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Genetics

Regulation of SOS mutagenesis by proteolysis

(UmuD/D′/UmuC/Lon/ClpXP/protein–protein interactions)

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ABSTRACT **DNA damage-inducible mutagenesis in *Escherichia coli* is largely dependent upon the activity of the UmuD (UmuD′) and UmuC proteins. The intracellular level of these proteins is tightly regulated at both the transcriptional and the posttranslational levels. Such regulation presumably allows cells to deal with DNA damage via error-free repair pathways before being committed to error-prone pathways. We have recently discovered that as part of this elaborate regulation, both the UmuD and the UmuC proteins are rapidly degraded *in vivo*. We report here that the enzyme responsible for their degradation is the ATP-dependent serine protease, Lon. In contrast, UmuD′ (the posttranslational product and mutagenically active form of UmuD) is degraded at a much reduced rate by Lon, but is instead rapidly degraded by another ATP-dependent protease, ClpXP. Interestingly, UmuD′ is rapidly degraded by ClpXP only when it is in a heterodimeric complex with UmuD. Formation of UmuD/UmuD′ heterodimers in preference to UmuD′ homodimers therefore targets UmuD′ protein for proteolysis. Such a mechanism allows cells to reduce the intracellular levels of the mutagenically active Umu proteins and thereby return to a resting state once error-prone DNA repair has occurred. The apparent half-life of the heterodimeric UmuD/D′ complex is greatly increased in the *clpX*::Kan and *clpP*::Kan strains and these strains are correspondingly rendered virtually UV nonmutable. We believe that these phenotypes are consistent with the suggestion that while the UmuD/D′ heterodimer is mutagenically inactive, it still retains the ability to interact with UmuC, and thereby precludes the formation of the mutagenically active UmuD′$_2$C complex.**

Exposure of *Escherichia coli* to many DNA-damaging agents that cause bulky adducts often results in a significant increase in the mutation frequency. While many DNA repair and recombination proteins are induced as a consequence of DNA damage, only a few gene products are required for this so-called "SOS mutagenesis" (for review, see ref. 1). Key participants in this process are the UmuD and UmuC proteins, which are thought to interact with RecA and DNA polymerase III in such a way as to facilitate translesion DNA synthesis (2–5). Perhaps because the mutagenic pathway is, by definition, error-prone, the activity of the Umu proteins is tightly regulated. For example, (*i*) the *umuDC* operon is tightly repressed by LexA protein and would be expected to be only fully derepressed under conditions of severe DNA damage (6); (*ii*) even when fully expressed, both the UmuD (7) and UmuC (7–9) proteins are labile and are rapidly degraded *in vivo*; (*iii*) UmuD protein is functionally inactive until it undergoes an inefficient RecA-mediated autoproteolytic cleavage reaction that generates the mutagenically active UmuD′ protein (10–12); and (*iv*) UmuD′ interacts with UmuD$^+$ to generate a mutagenically inactive UmuD$^+$/UmuD′ heterodimer in preference to an active UmuD′ homodimer (13).

Given the elaborate mechanisms that *E. coli* uses to keep the activity of the Umu proteins to a minimum, avoiding gratuitous mutagenesis, we were perplexed by the apparent stability of UmuD′$_2$C (7) and wondered if a mechanism existed to quickly reduce the level of the uncomplexed Umu proteins. One feasible mechanism to achieve this goal would be to target the Umu proteins for proteolytic degradation. *E. coli* possesses a number of proteases (for reviews, see refs. 14 and 15), with the best characterized being the ATP-dependent serine proteases, Lon and Clp. Lon is a homotetramer of ≈450 kDa that is known to degrade a number of cellular proteins including SulA and RcsA (14, 15). The Clp protease, in contrast, is a large heterooligomeric structure that consists of a protease subunit (ClpP) and a specificity subunit consisting of ClpA, ClpB, ClpX, or ClpY (14, 15). ClpAP has previously been shown to degrade a number of substrates (16) including ClpA itself (17) and the bacteriophage P1 RepA protein (18). By comparison, studies have shown that substrates of ClpXP include the bacteriophage-encoded proteins such as PhD (phage P1) (19), O protein (phage λ) (20), and MuA (phage Mu) (21–23), and the chromosomally expressed starvation sigma factor, RpoS (σ^S) (24). In addition to acting in concert with ClpP to promote proteolysis, both ClpA and ClpX have been shown to possess chaperonin-like activity independent of ClpP (18, 25, 26).

We were interested in determining what role proteolysis might play in regulating the intracellular levels of the Umu proteins and have therefore determined the *in vivo* stability of the UmuD, UmuD′ and UmuC proteins in various *lon*, *clpA*, *clpB*, *clpX*, and *clpP* strains.

Analysis revealed that the Umu proteins are indeed substrates of the Lon and ClpXP proteases. Lon appears to act by rapidly degrading UmuD before it is converted to the mutagenically active form of UmuD′. Likewise, Lon also degrades UmuC before it becomes stabilized by UmuD′ (7, 8). In contrast, ClpXP acts at a later stage (after activation of UmuD′) by specifically degrading UmuD′ when it is associated with UmuD in a UmuD/UmuD′ heterodimer. The implications and consequences of these proteolytic pathways for SOS mutagenesis are discussed.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The *E. coli* K-12 strains used in this study are listed in Table 1. All new strains were constructed by standard methods of generalized P1 transduction (30). Plasmids encoding UmuD (pRW362) (7), UmuD′

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Table 1. *E. coli* K-12 strains used in this study

Strain	Relevant genotype	Ref. or source
TK603	*recA*$^+$ *lexA*$^+$ *hisG4*	27
SG22099	*clpA319*::Kan	28
SG22100	*clpB*::Kan	25
TS356	*clpP*::Kan	T. Shrader
SG22101	*clpX*::Kan	28
SG12047	*lon146*::Tn*10*	29
SG22094	*rcsA166*::Kan Δ*lon clpP1*::*cat*	S. Gottesman
RW110	*recA*$^+$ *lexA*$^+$ *hisG4 sulA211*	Section on DNA Replication, Repair and Mutagenesis lab collection
EC8	*recA*$^+$ *lexA*$^+$ *uvrA6 hisG4* Δ(*umuDC*)*596*::*ermGT*	7
EC10	*recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT*	7
EC18	*recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT lon146*::Tn*10*	P1.SG12047 × EC10
EC22	*recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT clpP*::Kan	P1.TS356 × EC10
EC24	*recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT clpA319*::Kan	P1.SG22099 × EC10
EC26	*recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT clpB*::Kan	P1.SG22100 × EC10
EC28	*recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT clpX*::Kan	P1.SG22101 × EC10
EC34	*recA*$^+$ *lexA*$^+$ *uvrA6 hisG4 clpP*::Kan	P1.TS356 × TK603
EC202	*recA*$^+$ *lexA*$^+$ *uvrA6 hisG4 clpA319*::Kan	P1.SG22099 × TK603
EC204	*recA*$^+$ *lexA*$^+$ *uvrA6 hisG4 clpB*::Kan	P1.SG22100 × TK603
EC206	*recA*$^+$ *lexA*$^+$ *uvrA6 hisG4 clpX*::Kan	P1.SG22101 × TK603
EC210	*recA*$^+$ *lexA*$^+$ *uvrA6 hisG4 sulA211 rcsA166*::Kan	P1.SG22094 × RW110
EC212	*recA*$^+$ *lexA*$^+$ *uvrA6 hisG4 sulA211 rcsA166*::Kan *lon146*::Tn*10*	P1.SG12047 × EC210

(pRW66) (31), and UmuC (pRW124) (31) have been described. Plasmid pRW366, which expresses UmuD′ and a noncleavable UmuD1 protein in cis (from a low-copy-number plasmid), was constructed by cloning a 1631-bp *Bgl*II–*Eco*RI fragment from pGW2053 (13) into the similarly digested vector, pRW134 (32). Although UmuD1 carries a mutation that changes $Pro^{27} \rightarrow Ser^{27}$ and renders the protein noncleavable (33), the mutant protein is expressed at similar steady-state levels (13, 33) and has the same stability as the wild-type UmuD protein (unpublished observations). While both the UmuD′ and UmuD1 proteins are expressed from their natural LexA-regulated operator/promoter sequences, previous experiments have shown that UmuD (D1) is produced at somewhat higher levels than UmuD′ (8). Recent studies suggest, however, that UmuD′ is much more stable than UmuD (7). As a consequence of this differential expression and stability, we expect the steady-state levels of the pRW366-encoded UmuD1 and UmuD′ proteins to be approximately stoichiometric.

***In Vivo* Stability of the Umu Proteins in Various Genetic Backgrounds.** The stability of the Umu proteins in the Δ(*umuDC*)*596*::*ermGT recA*$^+$ *lexA51*(Def) strain, EC10, and its isogenic protease-deficient derivatives was studied as described previously (7). This assay allows us to follow the stability of the Umu proteins after protein synthesis is inhibited by the addition of chloramphenicol. Similar assay conditions have been previously used in studying the stability of many proteins, including LexA and the bacteriophage Mu repressor proteins (21, 34). Briefly, cells were grown at 37°C in Luria–Bertani media until the early exponential phase. At time zero, 100 μg/ml chloramphenicol was added to the culture and aliquots were removed at various times thereafter. Cells were harvested by centrifugation and the resulting cell pellet was resuspended in electrophoresis sample buffer. Cells were lysed by repeated freeze-thaw cycles and the resulting extract was subjected to electrophoresis in either SDS/15% PAGE gels (for UmuC) or SDS/17% PAGE gels (for UmuD and UmuD′). Proteins were electrotransferred to an Immobilon P membrane (Millipore) and subsequently probed either with a 1:10,000 dilution of affinity-purified polyclonal antisera raised against UmuD/UmuD′ or with a 1:20,000 dilution of polyclonal antisera raised against UmuC (31, 35). The Umu proteins were subsequently visualized using the CSPD-Western light chemiluminescent assay (Tropix, Bedford, MA). Membranes were exposed to Kodak X-Omat or Bio-Max film for periods of 1–20 min and the film subjected to densitometric analysis using the software NIH IMAGE (version 1.59; National Institutes of Health, Bethesda). While the estimated half-lives of the Umu proteins determined by this method have been found to be shorter than those previously described (7, 8), the same relative stability of the Umu proteins is, however, observed and we believe that this approach is a valid representation of the stability of the Umu proteins *in vivo* (7).

The UmuD/D′ antisera is very specific and only recognizes the UmuD and UmuD′ proteins in whole-cell *E. coli* extracts. The UmuC antisera is also specific but cross-reacts with another protein that is ≈5 kDa smaller than UmuC. While the identity of this protein is unknown, it serves as a useful internal control ensuring that equal amounts of protein extract have been applied to the gel (7).

Mutagenesis Assays. UV-induced reversion of the *hisG4*(Oc) allele found in TK603 and its protease-deficient, isogenic derivatives was studied as previously described (31). Briefly, cells were grown in Luria–Bertani medium until early-log phase, at which point they were harvested and resuspended in SM buffer (30). Cells were exposed to UV light and appropriate dilutions were plated on minimal agar plates supplemented with a trace amount of histidine. Mutations were scored after 4 days incubation at 37°C and mutation frequencies calculated as described by Sedgwick and Bridges (36).

RESULTS

Degradation of UmuD and UmuC Proteins by the Lon Protease. We have recently demonstrated that the UmuD and UmuC proteins are labile *in vivo* (7). We were therefore interested in determining which of *E. coli*'s many proteases was responsible for their degradation. Clues to the identity of the protease came from observations that certain strains exhibiting extremely stable levels of UmuC also exhibited a mucoid phenotype (unpublished observations). Such a phenotype is often associated with defects in the Lon protease [because of an increase in capsule synthesis mediated by the positive regulator, RcsA (37–39)]. To test the hypothesis that Lon may be involved in the degradation of the Umu proteins, we compared the stability of each of the separately plasmid-

encoded Umu proteins in isogenic Δ*umuDC lexA51*(Def) strains carrying either *lon*$^+$ or *lon*$^-$ alleles (Fig. 1). As clearly seen, the presence of the *lon146*::Tn*10* mutation had a dramatic effect on the stability of both the UmuD and the UmuC proteins. This observation strongly suggests that the UmuD and UmuC proteins are substrates of the Lon protease. In contrast, there was not a dramatic difference in UmuD′ stability between the *lon*$^-$ and *lon*$^+$ strains. This result seems to indicate that UmuD′ may also be a substrate of Lon but to a much lesser extent.

While the experiments reported here have, for simplicity, followed the stability of the UmuD, UmuD′, or UmuC proteins when expressed alone, similar results were obtained when we coexpressed the UmuDC or UmuD′C proteins (unpublished results). Under these conditions, the initial stability of the Umu proteins depends upon the presence of its cognate partner in the *lon*$^+$ background (7), but in the *lon*$^-$ strain, the

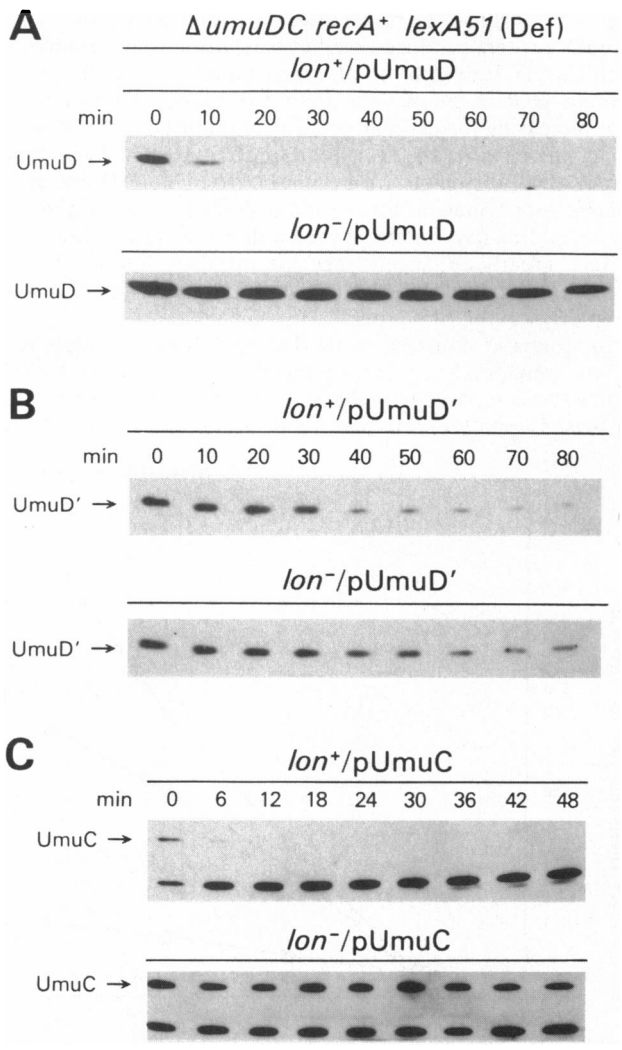

FIG. 1. Stability of UmuD, UmuD′, and UmuC proteins in isogenic *lon*$^+$ and *lon*$^-$ strains. Plasmids expressing UmuD (pRW362) (*A*), UmuD′ (pRW66) (*B*), and UmuC (pRW124) (*C*) were introduced into the Δ*(umuDC)596*::*ermGT recA*$^+$ *lexA51*(Def) *lon*$^+$ strain EC10 and the Δ*(umuDC)596*::*ermGT recA*$^+$ *lexA51*(Def) *lon*$^-$ strain EC18, and the relative stability of the Umu proteins was measured after protein synthesis was inhibited by the addition of chloramphenicol (100 μg/ml) at time zero. Additional aliquots were removed at 10-min intervals for the UmuD and UmuD′ experiments and at 6-min intervals for UmuC. Approximately 100 μg of extract was used to visualize UmuC in the *lon*$^+$ strain, while ≈40 μg of extract was used to visualize UmuC in the *lon*$^-$ strain and the UmuD and UmuD′ proteins in the *lon*$^+$ and *lon*$^-$ strains.

stability of UmuD, UmuD′, and UmuC was identical to that when expressed alone (Fig. 1). This might be expected for UmuD since it is naturally expressed in a 12-fold excess over UmuC (40), but it suggests that UmuC protein is also degraded when uncomplexed and that by forming a UmuD′$_2$C complex, UmuC is partially protected from Lon-dependent degradation.

It has previously been suggested that LexA51 protein is also stabilized in a *lon*$^-$ strain (41). However, all of the vectors used in this study express the Umu proteins from their LexA-regulated promoter. If such a stabilization of LexA51 protein does occur in a *lon*$^-$ strain, it does not apparently affect the expression of the Umu proteins (Fig. 1 and unpublished observations)

We have also analyzed the half-life of the UmuD, UmuD′, and UmuC proteins when expressed separately in a set of isogenic strains that were *lon*$^+$ but carried mutations in *clpA*, *clpB*, *clpX*, or *clpP*. Under these conditions, the stability of all three proteins remained essentially unchanged when compared with their parental *clp*$^+$ control, which suggests that homodimeric UmuD or UmuD′ proteins and the monomeric UmuC protein are not substrates of the ClpAP, ClpBP, or ClpXP proteases (unpublished observations).

Degradation of UmuD′ in a UmuD/UmuD′ Heterodimer by the ClpXP Protease. As noted above, in the Δ*umuDC* background, UmuD′ is moderately stable and is relatively insensitive to the actions of the Lon, ClpAP, ClpBP, or ClpXP proteases. Furthermore, in the Δ*umuDC* background, UmuD′ interacts with UmuC to form a very stable and mutagenically active UmuD′$_2$C complex (7, 8). Given the tight regulation that *E. coli* appears to have evolved to keep the mutagenically active UmuD′C complex to a minimum, it seems paradoxical that a cell would maintain high steady-state levels of the mutagenically active UmuD′C complex long after most DNA damage would be expected to have been repaired. We therefore hypothesized that there must be some mechanism available that would regulate the intracellular levels of the UmuD′C proteins. We considered the possibility that the formation of UmuD$^+$/UmuD′ heterodimers might provide such a regulatory function. In the experiments described above, the UmuD′ protein is expressed in a Δ*umuDC* background and can therefore only form homodimers. This situation rarely occurs in *umu*$^+$ cells, as UmuD′ is normally generated by the posttranslational processing of UmuD protein. Furthermore, previous studies have shown that *in vitro*, UmuD$^+$ and UmuD′ (which both form homodimers) preferentially form heterodimers (13). Since conversion of UmuD to UmuD′ is inefficient *in vivo* (40), it seems likely that at any given time, most of the UmuD′ will be complexed with UmuD in a UmuD/UmuD′ heterodimer. We were therefore interested in analyzing the stability of UmuD′ under conditions where it might be expected to be in a heterodimeric complex with UmuD rather than in a homodimer with itself. To do so, we used the Δ*umuDC* strains harboring a low-copy-number plasmid, pRW366, that expresses UmuD′ and a noncleavable UmuD1 protein (13, 33) in cis (Fig. 2*A*). (The use of a noncleavable UmuD protein reduced the possibility that there may be some gratuitous generation of UmuD′ from UmuD.) As noted in *Materials and Methods*, this plasmid should, in theory, express approximately equimolar steady-state levels of UmuD′ and UmuD1 proteins. While the stability of the mutant UmuD1 protein was comparable to the wild-type protein (compare Fig. 1*A* with Fig. 2*B*), analysis revealed that in a wild-type background, steady-state levels of UmuD′ were, in stark contrast to the homodimeric UmuD′ protein, barely detectable (compare Fig. 1*B* with Fig. 2*B*). Similar results were also obtained when pRW366 was introduced into the *clpA319*::Kan and *clpB*::Kan strains (Fig. 2*B*). In contrast, when the very same plasmid was introduced into a *clpX*::Kan or *clpP*::Kan strain, UmuD′ was expressed and was apparently very stable (Fig. 2*B*). Interestingly, UmuD1 protein also

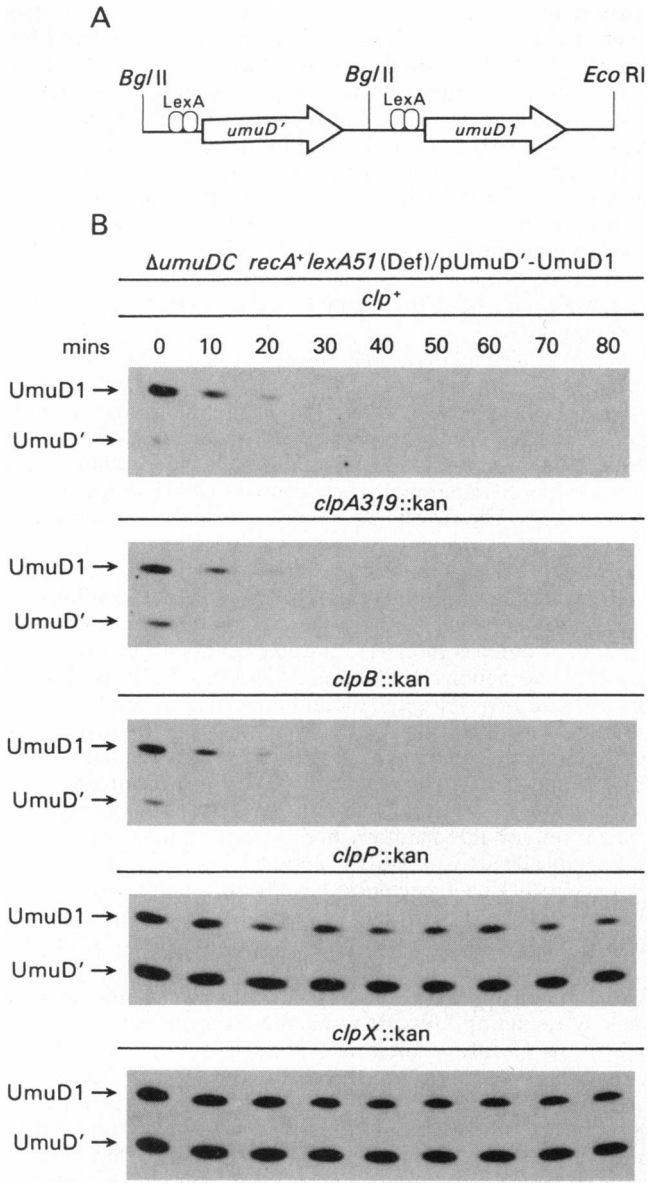

FIG. 2. Stability of UmuD′ and UmuD1 proteins in various *clp* strains. (*A*) Arrangement of the *umuD′* and *umuD1* genes in pRW366; a full description of pRW366 can be found in the text. Since the *umuD′* and *umuD1* genes contain identical operator/promoter regions, the UmuD′ and UmuD1 proteins are expected to be produced in equimolar amounts. (*B*) The stability of the UmuD1 and the UmuD′ proteins analyzed by introducing plasmid pRW366 into a set of Δ(*umuDC*)*596*::*ermGT recA*$^+$ *lexA51*(Def) isogenic strains: EC10 (*clp*$^+$), EC24 (*clpA319*::Kan), EC26 (*clpB*::Kan), EC28 (*clpX*::Kan), and EC22 (*clpP*::Kan). The relative stability of UmuD1 and UmuD′ proteins was measured after protein synthesis was inhibited by the addition of chloramphenicol (100 μg/ml) at time zero. Additional aliquots were removed at 10-min intervals.

appears to be dramatically stabilized in the *clpX*::Kan and *clpP*::Kan strains (Fig. 2*B*).

Together, these observations suggest to us that (*i*) UmuD′ is only rapidly degraded by the ClpXP protease when it is complexed with UmuD; and (*ii*) that UmuD is normally degraded by Lon when it is either monomeric or homodimeric, but not when it is in a heterodimer with UmuD′. Furthermore, the fact that there appears to be some limited degradation of UmuD1 in the *clpP* strain but not the *clpX* strain indicates that the ClpX chaperone might play a role in the assembly/disassembly of the heterodimeric UmuD/D′ complex. A simpler interpretation is that the UmuD1/UmuD′ heterodimer is a substrate of ClpXP. This seems unlikely, however, since only UmuD′ appears to be rapidly degraded in *clpXP*$^+$ strains (Fig. 2*B*), and if the heterodimer were a substrate of ClpXP, both UmuD1/UmuD′ might be expected to be degraded at equal efficiencies.

Effects of *lon* and *clpXP* Mutations on UV-Induced Mutagenesis. Given the effects that Lon and ClpXP play in the degradation of the Umu proteins, we were interested in determining the UV-mutagenesis phenotype of *lon* and *clpXP* strains. Although the *lon* mutant exhibits greatly elevated steady-state levels of both UmuD and UmuC proteins, this does not seem to have a dramatic effect on UV-induced mutagenesis (Fig. 3). This observation is consistent with earlier studies on *E. coli* B/r strains that are phenotypically Lon$^-$ but are perfectly UV mutable (42). At first glance, this might seem paradoxical, especially given the observation that the mutagenically inactive UmuD protein is dramatically stabilized in the *lon*$^-$ strains. Furthermore, any mutagenically active UmuD′ protein produced might be expected to be complexed with UmuD. It must be emphasized, however, that all of these protein–protein interactions are in a dynamic equilibrium and that increasing the cellular concentration of UmuD also leads to an increase in the level of UmuD′. At any given time, UmuD′ will dissociate from UmuD$^+$ (or UmuD1) and reassociate with UmuC to form a mutagenically active UmuD′$_2$C complex. In a *lon*$^-$ strain, it seems that such interactions are, in fact, greatly enhanced since the normally limiting UmuC protein is much more abundant than in the *lon*$^+$ background (Fig. 1*C*).

In dramatic contrast, both the *clpX*::Kan and *clpP*::Kan strains were rendered phenotypically poorly mutable by UV light when compared with the isogenic *clp*$^+$ strain, TK603 (Fig. 3). Based upon the data presented in Fig. 2*B*, we expect that

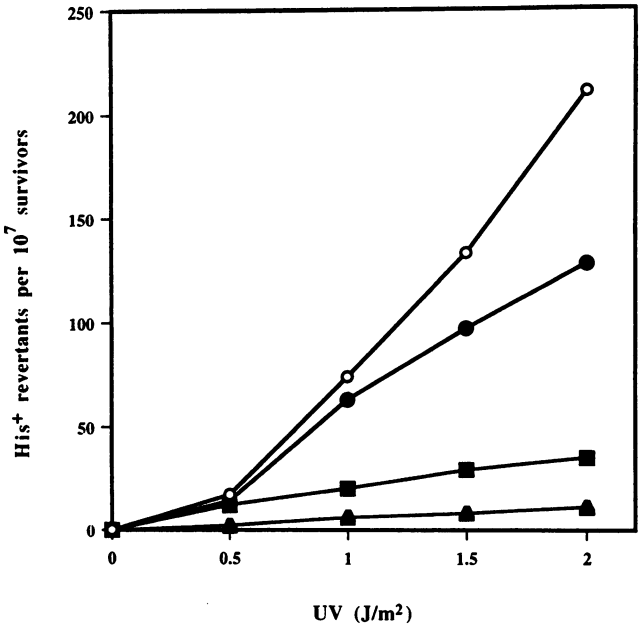

FIG. 3. Ability of various protease-proficient and protease-deficient strains to promote UV-induced mutagenesis. TK603 (*clp*$^+$) (●), EC34 (*clpP*::Kan) (▲), EC206 (*clpX*::Kan) (■), and EC212 (*lon146*::Tn*10*) (○). (EC212 also carries mutations in *sulA* and *rcsA*, which avoid the UV-induced lethal filamentation and mucoid colony formation associated with mutations in *lon*.) For all of the strains shown, the data represent the means from at least three independent experiments. Experiments with EC202 (*clpA319*::Kan) and EC204 (*clpB*::Kan) revealed that they exhibited identical UV-induced mutagenesis phenotypes when compared with their *clp*$^+$ parent, TK603, and are not shown for simplicity.

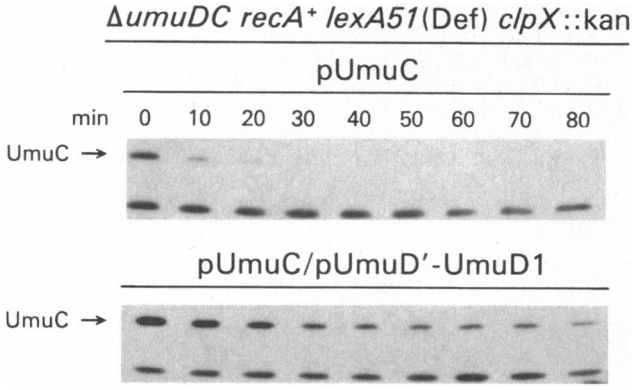

FIG. 4. Effect of coexpressing UmuD′ and UmuD1 proteins on UmuC stability. Plasmids expressing UmuC alone (pRW124) or coexpressing UmuD′/D1 (pRW366) were introduced into the Δ(*umuDC*)596::*ermGT recA*$^+$ *lexA51*(Def) strain EC10 and its *clpX*::Kan derivative, EC28. The relative stability of UmuC was assayed after protein synthesis was inhibited by the addition of chloramphenicol (100 μg/ml) at time zero. Additional aliquots were removed at 10-min intervals. Approximately 100 μg of cell extract was used to visualize UmuC when expressed on its own from pRW124, whereas only ≈40 μg of extract was used to visualize UmuC when coexpressed with UmuD′/UmuD1. The position of UmuC is indicated by an arrow at the left of the Fig.

the chromosomally encoded UmuD$^+$ and UmuD′ proteins are more likely to exist as a heterodimer in these strains; we believe that this observation is consistent with the hypothesis that heterodimers are mutagenically inactive (13). The poor mutability of the *clpX* and *clpP* strains also suggests that the wild-type ClpXP proteins may actually play an indirect role in promoting mutagenesis. Alternatively, ClpXP might affect some other protein required for SOS mutagenesis. This latter hypothesis seems unlikely, however, given that Δ*umuDC clpXP* strains are perfectly mutable if provided plasmid-encoded levels of Umu proteins (unpublished observations).

Effects of Stable UmuD1/UmuD′ Heterodimer Formation on the *in Vivo* Stability of UmuC Protein. It has previously been demonstrated that UmuD′ homodimers are much more efficient at stabilizing UmuC *in vivo* than UmuD homodimers (7, 8). One possible explanation for the poor mutability of the *clpXP* strains is that the stably maintained but mutagenically inactive UmuD$^+$/UmuD′ heterodimer can also interact with UmuC *in vivo,* thereby precluding any UmuD′$_2$C interactions. This hypothesis is strengthened by the *in vitro* studies of Woodgate *et al.* (35), who found that UmuC protein was retained on a UmuD$^+$/UmuD′ protein affinity column, a property that facilitated UmuC purification. We have tested this hypothesis by analyzing the *in vivo* stability of a plasmid-encoded UmuC protein in a *recA*$^+$ *lexA51*(Def) Δ(*umuDC*)596::*ermGT clpX*::Kan strain in the absence and presence of the plasmid-encoded UmuD1/UmuD′ proteins (Fig. 4). In the absence of the UmuD1/D′ proteins, UmuC remained labile in the *clpX* strain and exhibited similar stability to the *clp*$^+$ control strain (compare with Fig. 1*C*). In contrast, however, UmuC was greatly stabilized in the presence of the UmuD1/UmuD′ proteins. Indeed, the extent of UmuC stabilization was similar to that seen when coexpressed with homodimeric UmuD′ protein (7). We believe that such an observation is indicative of an interaction between the UmuD1/UmuD′ heterodimer and UmuC proteins *in vivo*.

DISCUSSION

Regulation of the Intracellular Levels of UmuD, UmuD′, and UmuC by the Lon and ClpXP Serine Proteases. As noted in the Introduction, previous experiments have suggested that the activity of the *E. coli* Umu proteins is tightly regulated at several steps. As part of this complex regulation, we have identified Lon as the protease that degrades the UmuD and UmuC proteins before they become mutagenically active. Our observation that the ClpXP protease acts on UmuD′ primarily when it is dimerized with UmuD$^+$ (or UmuD1) protein adds yet another level of complexity.

It is becoming increasingly clear that the level of SOS mutagenesis permitted in a cell exposed to any given mutagen is dependent upon a series of intimate protein–protein interactions and the association/dissociation constants that determine these interactions. For example, although UmuD is thought to primarily exist in a dimeric state in solution, recent structural analysis suggests that for it to undergo autocatalytic cleavage, it must be in a monomeric state (43). Thus, the ability of a cell to produce UmuD′ is the product of several reactions (Fig. 5): (*i*) The efficiency of the UmuD-Lon interaction; (*ii*) the ability of the dimeric UmuD protein to dissociate and form monomers; and (*iii*) the efficiency of the UmuD monomer's interaction with a RecA nucleoprotein filament (itself a complex reaction). Once generated, the monomeric UmuD′ protein faces at least two options: (*i*) it can interact with itself to form UmuD′ homodimers, which can then interact with, and stabilize, UmuC to form the mutagenically active UmuD′$_2$C complex; or (*ii*) it can heterodimerize with UmuD (since UmuD is, in most situations, in a vast excess over UmuD′). If it chooses the latter pathway, UmuD′ is rapidly degraded by ClpXP, thereby releasing UmuD protein so that it too, can be degraded either in a monomeric or in a dimeric state by Lon.

In a wild-type cell, all of these reactions are undoubtedly in a dynamic equilibrium, which, although favoring degradation

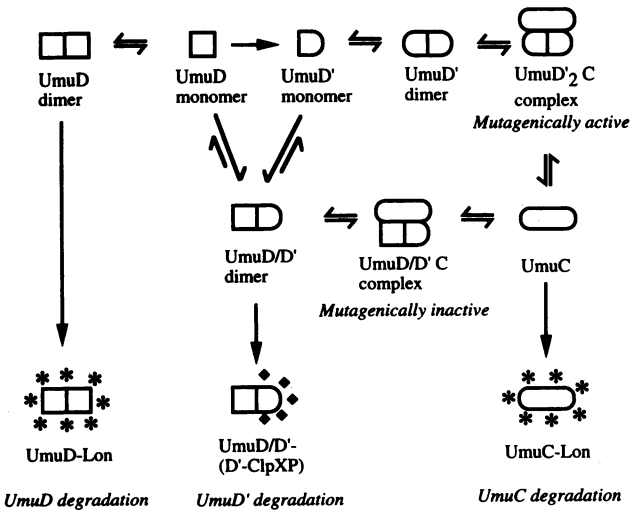

FIG. 5. Protein–protein interactions that result in degradation of the Umu proteins and thereby affect SOS mutagenesis. A full description of the numerous and complex interactions is described in the *Discussion*. Virtually all of these reactions are reversible, although, where noted, some are clearly more favored than others. The obvious exceptions are those interactions that lead to proteolysis, such as conversion of UmuD to UmuD′ or those leading to the complete degradation of the UmuD, UmuD′, and UmuC proteins. For simplicity, we have not depicted the additional protein–protein interactions between UmuD–RecA, UmuD–UmuC, UmuD′–RecA, UmuD′$_2$C–RecA, and UmuC–Hsp60/Hsp70 that also modulate the level of SOS mutagenesis. See ref. 7 for a full description of these interactions. Although we have depicted Lon as interacting with a dimer of UmuD, it is equally plausible that Lon interacts with UmuD when it is in a monomeric state. Mutations in either *clpX* or *clpP* result in a dramatic shift in the equilibrium of these interactions. As a result, the mutagenically inactive UmuD/UmuD′ heterodimer is maintained, and presumably renders the cell phenotypically nonmutable by successfully competing with homodimeric UmuD′ for a binding site on UmuC.

of the Umu proteins, still allows them to promote SOS mutagenesis (see below). As a consequence, it is not surprising that by moderately overproducing the Umu proteins from low- or medium-copy number plasmids, the equilibrium of these protein–protein interactions can be shifted to favor one interaction over another. Such shifts can also be manifested in the chromosomally expressed Umu proteins in *clpX* and *clpP* strains so that heterodimeric $UmuD^{+}/D'$ formation is greatly favored over the homodimeric complex. As demonstrated above (Fig. 4), although this complex appears to be mutagenically inactive, it is still able to interact with UmuC in such a way as to stabilize UmuC. Such an interaction presumably precludes the formation of an active $UmuD'_2C$ complex and thereby renders the cell phenotypically nonmutable. Thus, mutations that interfere with degradation of the mutagenically active UmuD′ protein result in a nonmutable phenotype. Intriguingly, such phenotypes may be common in other organisms. For example, the *Saccharomyces cerevisiae* RAD6 protein is a ubiquitin-conjugating enzyme that targets proteins for degradation (44) and mutations in *RAD6* render *S. cerevisiae* nonmutable (45).

SOS Mutagenesis, Despite Elaborate Mechanisms To Avoid it! Given what now appears to be an even more elaborate mechanism to keep the level of the *E. coli* UmuD′C proteins to a minimum in wild-type cells, why is there SOS mutagenesis after *E. coli* is exposed to a variety of chemical agents? One would have to argue that only a small fraction of the total UmuD′C proteins that are produced within the cell are actually required for SOS mutagenesis. We have previously estimated that there may only be 200 UmuC molecules (and therefore UmuD′C complexes) in a fully induced cell (40). Even if 90% of the UmuD′C complex were removed by proteolysis, there would still be roughly stoichiometric amounts of UmuD′C and DNA polymerase III holoenzyme within the cell. Since the exact nature of the $UmuD'_2C$–polymerase III interaction that results in error-prone, translesion DNA synthesis still remains to be elucidated, it is conceivable that the limited number of UmuD′C molecules that escape degradation are sufficient to promote mutagenesis, especially if they are protected from proteolysis by the formation of the "mutasome" (7, 35, 46). With the advent of a strategy to purify a soluble UmuD′C complex (47), these hypotheses can now be addressed biochemically.

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