain, EC28. The relative stability of UmuD1 and the UmuD′ deletion mutants were measured after chloramphenicol (100 μg/ml) inhibition of protein synthesis at time zero. Additional aliquots were removed at the indicated times.

Effect of an amino-terminal truncation on UmuD′ stability

The results reported above demonstrate that carboxyl-terminal truncations of up to four amino acids appear to have little effect on the ability of the protein to form heterodimeric complexes. We were therefore interested in assessing the potential role of the amino-terminus of UmuD′ in heterodimer formation. To do so, we utilized a previously de-

scribed amino-terminal deletion of UmuD' designated UmuD'302 (or ΔN-UmuD'). The UmuD'302 mutant lacks the 20 amino acid amino-terminal tail of UmuD'. This mutant protein is able to form homodimers similar to wild-type UmuD and UmuD', yet it is functionally defective in SOS-dependent spontaneous or MMS induced mutagenesis [11]. We transferred a low-copy-number plasmid which co-expresses UmuD'302 and UmuD1 *in cis* into a *lexA*(Def) Δ*umuDC* strain either proficient or deficient for ClpX activity (Fig. 4). In the *clp*$^+$ strain, UmuD'302 and UmuD1 appear to form heterodimers at a much reduced efficiency. The levels of UmuD'302 in the presence of UmuD1, are considerably higher at each time point than that seen for wild-type UmuD'. This is most likely due to the inability of UmuD'302 to form efficient heterodimers with UmuD1 resulting in an appreciable decrease in the ClpXP-mediated degradation of UmuD'302. This conclusion is further supported by our observation that UmuD'302 was not greatly stabilized in a *clpX* background. In contrast, however, UmuD1 (in the presence of UmuD'302) demonstrated a marked stabilization in the *clpX*::kan strain (Fig. 4).

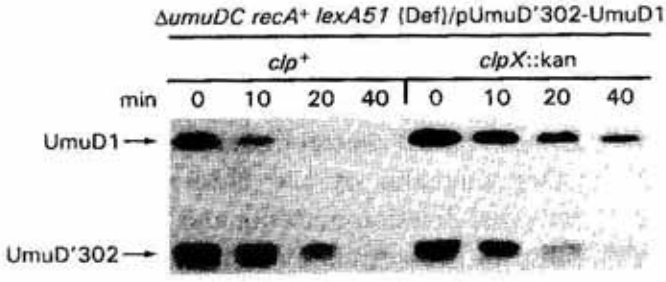

Figure 4. Role of the amino terminus in UmuD'/UmuD1 stability.

Plasmid pKSD7 co-expressing UmuD'302 and UmuD1 was introduced into the *recA*$^+$ *lexA51*(Def) Δ*(umuDC)596::ermGT clp*$^+$ strain, EC10, and the *recA*$^+$ *lexA51*(Def) Δ*(umuDC)596::ermGT clpX*::kan strain, EC28. The relative stability of UmuD1, and the amino-terminal 20 amino acid deletion of UmuD' (UmuD'302), were measured after chloramphenicol (100 μg/ml) inhibition of protein synthesis at time zero. Additional aliquots were removed at the indicated times.

DISCUSSION

Proteolysis plays a vital role in the regulation of many processes. In view of the many protein interactions necessary for SOS mutagenesis to proceed, it is no wonder that proteolysis is so vital to the regulation of the mutagenic response after DNA damage. The *E. coli* Lon protease contributes to this complex regulatory pathway by degrading both the UmuD and UmuC proteins [3]. By comparison, UmuD' is relatively insensitive to proteolysis by Lon [3]. This observation alone is highly suggestive that the Lon degradation signal is located within the N-terminal amino acids of UmuD which are absent in UmuD'. In this report, we also identify the *S. typhimurium* UmuD protein as a substrate of the *E. coli* Lon protease. If our conclusions about the Lon recognition signal being located in the N-terminus of UmuD are correct, one could argue that such recognition sites would be shared by both the *E. coli* and *S. typhimurium* UmuD proteins. Interestingly, while the two proteins are 73% identical over the entire protein, the N-terminal tails are more diverged, sharing only 13/24 identical residues [12, 13]. Experiments designed to pin-point the Lon degradation signal will be reported elsewhere.

In order to form the mutagenically active UmuD' protein, UmuD must undergo a RecA-mediated self-cleavage reaction [7]. The UmuD cleavage reaction is relatively inefficient *in vivo* [4] and following limited cleavage one could speculate that a number of UmuD (UmuD') dimer species might exist. The two prominent complexes would be the UmuD homodimer and the UmuD/UmuD' heterodimer. Because of the preferential formation of UmuD/UmuD' heterodimers [9], it seems

highly unlikely that a UmuD′ homodimer would exist under conditions of limited cleavage. Furthermore, formation of a stable mutagenically active UmuD′ homodimer in an undamaged cell may lead to high levels of spontaneous mutagenesis. *E. coli* has clearly gone to great lengths to ensure that the levels of the Umu proteins remain in check during normal cell growth. The UmuD protein is present at about 180 copies per uninduced cell [4] and despite the inefficient nature of the cleavage reaction, some UmuD′ is generated, even in undamaged cells (Fig. 2). Evidence of the formation of UmuD′ in an uninduced cell has not, in all likelihood, been reported previously because it appears that UmuD′ is specifically targeted for ClpXP-mediated degradation when complexed with UmuD and thus can only be visualized in an ClpXP-deficient strain (Fig. 2). We believe, therefore, that the ClpXP protease plays dual roles in regulating SOS mutagenesis: it keeps the basal levels of UmuD′ to a minimum in undamaged cells and thereby postpones the likelihood of aberrant error-prone DNA synthesis. ClpXP also acts in damaged cells to reduce the elevated levels of mutagenically active UmuD′ protein thereby returning the cell to a resting non-mutable state [3].

Most protein interactions tend to stabilize otherwise labile proteins. The normally stable UmuD′ protein, in contrast, has been shown (*in vivo*) to be a substrate of the ClpXP protease only when complexed in a heterodimer with UmuD [3]. Known substrates of ClpXP are postulated to be degraded *via* recognition of a carboxyl-terminal recognition or degradation signal [16]. Deletion of the four extreme carboxyl-terminal amino acids of the Mu transposase, MuA, results in a protein less sensitive to the action of ClpXP [16, 21]. Mutant derivatives of the otherwise stable Mu *c* repressor (designated vir) have modified carboxyl-termini which impart ClpXP-mediated instability [22, 23]. A single amino acid substitution in the carboxyl-terminal 7 amino acids of the vir repressor protein increases the stability of the mutant [18]. In contrast, however, carboxyl-terminal deletions of UmuD′ (up to four amino acids) had little effect on the apparent ability of the mutants to heterodimerize with UmuD1 and did not appear to change the susceptibility of the mutant UmuD′/UmuD1 heterodimer to degradation by ClpXP. Analysis of the large amino-terminal deletion mutant (UmuD′302) suggests that it can still form heterodimers with UmuD but with a greatly reduced affinity. This conclusion was based upon the increased stability of UmuD′302 compared to UmuD′ when co-expressed with UmuD1 in a clp^+ background and the lack of UmuD′302 stabilization in a *clpX* strain (although UmuD1 was stabilized). Moreover, *in vitro* results did not reveal a UmuD′302 /UmuD interaction (E.G. Frank, unpublished results). These observations strongly suggest that the 20 N-terminal residues of UmuD′ play an important role in UmuD/UmuD′ heterodimer formation.

Our analysis of the N-terminal and C-terminal deletion mutants and their susceptibility to proteolysis by ClpXP, together with recent data suggesting that an internal sequence of the σ^S starvation-transcription factor is required for ClpXP mediated degradation [24], indicate that ClpXP recognition of potential substrates will involve sequences, or more likely structures, that span the entire length of the protein.

Clearly, proteolysis is a major contributing factor in many biological processes. With all of the intricate protein interactions required for maintaining proper levels of the mutagenesis proteins, the SOS response remains a valuable system in which to study the mechanism(s) driving regulated proteolysis.

REFERENCES

1. Friedberg, E.C., Walker, G.C. & Siede, W. (1995) *DNA Repair and Mutagenesis*. American Society of Microbiology, Washington, DC.

2. Woodgate, R. & Levine, A.S. (1996) Damage inducible mutagenesis: Recent insights into the activities of the Umu family of mutagenesis proteins; in *Cancer Surveys: Genetic Instability in Cancer* (Lindahl, T., ed.) pp. 117–140, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

3. Frank, E.G., Ennis, D.G., Gonzalez, M., Levine, A.S. & Woodgate, R. (1996) Regulation of SOS mutagenesis by proteolysis. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10291–10296.

4. Woodgate, R. & Ennis, D.G. (1991) Levels of chromosomally encoded Umu proteins and requirements for *in vivo* UmuD cleavage. *Mol. Gen. Genet.* **229**, 10–16.

5. Shinagawa, H., Iwasaki, H., Kato, T. & Nakata, A. (1988) RecA protein-dependent cleavage of UmuD protein and SOS mutagenesis. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1806–1810.

6. Burckhardt, S.E., Woodgate, R., Scheuermann, R.H. & Echols, H. (1988) UmuD mutagenesis protein of *Escherichia coli*: Overproduction, purification and cleavage by RecA. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1811–1815.

7. Nohmi, T., Battista, J.R., Dodson, L.A. & Walker, G.C. (1988) RecA-mediated cleavage activates UmuD for mutagenesis: Mechanistic relationship between transcriptional derepression and posttranslational activation. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1816–1820.

8. Frank, E.G., Gonzalez, M., Ennis, D.G., Levine, A.S. & Woodgate, R. (1996) *In vivo* stability of the Umu mutagenesis proteins: A major role for RecA. *J. Bacteriol.* **178**, 3550–3556.

9. Battista, J.R., Ohta, T., Nohmi, T., Sun, W. & Walker, G.C. (1990) Dominant negative *umuD* mutations decreasing RecA-mediated cleavage suggest roles for intact UmuD in modulation of SOS mutagenesis. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7190–7194.

10. Churchward, G., Belin, D. & Nagamine, Y. (1984) A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors. *Gene* **31**, 165–171.

11. Peat, T.S., Frank, E.G., McDonald, J.P., Levine, A.S., Woodgate, R. & Hendrickson, W.A. (1996) The UmuD' protein filament and its potential role in damage induced mutagenesis. *Structure* **4**, 1401–1412.

12. Thomas, S.M., Crowne, H.M., Pidsley, S.C. & Sedgwick, S.G. (1990) Structural characterization of the *Salmonella typhimurium* LT2 *umu* operon. *J. Bacteriol.* **172**, 4979–4987.

13. Smith, C.M., Koch, W.H., Franklin, S.B., Foster, P.L., Cebula, T.A. & Eisenstadt, E. (1990) Sequence analysis of the *Salmonella typhimurium* LT2 *umuDC* operon. *J. Bacteriol.* **172**, 4964–4978.

14. Sedgwick, S.G., Lodwick, D.L., Doyle, N., Crowne, H.M. & Strike, P. (1991) Functional complementation between chromosomal and plasmid mutagenic DNA repair genes. *Mol. Gen. Genet.* **229**, 428–436.

15. Koch, W.H., Kopsidas, G., Meffle, B., Levine, A.S. & Woodgate, R. (1996) Analysis of chimeric UmuC proteins: Identification of regions in *Salmonella typhimurium* UmuC important for mutagenic activity. *Mol. Gen. Genet.* **251**, 121–129.

16. Levchenko, I., Luo, L. & Baker, T.A. (1995) Disassembly of the Mu transposase tetramer by the ClpX chaperone. *Genes & Dev.* **9**, 2399–2408.

17. Geuskens, V., Vogel, J.L., Grimaud, R., Desmet, L., Higgins, N.P. & Toussaint, A. (1991) Frameshift mutations in the bacteriophage Mu repressor gene can confer a *trans*-dominant virulent phenotype to the phage. *J. Bacteriol.* **173**, 6578–6585.

18. Laachouch, J.E, Desmet, L., Geuskens, V., Grimaud, R. & Toussaint, A. (1996) Bacteriophage Mu repressor as a target for the *Escherichia coli* ATP-dependent Clp protease. *EMBO J.* **15**, 437–444.

19. Wojtkowiak, D., Georgopoulos, C. & Zylicz, M. (1993) Isolation and characterization of ClpX, a new ATP-dependent specificity component of the Clp protease of *Escherichia coli*. *J. Biol. Chem.* **268**, 22609–22617.

20. Lehnherr, H. & Yarmolinsky, M.B. (1995) Addiction protein Phd of plasmid prophage P1 is a substrate of the ClpXP serine protease of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 3274–3277.

21. Levchenko, I., Yamauchi, M. & Baker, T.A. (1997) ClpX and MuB interact with overlapping regions of Mu transposase: Implications for control of the transposition pathway. *Genes & Dev.* **11**, 1561–1572.

22. Geuskens, V., Mhammedi-Alaoui, A., Desmet, L. & Toussaint, A. (1992) Virulence in bacteriophage Mu: A case of *trans*-dominant proteolysis by the *Escherichia coli* Clp serine protease. *EMBO J.* **11**, 5121–5127.

23. Mhammedi-Alaoui, A., Pato, M., Gama, M.-J. & Toussaint, A. (1994) A new component of bacteriophage Mu replicative transposition machinery: The *Escherichia coli* ClpX protein. *Mol. Microbiol.* **11**, 1109–1116.

24. Schweder, T., Lee, K.H., Lomovskaya, O. & Matin, A. (1996) Regulation of *Escherichia coli* starvation sigma factor (σ^S) by ClpXP protease. *J. Bacteriol.* **178**, 470–476.