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In Vivo Stability of the Umu Mutagenesis Proteins: a Major Role for RecA

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The *Escherichia coli* Umu proteins play critical roles in damage-inducible SOS mutagenesis. To avoid any gratuitous mutagenesis, the activity of the Umu proteins is normally kept to a minimum by tight transcriptional and posttranslational regulation. We have, however, previously observed that compared with an isogenic *recA*$^+$ strain, the steady-state levels of the Umu proteins are elevated in a *recA730* background (R. Woodgate and D. G. Ennis, Mol. Gen. Genet. 229:10–16, 1991). We have investigated this phenomenon further and find that another coprotease-constitutive (*recA) mutant, a *recA432* strain, exhibits a similar phenotype. Analysis revealed that the increased steady-state levels of the Umu proteins in the *recA** strains do indeed reflect an in vivo stabilization of the proteins. We have investigated the basis for the phenomenon and find that the mutant RecA* protein stabilizes the Umu proteins by not only converting the labile UmuD protein to the much more stable (and mutagenically active) UmuD′ protein but by directly stabilizing UmuD′ itself. In contrast, UmuC does not appear to be directly stabilized by RecA* but is instead dramatically stabilized in the presence of UmuD′. On the basis of these observations, we suggest that formation of a UmuD′C-RecA*-DNA quaternary complex protects the UmuD′C proteins from proteolytic degradation and as a consequence helps to promote the switch from error-free to error-prone mechanisms of DNA repair.**

Escherichia coli has equipped itself with an array of DNA repair enzymes to deal with a variety of lesions produced upon exposure to UV light and many chemical agents (reviewed in reference 17). Certain situations arise, however, whereby the damage fails to be repaired by error-free repair pathways and is instead processed via error-prone repair pathways. It is believed that this so-called "SOS mutagenesis" occurs directly as a result of the ability of UmuD′C and RecA proteins to coerce DNA polymerase III to replicate across damage-induced misinstructional lesions with a concomitant reduction in replication fidelity (3, 24, 28, 32, 41, 46).

Various studies have indicated that in *E. coli* cells, the SOS response has evolved so that the error-prone Umu-dependent pathway is manifested only as a last resort to permit cell survival in response to the otherwise fatal consequences of DNA damage. For example, the UmuDC proteins are tightly regulated at the transcriptional level by the LexA repressor. On the basis of the determined K_d for the LexA repressor and its binding site in the *umu* operator sequence, it seems likely that *umuDC* is one of the last SOS-regulated operons to be induced during the SOS response (23). Even when fully derepressed in *lexA*(Def) cells, the Umu proteins are expressed at levels only ~12-fold higher than those in the repressed state, with ~2,400 molecules of UmuD and ~200 molecules of UmuC per cell (44). The mutagenic response is also regulated at the posttranslational level because the UmuD protein is functionally inactive until it undergoes a RecA-mediated cleavage reaction generating the mutagenically active UmuD′ protein (4, 29, 37). Again, both in vitro (4) and in vivo (44) experiments have demonstrated that the cleavage reaction is inefficient and is likely to occur only when cells are under severe environmental stress. Finally, the mutagenic potential of these proteins may be further regulated by the preferential formation of heterodimers between UmuD and UmuD′, thereby depleting the cell of mutagenically active UmuD′ homodimers (2).

It is clear that quite elaborate mechanisms have evolved to keep the cellular concentration of the Umu proteins within a narrowly defined range. Indeed, relatively small variations in their cellular levels can result in a variety of phenotypes. For example, too little or too much UmuC renders cells nonmutable (47), and modest overexpression of UmuDC results in a cold-sensitive phenotype associated with a rapid cessation of DNA replication (26). Furthermore, it has been hypothesized that a slight increase in the cellular levels of UmuD′C is all that is necessary to result in a switch from error-free recombinational repair to error-prone SOS mutagenesis (38). This suggestion is particularly intriguing in light of our previous observation that a *recA730 lexA*(Def) strain, which exhibits a spontaneous SOS mutator activity, also exhibits three- to five-fold higher steady-state levels of the Umu proteins than does the isogenic *recA*$^+$ *lexA*(Def) strain (44). We were therefore interested in determining the molecular basis for this phenotype. To achieve this goal, we have examined the stability of both chromosomally encoded and plasmid-encoded Umu proteins under a variety of conditions and have identified conditions that lead to their in vivo stabilization. We hypothesize that the stabilization occurs through favorable in vivo protein interactions that protect the proteins from degradation, and as a consequence, this stabilization most likely helps to promote the switch from error-free to error-prone repair pathways.

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

Strain or plasmid	Relevant genotype or characteristics	Source or reference
Strains		
DE190	*recA*$^+$ *lexA51*(Def) *umuDC*$^+$	9
DM2572	*recA430 lexA51*(Def) *umuDC*$^+$	9
DE272	*recA730 lexA51*(Def) *umuDC*$^+$	9
DE860	*recA432 lexA51*(Def) *umuDC*$^+$	12
RW86	*recA lexA51*(Def) Δ(*umuDC*)*595*::*cat*	43
EC8	*recA*$^+$ *lexA*$^+$ *uvrA6 hisG4* Δ(*umuDC*)*596*::*ermGT*	This study
EC10	*recA*$^+$ *lexA51*(Def) Δ(*umuDC*)*596*::*ermGT*	This study
EC12	*recA730 lexA51*(Def) Δ(*umuDC*)*596*::*ermGT*	This study
Plasmids		
pRW124	Amp^r, medium-copy-number, pBR322-based plasmid that expresses close to physiological levels of UmuC	47
pRW66	Spc^r, low-copy-number, pGB2-based plasmid expressing UmuD′	47
pRW362	Spc^r, low-copy-number, pGB2-based plasmid expressing UmuD	This study
pRW154	Spc^r, low-copy-number, pGB2-based plasmid expressing UmuDC	19
pRW134	Spc^r, low-copy-number, pGB2-based plasmid expressing UmuD′C	10

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* strains and plasmids used in this study are listed in Table 1. Most have been reported elsewhere. The exceptions are described below. The Δ(*umuDC*)*596*::*ermGT* mutation (which confers resistance to erythromycin) was constructed in a K-12 *uvrA6* strain, TK603 (22), with the same strategy used to generate the original Δ(*umuDC*)*595*::*cat* mutation (43). Briefly, a 2.2-kb *Bam*HI restriction fragment carrying the *ermGT* gene was obtained from plasmid pTRK95 (42) and was ligated into the unique *Bam*HI site of plasmid pRW52 (43), generating plasmid pEC65. This plasmid carries the *ermGT* gene flanked by approximately 770 bp of the chromosomal *E. coli* sequences located immediately upstream of the *umuDC* operon and ~300 bp of downstream sequence. An ~3.3-kb *Eco*RI fragment was isolated from pEC65 and was ligated into an *Eco*RI-digested λ vector, λgt1-λB (21). The ligation mix was packaged with Gigapack II Gold (Stratagene) and used to infect TK603 cells (22). Recombinant phages carrying the *Eco*RI *ermGT* insert were identified with standard screening techniques (35) and a radiolabelled *ermGT* probe. The structure of one of the recombinant phages, λEC01, was subsequently verified by restriction enzyme analysis. Lysogens of λEC01 were obtained by selection for colonies that were immune to λch80del9 (21) and screened for erythromycin resistance (100 μg/ml) at 30°C. Lysogens were cured of λEC01 by overnight incubation on Luria-Bertani plates supplemented with 1 mM EDTA at 42°C. One of the isolates, EC8, which was erythromycin resistant and exhibited the correct restriction pattern expected for the Δ(*umuDC*)*596*::*ermGT* substitution mutation (as identified by Southern analysis), was used for further studies.

Strain EC10 was constructed by subsequent transduction (via generalized P1 transduction) of the Δ(*umuDC*)*596*::*ermGT* mutation into RW86 (43) and selection for erythromycin resistance (100 μg/ml) as well as chloramphenicol sensitivity. Strain EC20 was made by similar transduction of the Δ(*umuDC*)*596*::*ermGT* mutation into DE272 (9) and selection for erythromycin resistance.

Plasmid pRW362 was constructed by digestion of the low-copy-number *umuDC* plasmid, pRW155, with *Mlu*I and *Bam*HI restriction enzymes, filling in of the ends with DNA polymerase I (Klenow fragment), and subsequent religation of the plasmid with T4 DNA ligase. This procedure removed most of the *umuC* coding region and resulted in a low-copy-number plasmid that expresses a LexA-regulated UmuD protein.

Stability of the Umu mutagenesis proteins. The assay used to determine the in vivo stability of the Umu proteins was adapted from that previously employed to analyze the stability of the LexA (36) and bacteriophage Mu repressor proteins (18). Briefly, fresh overnight cultures were diluted 1:100 in Luria-Bertani media (27) and grown at 37°C. At a cell density of approximately 1×10^8 to 2×10^8 cells per ml, chloramphenicol was added to a final concentration of 100 μg/ml. The culture was incubated at 37°C for the duration of the experiment, and 1.5-ml aliquots were removed at the various times noted. 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, 2.3% sodium dodecyl sulfate [SDS], 0.1% bromophenol blue, 10 mM dithiothreitol). An equal amount of whole-cell extract, equivalent to approximately 10^8 cells, was subjected to electrophoresis in SDS-polyacrylamide gel electrophoresis (PAGE) gels containing 15% polyacrylamide for UmuC or 17% polyacrylamide for UmuD and UmuD′. Proteins were transferred to an Immobilon P membrane (Millipore), and the membrane was subsequently probed with a 1:10,000 dilution of polyclonal antisera raised against UmuD and UmuD′ or a 1:20,000 dilution of polyclonal antisera raised against UmuC (45, 47). The Umu proteins were detected with the chemiluminescent disodium-3-(4-methoxyspiro [1,2-dioxetane-3,2′ (5′-chloro) tricyclo (3.3.1.1.^{3,7}) decan]-4-yl) phenyl-phosphate (CSPD)-Western light assay (Tropix, Bedford, Mass.). Membranes were exposed to Kodak X-Omat or Bio-Max films for periods of 1 to 20 min. The half-life of the Umu proteins was determined from these exposures after they were subjected to densitometric analysis with the software *NIH Image* (version 1.59) and a Macintosh computer equipped with a Sierra Scientific MS-4030 high-resolution video camera and a Data Translation Quick Capture DT2255 Frame Grabber Board.

In some experiments, the half-life of the Umu proteins was independently determined with radiolabeling and immunoprecipitation assays as described previously (39). Briefly, exponential-phase cultures (optical density at 600 nm, ~0.3) grown in M9 salts medium plus supplements (20) were labelled with 75 μCi of [^{35}S]methionine per ml for 3 min and chased with excess unlabelled methionine (1 mg/ml, final concentration), and 1-ml aliquots were sampled directly in 0.11 ml of 50% trichloroacetic acid. UmuC was immunoprecipitated from aliquots of each sample containing equal counts per minute. The immunoprecipitates were subjected to electrophoresis in an SDS–12% polyacrylamide gel. After electrophoresis, the gels were dried, autoradiographed, and quantitated with a PhosphorImager (Molecular Dynamics).

RESULTS

Stability of the Umu proteins in various *recA* strains. We have previously noted that a *lexA*(Def) *recA730* strain exhibited three- to fivefold higher steady-state levels of the Umu mutagenesis proteins than did the isogenic *recA*$^+$ strain (44). On the basis of this observation, we hypothesized that this might reflect a stabilizing interaction between the Umu proteins and the RecA730 protein (44). RecA730 protein differs from wild-type RecA in that it is considered to be a coprotease constitutive mutant. To determine whether this property was important for Umu stabilization, we compared the stabilities of the Umu proteins in various *recA* strains that varied in their coprotease activity. RecA432 is like RecA730 in that it is coprotease constitutive when fully derepressed (10, 11); wild-type RecA is damage inducible for coprotease functions (5, 6), while RecA430 exhibits greatly reduced coprotease activity, even after DNA damage (12, 33, 37). All of these phenotypes are thought to reflect the ability of the particular mutant RecA protein to form a RecA*-nucleoprotein filament. (Such a phenotype can easily be distinguished by the constitutive conversion of UmuD to UmuD′ in the absence of cellular damage [Fig. 1].) Analysis revealed that like the *recA730* strain, the *recA432* strain exhibited elevated steady-state levels of UmuC. In comparison, neither the *recA*$^+$ or *recA430* strains showed elevated steady-state levels of UmuC (Fig. 1). Preliminary studies indicated that the increased steady-state levels of the Umu proteins observed in these experiments indeed reflect an increase in the stability of the proteins (unpublished obser-

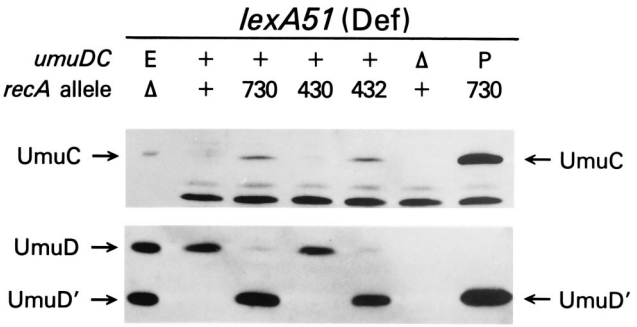

FIG. 1. Steady-state levels of the chromosomally encoded Umu proteins in various *recA* strains. The UmuD, UmuD′, and UmuC proteins were detected in extracts from various *recA lexA51*(Def) strains of *E. coli* with the chemiluminescent CSPD-Western light assay. Whole-cell extracts (~40 μg of protein) were obtained from DE192 (*recA*$^+$), DE272 (*recA730*), DM2572 (*recA430*), DE860 (*recA432*), and RW86 (*recA*$^+$ Δ*umuDC*). The *recA* allele of the strain is indicated above its appropriate track. Lanes E and P are our Umu standards. Lane E was obtained by combination of partially purified Umu proteins obtained from a Δ*recA* strain, while lane P was a whole-cell extract obtained from DE272/pRW134. The positions of UmuD, UmuD′, and UmuC are indicated by arrows on the right and left of the figure. As can be seen, the UmuC antiserum recognizes another cellular protein in addition to UmuC. The identity of this protein is unknown, but it serves as a useful internal control, ensuring that equal amounts of protein extract have been applied to the gel. In this exposure, the lower steady-state levels of the chromosomally expressed UmuC protein are not detectable in the *recA*$^+$ and *recA430* cell extracts.

vations), which prompted us to investigate this phenomenon further.

Stabilization of UmuC by coexpression with UmuD or UmuD′. Donnelly and Walker have previously noted that in a *groE* background, UmuD′ is much more effective than UmuD in stabilizing UmuC (7), although no such difference was observed in a *groE*$^+$ background. Despite the fact that all of the strains used in this study are *groE*$^+$, we wanted to investigate the possibility that the increased steady-state levels of UmuC might indeed result from the presence of UmuD′ rather than from the direct activity of the RecA* protein. To assess the relative contribution that UmuD or UmuD′ might play in stabilizing UmuC, we introduced a plasmid encoding UmuC alone (pRW124) or a plasmid coexpressing UmuDC (pRW154) or UmuD′C (pRW134) into the fully SOS-derepressed Δ*umuDC* strain EC10 and analyzed the stability of UmuC (Fig. 2). When expressed in the absence of either UmuD or UmuD′, UmuC appears to be extremely labile (Fig. 2). Indeed, the steady-state levels of UmuC were much lower than when coexpressed with UmuD or UmuD′, and as a result, approximately 2.5-fold more cell extract was necessary even to visualize UmuC at the zero time point. Ten minutes after protein synthesis was inhibited by the addition of chloramphenicol, UmuC was undetectable. On the basis of additional experiments in which shorter intervals were used, the half-life of UmuC was estimated to be ~6 min (Fig. 3). This value is somewhat smaller than that previously observed by Donnelly and Walker (7), who estimated that the half-life of UmuC in a *groE*$^+$ strain is 18 min. These discrepancies probably reflect the differences in the experimental protocols used to determine the half-life of the protein. Indeed, with a pulse-chase–immunoprecipitation protocol that does not utilize chloramphenicol to inhibit protein synthesis, the half-life of UmuC was estimated to be ~16 min. While there appear to be slight variations in the determined half-life of UmuC, it is important to note that the same relative instability of UmuC is observed with all of the protocols used to determine its stability.

In our chemiluminescent assay, coexpression of UmuC to-

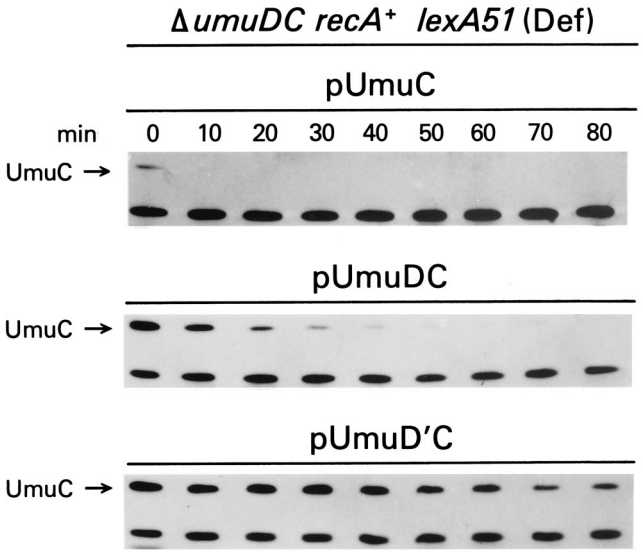

FIG. 2. Effects of coexpressing UmuDC or UmuD′C on the stability of UmuC. Plasmids expressing UmuC alone (pRW124) or coexpressing UmuDC (pRW154) or UmuD′C (pRW134) were introduced into the Δ(*umuDC*)*596*::*ermGT recA*$^+$ *lexA51*(Def) strain EC10, and the relative stability of UmuC was measured after protein synthesis was inhibited by the addition of chloramphenicol (100 μg/ml) at time zero. Additional aliquots were removed at 10-min intervals. Approximately 100 μg of cell extract was used to visualize UmuC when expressed on its own from pRW124, while only ~40 μg of extract was used to visualize UmuC when coexpressed with either UmuD or UmuD′. The positions of UmuC are indicated by arrows on the left of the figure.

gether with UmuD resulted in a modest stabilization of UmuC protein. Under these conditions, UmuC has an estimated half-life of ~9 min (Fig. 2 and 3). In contrast, coexpression of UmuC with UmuD′ greatly stabilized UmuC and resulted in a

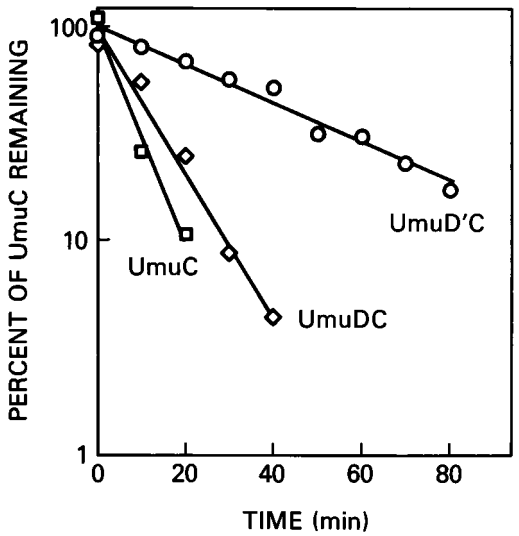

FIG. 3. Estimated half-life of UmuC determined with the chemiluminescent detection assay. Densitometric analyses of several experiments similar to that shown in Fig. 2 (particularly those measuring UmuC stability, in which aliquots were taken at much shorter time intervals) have allowed us to determine the relative stability of the UmuC protein under our assay conditions. When expressed on its own, UmuC has an estimated half-life of ~6 min. Coexpression with UmuD resulted in partial stabilization with a half-life of ~9 min. In contrast, UmuC was greatly stabilized by coexpression with UmuD′, with an apparent half-life of ~33 min.

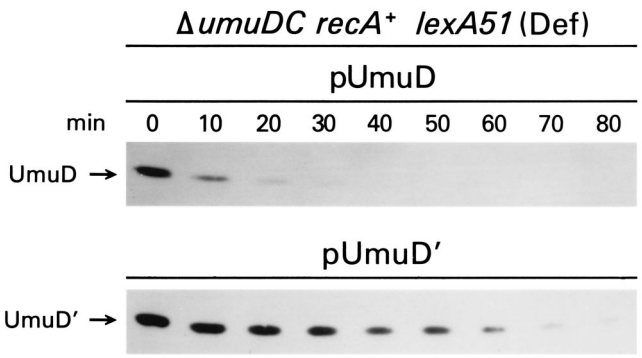

FIG. 4. Stability of UmuD and UmuD′ in a *recA*$^+$ strain. Plasmids expressing UmuD alone (pRW155) or UmuD′ alone (pRW66) were introduced into the Δ(*umuDC*)*596*::*ermGT recA*$^+$ *lexA51*(Def) strain EC10, and the relative stability of the UmuD and UmuD′ proteins was measured after protein synthesis was inhibited by the addition of chloramphenicol (100 μg/ml) at time zero. Additional aliquots were removed at 10-min intervals. Approximately 40 μg of extract was used to visualize the UmuD and UmuD′ proteins.

half-life of ~33 min (Fig. 2 and 3). Similar results were obtained when UmuD′ and UmuC were expressed from an F′ episomal plasmid or were expressed in *trans* from two compatible plasmids (data not shown). The half-life of UmuC as determined by the pulse-chase–immunoprecipitation method was qualitatively similar. When coexpressed with UmuD, the half-life of UmuC was ~20 min, while when coexpressed with UmuD′, it was >60 min. Given that both protocols reflect the same basic phenomenon, i.e., coexpression of UmuD′ and UmuC leading to dramatic UmuC stabilization (even in a *groE*$^+$ background), further estimations of Umu half-lives were determined only with the nonisotopic chemiluminescent assay.

Stability of the UmuD and UmuD′ proteins in a *recA*$^+$ strain. We were interested in further investigating the differential effect of coexpressing UmuD or UmuD′ on UmuC stability and turned our attention to the UmuD and UmuD′ proteins themselves. Somewhat surprisingly, UmuD appeared to be labile, with an estimated half-life of 7 min (Fig. 4). In comparison, its posttranslational cleavage product, UmuD′, was much more stable than its precursor, with an estimated half-life of 18 min (Fig. 4).

The fact that UmuD is much less stable than UmuD′ might also explain the limited stabilizing effect that it has on UmuC; it simply is not long-lived enough to protect UmuC from proteolysis, although it is equally conceivable that the natures of the UmuD-UmuC and UmuD′-UmuC interactions are intrinsically different. Support for the last hypothesis comes from our observation that when coexpressed with UmuC in roughly stoichiometric amounts, the half-life of UmuD′ increases from 18 min to 33 min. In contrast, the half-life of UmuD did not change in either the presence or the absence of UmuC (data not shown).

Stability of UmuD′ in *recA*$^+$ and *recA730* strains. On the basis of the studies described above, it appeared that the initial observation of greater UmuC stability in the *recA** strains occurs primarily as a result of constitutive and efficient conversion of UmuD to UmuD′. Studies in which the various *umu* plasmids were introduced into a *recA730* strain revealed, however, that RecA730 could play a more direct stabilizing role. While the half-lives of UmuC in the absence of either UmuD or UmuD′ remained virtually identical in both the *recA*$^+$ and *recA730* strains (data not shown), the half-life of UmuD′ (in the absence of UmuC) increased from 18 min in a *recA*$^+$ strain to 60 min in the *recA730* strain (Fig. 5). With a modified DNA band-shift assay, we have previously shown that UmuD′ physically interacts with an activated RecA*-nucleoprotein filament (15), and we believe that the increased half-life of UmuD′ in the *recA730* background represents such an interaction in vivo.

While we find that the stability of the Umu proteins increases in a coprotease constitutive *recA* mutant, we believe that a similar stabilization occurs in a wild-type cell that has received cellular DNA damage. The only difference is that the stabilizing effect will be more transient. Presumably, once the damage has been repaired (and the Umu proteins are no longer required for error-prone translesion DNA synthesis), wild-type RecA will return to its nonactivated state. In the absence of a stabilizing RecA*-nucleoprotein filament, we hypothesize that the Umu proteins are more likely to be targeted for proteolysis. Such a process would provide a mechanism whereby a cell could return to a resting nonmutable state (14).

DISCUSSION

The susceptibility of the UmuD and UmuC proteins to proteolysis provides another mechanism by which SOS mutagenesis can be regulated. As outlined in the Introduction, the mutagenic process is regulated at a number of levels. Our observation that both UmuD and UmuC are labile, even when coexpressed, probably represents yet another level of regulation. One can imagine a scenario wherein modest DNA damage leads to partial derepression of the SOS-regulated operons, and because the *umu* operon appears to be one of the most tightly regulated, it would only become derepressed after those genes involved in error-free mechanisms of DNA repair. Even after it is translated, intact UmuD remains mutagenically inactive and by rapidly targeting both the UmuD and UmuC proteins for proteolytic degradation, cells temporarily postpone being committed to SOS mutagenesis. Under conditions in which cellular DNA damage is more extensive, lesions are likely to arise that cannot be repaired via error-free mechanisms. At this point, the availability of the Umu proteins and a translesion polymerase bypass mechanism to avoid the immediate fatal consequences of DNA damage (even though the bypass event might be ultimately mutagenic) is an obvious evolutionary advantage. This appears to be achieved by conversion of UmuD to the more stable UmuD′, which in turn, stabilizes UmuC. Such regulation thereby keeps the level of functionally active Umu proteins to a minimum until they are

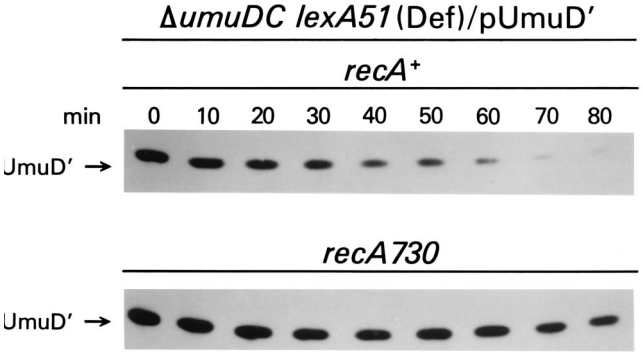

FIG. 5. Stability of UmuD′ in a *recA*$^+$ strain and a *recA730* strain. A plasmid expressing UmuD′ alone (pRW66) was introduced into the two isogenic strains EC10 [Δ(*umuDC*)*596*::*ermGT recA*$^+$ *lexA51*(Def)] and EC20 [Δ(*umuDC*)*596*::*ermGT recA730 lexA51*(Def)], and the relative stability of UmuD′ 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. Approximately 40 μg of extract was used to visualize UmuD′ in the two strains.

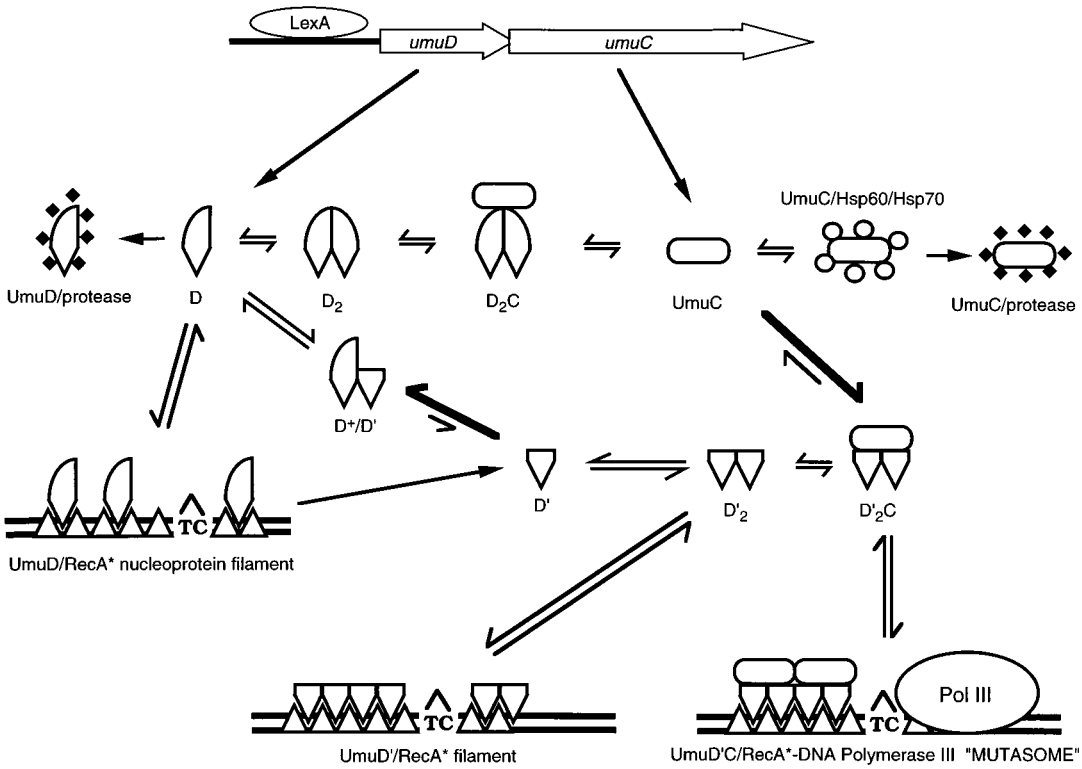

FIG. 6. Protein-protein interactions that affect SOS mutagenesis. A full description of the numerous and complex interactions is described in the Discussion section. Virtually all of these reactions are reversible, although where noted, some are clearly more favored than others. The obvious exceptions are those interactions that lead to proteolysis, leading to either the complete degradation of the UmuD and UmuC proteins or, in the case of UmuD, its conversion to UmuD′. Although RecA binds more avidly to regions of single-stranded DNA (which leads to the stabilization of the Umu proteins), only complexes that are formed on lesion-containing DNA will result in mutagenesis. As a consequence, we have depicted that the RecA*-nucleoprotein filament has formed at the site of a TC lesion in double-stranded DNA (34). We envision that the UmuD and UmuD′ proteins will bind to the entire length of the RecA*-nucleoprotein filament, although it is conceivable that they only bind to the very end of it (38). The stability of the Umu proteins increases by forming a UmuD′$_2$C-RecA-DNA complex, and upon the arrival of DNA polymerase III, a "mutasome" is formed (8, 45) that ultimately leads to error-prone translesion DNA synthesis.

needed to facilitate translesion DNA synthesis (described below).

Posttranslational processing of UmuD to UmuD′ not only converts it to a mutagenically active species but also results in a form that is more resistant to cellular proteolysis. What is the molecular basis for this difference? One hypothesis is that the initial protease recognition site lies within the first 24 amino acid residues of UmuD that are discarded when it is converted to UmuD′. Alternatively, recent structural analysis of UmuD′ (30), which suggests that UmuD and UmuD′ might adopt somewhat different structural conformations, leads to the notion that the cellular protease that degrades UmuD might simply not recognize the tertiary structure of the UmuD′ homodimer. Studies are currently in progress to address these different hypotheses.

Why was such a difference in the stability of the two proteins not observed before? One possible explanation comes from the observation that when greatly overproduced, a portion of UmuD is sequestered in insoluble inclusion bodies (4) and therefore might not be accessible for proteolysis. Alternatively, UmuD overproduction might simply overwhelm the protease(s) that normally maintains UmuD at low cellular levels. Our plasmid system avoids both problems because the UmuD and UmuD′ proteins are expressed from their natural LexA-regulated promoter on a low-copy-number plasmid, and therefore their cellular concentrations more likely resemble physiological levels.

Protein-protein interactions that affect the stability of the Umu proteins and lead to SOS mutagenesis. The exact process by which mutations are introduced into the *E. coli* genome as a consequence of cellular DNA damage remains to be resolved. It is clear, however, that this process only occurs after a series of intricate protein-protein interactions. While a variety of molecular and biochemical techniques have been used to investigate such interactions, all have their limitations. For example, although we have previously demonstrated that the UmuD′ and UmuC proteins physically interact in vitro (45), further studies of this and other interactions have been hindered because the characteristics of UmuC make its purification difficult. The two-hybrid system developed by Fields and Song (13) has often proven useful to investigate protein-protein interactions, but negative results are not easy to interpret. In this report, we have identified conditions that lead to the in vivo stabilization of the Umu proteins. We hypothesize that this stabilization occurs via favorable protein-protein interactions in vivo that protect the Umu proteins from proteolysis. If this is indeed the case, then certain protein combinations that fail to lead to stabilization should reflect the fact that the proteins do not physically interact in vivo, or if they do, the interaction may be more transient than those that do lead to stabilization. Incorporating our new observations with those previously reported, it is possible to hypothesize about the order and nature of the protein-protein interactions that lead to the formation of the "mutasome" (Fig. 6) (8, 45).

Once the *umu* operon is derepressed and the Umu proteins are translated, their stability and fate vary considerably and depend upon a series of dynamic protein-protein interactions. UmuD, for example, appears to exist as a dimer under physi-

ological conditions (2, 4), yet is probably converted to UmuD′ via a RecA-mediated reaction when it is in a monomeric form (25, 30). Once converted to UmuD′, it can form either homodimers (described below) or, especially under conditions of limiting cleavage, UmuD-UmuD′ heterodimers (2, 45). In its uncleaved state, UmuD (either the monomeric or dimeric form) is recognized by one of the many *E. coli* proteases (14) and targeted for degradation (Fig. 4). UmuD (probably the dimeric form) also interacts with UmuC in such a way as to at least partially protect UmuC from degradation (Fig. 2 and 3).

Although coordinately expressed with UmuD, it is likely that for some period of time, UmuC exists in its monomeric form (45). In this state, it is extremely labile and is quickly degraded by a cellular protease (7, 14) (Fig. 2 and 3). Association with the Hsp60 and Hsp70 chaperones appears to partially stabilize UmuC (7, 31) until it can interact with either UmuD or, more favorably, UmuD′ (7) (Fig. 2 and 3). Such a situation is only likely to occur under severe environmental conditions when an excess of UmuD′ homodimers are produced, thus generating a UmuD′$_2$C complex (45). Although UmuC and RecA have been shown to interact in vitro (16), if these interactions occur in vivo, they do not appear to result in stabilization of UmuC (unpublished observations). On the basis of the studies presented here, we favor the hypothesis that UmuC interacts with activated RecA primarily via its interaction with UmuD′ (described below).

In addition to interacting with UmuC, UmuD′ homodimers also interact with a RecA*-nucleoprotein filament (15). Given that we have previously estimated that there is a 12-fold excess of UmuD′ over UmuC (44), it is likely that most of the UmuD′ is either free in solution or bound to a RecA*-nucleoprotein filament and that only a fraction exists in the UmuD′$_2$C complex. This complex is possibly targeted to the RecA*-nucleoprotein filament (1, 15, 40) by an exchange reaction between UmuC in the free UmuD′$_2$C complex and the UmuD′ bound to the RecA*-nucleoprotein filament or by the UmuD′$_2$C complex binding directly to the RecA*-nucleoprotein filament. Ultimately, these interactions lead to stabilization of the Umu proteins, thereby raising the steady-state levels within the cell (44) (Fig. 1) and, as a result, helping the cell survive the otherwise fatal consequences of DNA damage by promoting translesion DNA synthesis (even if it is error prone).

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REFERENCES

1. **Bailone, A., S. Sommer, J. Knezevic, M. Dutreix, and R. Devoret.** 1991. A RecA protein mutant deficient in its interaction with the UmuDC complex. Biochimie **73:**479–484.
2. **Battista, J. R., T. Ohta, T. Nohmi, W. Sun, and G. C. Walker.** 1990. Dominant negative *umuD* mutations decreasing RecA-mediated cleavage suggest roles for intact UmuD in modulation of SOS mutagenesis. Proc. Natl. Acad. Sci. USA **87:**7190–7194.
3. **Bridges, B. A., and R. Woodgate.** 1985. Mutagenic repair in *Escherichia coli*: products of the *recA* gene and of the *umuD* and *umuC* genes act at different steps in UV-induced mutagenesis. Proc. Natl. Acad. Sci. USA **82:**4193–4197.
4. **Burckhardt, S. E., R. Woodgate, R. H. Scheuermann, and H. Echols.** 1988. UmuD mutagenesis protein of *Escherichia coli*: overproduction, purification and cleavage by RecA. Proc. Natl. Acad. Sci. USA **85:**1811–1815.
5. **Craig, N. L., and J. W. Roberts.** 1980. *E. coli recA* protein-directed cleavage of phage lambda repressor requires polynucleotide. Nature (London) **283:**26–30.
6. **Craig, N. L., and J. W. Roberts.** 1981. Function of nucleoside triphosphate and polynucleotide in *Escherichia coli recA* protein-directed cleavage of phage lambda repressor. J. Biol. Chem. **256:**8039–8044.
7. **Donnelly, C. E., and G. C. Walker.** 1992. Coexpression of UmuD′ with UmuC suppresses the UV mutagenesis deficiency of *groE* mutants. J. Bacteriol. **174:**3133–3139.
8. **Echols, H., and M. F. Goodman.** 1990. Mutation induced by DNA damage: a many protein affair. Mutat. Res. **236:**301–311.
9. **Ennis, D. G., B. Fisher, S. Edmiston, and D. W. Mount.** 1985. Dual role for *Escherichia coli* RecA protein in SOS mutagenesis. Proc. Natl. Acad. Sci. USA **82:**3325–3329.
10. **Ennis, D. G., A. S. Levine, W. H. Koch, and R. Woodgate.** 1995. Analysis of *recA* mutants with altered SOS functions. Mutat. Res. **336:**39–48.
11. **Ennis, D. G., J. W. Little, and D. W. Mount.** 1993. Novel mechanism for UV sensitivity and apparent UV nonmutability of *recA432* mutants: persistent LexA cleavage following SOS induction. J. Bacteriol. **175:**7373–7382.
12. **Ennis, D. G., N. Ossanna, and D. W. Mount.** 1989. Genetic separation of *Escherichia coli recA* functions for SOS mutagenesis and repressor cleavage. J. Bacteriol. **171:**2533–2541.
13. **Fields, S., and O. K. Song.** 1989. A novel genetic system to detect protein-protein interactions. Nature (London) **340:**245–246.
14. **Frank, E. G., D. G. Ennis, M. Gonzalez, A. S. Levine, and R. Woodgate.** Regulation of SOS mutagenesis by proteolysis. Submitted for publication.
15. **Frank, E. G., J. Hauser, A. S. Levine, and R. Woodgate.** 1993. Targeting of the UmuD, UmuD′ and MucA′ mutagenesis proteins to DNA by RecA protein. Proc. Natl. Acad. Sci. USA **90:**8169–8173.
16. **Freitag, N., and K. McEntee.** 1989. "Activated"-RecA protein affinity chromatography of LexA repressor and other SOS-regulated proteins. Proc. Natl. Acad. Sci. USA **86:**8363–8367.
17. **Friedberg, E. C., G. C. Walker, and W. Siede.** 1995. DNA repair and mutagenesis. American Society for Microbiology, Washington, D.C.
18. **Geuskens, V., A. Mhammedi-Alaoui, L. Desmet, and A. Toussaint.** 1992. Virulence in bacteriophage Mu: a case of *trans*-dominant proteolysis by the *Escherichia coli* Clp serine protease. EMBO J. **11:**5121–5127.
19. **Ho, C., O. I. Kulaeva, A. S. Levine, and R. Woodgate.** 1993. A rapid method for cloning mutagenic DNA repair genes: isolation of *umu*-complementing genes from multidrug resistance plasmids R391, R446b, and R471a. J. Bacteriol. **175:**5411–5419.
20. **Hoyt, M. A., D. M. Knight, A. Das, H. I. Miller, and H. Echols.** 1982. Control of phage lambda development by stability and synthesis of cII protein: role of viral cIII and host *hflA*, *himA*, and *himD* genes. Cell **31:**565–573.
21. **Joyce, C. M., and N. D. F. Grindley.** 1984. Method for determining whether a gene of *Escherichia coli* is essential: application to the *polA* gene. J. Bacteriol. **158:**636–643.
22. **Kato, T., and Y. Shinoura.** 1977. Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. Mol. Gen. Genet. **156:**121–131.
23. **Kitagawa, Y., E. Akaboshi, H. Shinagawa, T. Horii, H. Ogawa, and T. Kato.** 1985. Structural analysis of the *umu* operon required for inducible mutagenesis in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82:**4336–4340.
24. **Lawrence, C. W., A. Borden, and R. Woodgate.** Analysis of the mutagenic properties of the UmuDC, MucAB and RumAB proteins, using a site specific abasic lesion. Mol. Gen. Genet., in press.
25. **Lee, M. H., T. Ohta, and G. C. Walker.** 1994. A monocysteine approach for probing the structure and interactions of the UmuD protein. J. Bacteriol. **176:**4825–4837.
26. **Marsh, L., and G. C. Walker.** 1985. Cold sensitivity induced by overproduction of UmuDC in *Escherichia coli*. J. Bacteriol. **162:**155–161.
27. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Murli, S., and G. C. Walker.** 1993. SOS mutagenesis. Curr. Opin. Genet. Dev. **3:**719–725.
29. **Nohmi, T., J. R. Battista, L. A. Dodson, and G. C. Walker.** 1988. RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and posttranslational activation. Proc. Natl. Acad. Sci. USA **85:**1816–1820.
30. **Peat, T. S., E. G. Frank, J. P. McDonald, A. S. Levine, R. Woodgate, and W. A. Hendrickson.** 1996. Structure of the UmuD′ protein and its regulation in response to DNA damage. Nature (London) **380:**727–730.
31. **Petit, M. A., W. Bedale, J. Osipiuk, C. Lu, M. Rajagopalan, P. McInerney, M. F. Goodman, and H. Echols.** 1994. Sequential folding of UmuC by the Hsp70 and Hsp60 chaperone complexes of *Escherichia coli*. J. Biol. Chem. **269:**23824–23849.
32. **Rajagopalan, M., C. Lu, R. Woodgate, M. O'Donnell, M. F. Goodman, and H. Echols.** 1992. Activity of the purified mutagenesis proteins UmuC, UmuD′ and RecA in replicative bypass of an abasic DNA lesion by DNA polymerase III. Proc. Natl. Acad. Sci. USA **89:**10777–10781.
33. **Rebollo, J. E., P. L. Moreau, M. Blanco, and R. Devoret.** 1984. Restoration of RecA protein activity by genetic complementation. Mol. Gen. Genet. **195:**83–89.
34. **Rosenberg, M., and H. Echols.** 1990. Differential recognition of ultraviolet lesions by RecA protein. J. Biol. Chem. **265:**20641–20645.
35. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Sassanfar, M., and J. W. Roberts.** 1990. Nature of the SOS-inducing signal in *Escherichia coli*: the involvement of DNA replication. J. Mol. Biol. **212:** 79–96.

37. **Shinagawa, H., H. Iwasaki, T. Kato, and A. Nakata.** 1988. RecA protein-dependent cleavage of UmuD protein and SOS mutagenesis. Proc. Natl. Acad. Sci. USA **85:**1806–1810.
38. **Sommer, S., A. Bailone, and R. Devoret.** 1993. The appearance of the UmuD′C protein complex in *Escherichia coli* switches repair from homologous recombination to SOS mutagenesis. Mol. Microbiol. **10:**963–971.
39. **Straus, D., W. Walter, and C. A. Gross.** 1990. DnaK, DnaJ, and GrpE heat shock proteins negatively regulate heat shock gene expression by controlling the synthesis and stability of σ^{32}. Genes Dev. **4:**2202–2209.
40. **Sweasy, J. B., E. M. Witkin, N. Sinha, and V. Roegner-Maniscalco.** 1990. RecA protein of *Escherichia coli* has a third essential role in SOS mutator activity. J. Bacteriol. **172:**3030–3036.
41. **Szekeres, E. S., Jr., R. Woodgate, and C. W. Lawrence.** 1996. Substitution of *mucAB* or *rumAB* for *umuDC* alters the relative frequencies of the two classes of mutations induced by a site-specific T-T cyclobutane dimer and the efficiency of translesion DNA synthesis. J. Bacteriol. **178:**2559–2563.
42. **Tannock, G. W., J. B. Luchansky, L. Miller, H. Connell, S. Thode-Andersen, A. A. Mercer, and T. R. Klaenhammer.** 1994. Molecular characterization of a plasmid-borne (pGT633) erythromycin resistance determinant (*ermGT*) from *Lactobacillus reuteri* 100-63. Plasmid **31:**60–71.
43. **Woodgate, R.** 1992. Construction of a *umuDC* operon substitution mutation in *Escherichia coli*. Mutat. Res. **281:**221–225.
44. **Woodgate, R., and D. G. Ennis.** 1991. Levels of chromosomally encoded Umu proteins and requirements for in vivo UmuD cleavage. Mol. Gen. Genet. **229:**10–16.
45. **Woodgate, R., M. Rajagopalan, C. Lu, and H. Echols.** 1989. UmuC mutagenesis protein of *Escherichia coli*: purification and interaction with UmuD and UmuD′. Proc. Natl. Acad. Sci. USA **86:**7301–7305.
46. **Woodgate, R., and S. G. Sedgwick.** 1992. Mutagenesis induced by bacterial UmuDC proteins and their plasmid homologues. Mol. Microbiol. **6:**2213–2218.
47. **Woodgate, R., M. Singh, O. I. Kulaeva, E. G. Frank, A. S. Levine, and W. H. Koch.** 1994. Isolation and characterization of novel plasmid-encoded *umuC* mutants. J. Bacteriol. **176:**5011–5021.