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Coexpression of UmuD′ with UmuC Suppresses the UV Mutagenesis Deficiency of *groE* Mutants

CAROLINE E. DONNELLY† AND GRAHAM C. WALKER*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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The GroE proteins of *Escherichia coli* are heat shock proteins which have also been shown to be molecular chaperone proteins. Our previous work has shown that the GroE proteins of *E. coli* are required for UV mutagenesis. This process requires the *umuDC* genes which are regulated by the SOS regulon. As part of the UV mutagenesis pathway, the product of the *umuD* gene, UmuD, is posttranslationally cleaved to yield the active form, UmuD′. In order to investigate what role the *groE* gene products play in UV mutagenesis, we measured UV mutagenesis in *groE*$^+$ and *groE* strains which were expressing either the *umuDC* or *umuD′C* genes. We found that expression of *umuD′* instead of *umuD* will suppress the nonmutability conferred by the *groE* mutations. However, cleavage of UmuD to UmuD′ is unaffected by mutations at the *groE* locus. Instead we found that the presence of UmuD′ increased the stability of UmuC in *groE* strains. In addition, we obtained evidence which indicates that GroEL interacts directly with UmuC.

The GroE proteins of *Escherichia coli* are members of a ubiquitous family of molecular chaperone proteins found in all organisms (20). The *groE* genes of *E. coli* cannot be deleted from the genome, and it is most likely that the *groES* and *groEL* gene products perform essential functions (10). The role(s) that the *groE* gene products play in the normal physiology of *E. coli* growth is unclear; however, recent evidence suggests that the GroE proteins may play a role in the secretion of certain proteins and protein folding (2, 23). The *groE* locus was identified by mutations that cause defects in phage morphogenesis (11, 25). Later this locus was shown to encode two proteins, GroES and GroEL (25). Mutations in *groES* and *groEL* are pleiotropic and apparently represent partial loss-of-function alleles.

In eukaryotic cells, a homolog of the GroEL protein, Hsp60, is present in chloroplasts (15) and mitochondria (5) and has been shown to be required for the proper assembly of proteins within the organelle (5, 15, 22). By analogy, the GroE proteins of *E. coli* may function in a similar fashion. Our previous work and the work of others have shown that the GroE proteins of *E. coli* are required for processes involved in DNA repair, i.e., UV mutagenesis (which is the specialized processing of UV-irradiated DNA that results in mutation introduction) (6) and Weigle reactivation (which is the repair of UV-irradiated bacteriophage) (17).

Treatment of *E. coli* cells with DNA-damaging agents results in the induction of the genes of the SOS response (26). UV mutagenesis and Weigle reactivation are components of the elaborately regulated SOS response and are dependent on the *umuDC* genes, which are regulated as part of this response (26).

We have been particularly interested in the various levels of regulation associated with UV mutagenesis. This SOS process requires the products of the *umuDC* operon as well as activated RecA protein (RecA*). RecA* mediates the proteolytic cleavage of LexA, the repressor of the genes in the SOS regulon, thus allowing the increased transcription of the *umuDC* genes (26). Subsequently, RecA* mediates the cleavage of UmuD to yield UmuD′, the active form of UmuD in UV mutagenesis (4, 21). Intact UmuD may also play another role in this complex regulatory pathway by acting as a dominant inhibitor of UV mutagenesis (1). Furthermore, RecA plays a third role in UV mutagenesis in addition to mediating the cleavage of LexA and UmuD, although this is not clearly defined (7, 9, 24).

A complex set of interactions between several gene products is thought to be required for UV mutagenesis. It has been suggested that UmuC and UmuD′ act in concert with DNA polymerase III (with perhaps other proteins) to allow the polymerase to bypass lesions in the DNA which block replication (3). The results reported here support a model in which the GroE proteins interact with UmuC prior to the formation of complexes of UmuC with UmuD and UmuD′. We propose that the function of this interaction is to protect UmuC from proteolysis until such time as UmuD′ becomes available and the formation of a functional complex between UmuC and UmuD′ allows repair and mutagenesis of damaged DNA.

MATERIALS AND METHODS

Bacterial strains and plasmids. Host strains and plasmids are described in Table 1. Plasmid pGW3750 was constructed by replacing the 5′ end of the *umuD* gene present on a 1-kb *Bgl*II fragment of pSE115 with the corresponding *umuD′*-specific *Bgl*II fragment from pGW2122 (21). Similarly, to construct pGW3751 the same *umuD′*-specific *Bgl*II fragment was used to replace the 5′ end of the *umuD* gene present on pSE117.

Cultures were grown as indicated. When appropriate for plasmid selection, the antibiotics ampicillin (200 μg/ml) and kanamycin (40 μg/ml) were added to the medium.

UV mutagenesis. Measurement of the frequency of UV mutagenesis has been described previously (6, 8). More specifically, a 5-ml culture was grown in LB broth, pelleted, and resuspended in an equal volume of 0.85% saline. The suspension was irradiated at a UV fluence of 1 J/m^2/s for various lengths of time. To measure UV mutagenesis, we measured the frequency of reversion of the *argE3* mutation

* Corresponding author.
† Present address: Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, MA 02115.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype	Reference or source
Strains		
AB1157	*argE3*	8
GW2730	*lexA71*::Tn*5*(Def) *sulA11*	16
GW7514	W3110 *lacI*^{q}	S. Elledge
Plasmids		
pSE115	*umuD*$^+$*C*$^+$; pSC101	S. Elledge
pGW3750	*umuD'C*$^+$; pSC101	This work
pGW2101	*umuD*$^+$*C*$^+$	21
pGW6050	PT7 *umuD*$^+$*C*$^+$; pBR322	J. Battista
pGW5013	PT7 *umuD'C*$^+$; pBR322	J. Battista
pGW2030	PT7 *umuC*$^+$; pBR322	6
pACT7	Encodes IPTG-inducible T7 RNA polymerase; pACYC184	S. Karnic
pSE117	*umuD*$^+$*C*$^+$; pBR322	19
pGW3751	*umuD'C*$^+$; pBR322	This work

present in these strains. The frequency of Arg$^+$ clones was determined by plating the irradiated cells on M9 medium containing the full complement of required amino acids (100 μg/ml) except arginine, which was added in limiting amounts (1 μg/ml), and incubating the plates at 37°C. Colonies growing on limiting arginine plates were counted after 48 h. To measure survival in these experiments, we also diluted the cells appropriately in saline and plated them on M9 medium containing excess arginine (100 μg/ml). Colonies growing on these plates were counted after 24 h.

UV survival. Cultures were grown and irradiated as described above for UV mutagenesis. To measure survival at high doses of UV, we plated the cultures on LB agar plates containing appropriate antibiotics and incubated at 37°C. Colonies growing on these plates were counted after 18 h.

In vivo cleavage of UmuD to UmuD'. Monitoring of the in vivo cleavage of UmuD to UmuD' has been described previously (21). Briefly, a 20-ml culture of AB1157 (pGW2101) was grown to an optical density at 600 nm of 0.5 in LB broth containing ampicillin at 37°C. Cells were collected by centrifugation, washed in 5 ml of saline, and resuspended in 20 ml of saline. Cells were irradiated at a fluence of 1 J/m^2/s for 35 s. The cells were collected and resuspended in 20 ml of LB broth (with antibiotics) and allowed to grow in the dark at 37°C. Samples were collected at 0, 15, 30, and 60 min after UV irradiation. Five-milliliter samples were placed on ice for 5 min, and the cells were collected and boiled in 50 μl of sample buffer (6). The entire sample was loaded onto a sodium dodecyl sulfate (SDS)–20% polyacrylamide gel (21). The gel was subjected to Western blot (immunoblot) analysis with anti-UmuD' antibodies (which also recognizes UmuD) (13, 21). UmuD and UmuD' were visualized by the reaction of horseradish peroxidase coupled to a second antibody with the substrate, 3,3'-diaminobenzidine.

Pulse-labelling of UmuC, UmuD, and UmuD' proteins with [^{35}S]methionine and chase. Cells containing plasmids were grown and labelled similarly to that previously described (6). GW7514(pGW6050)(pACT7) and GW7514(pGW5013)(pACT7) *groE*$^+$ and *groE* strains were grown overnight in M9 medium containing antibiotics. Cells were diluted 50-fold into 20 ml of fresh M9 medium and allowed to grow to an optical density at 600 nm of 0.5. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added (1 mM). Thirty minutes later, rifampin was added (400 μg/ml). After 45 min, the cells were pulse-labelled for 5 min with 0.2 μCi of [^{35}S]methionine per ml (>1,000 Ci/mmol). Cold methionine was then added (1 mg/ml). Five-milliliter samples were taken at 0, 10, 20, 30, and 40 min later and added to a equal volume of cold 10% trichloroacetic acid. Precipitated proteins were collected by centrifugation, and the pellet was washed in acetone and then washed in 0.1 M Tris hydrochloride, pH 8.0. The sample was suspended in 50 μl of sample buffer and boiled for 3 min.

Samples were run on SDS–20% polyacrylamide gels and analyzed by autoradiography (6). Dried gels were also analyzed on PhosphorImager (Molecular Dynamics). After bands representing UmuC were quantified, a semilog graph of the decay of UmuC yielded the half-life ($t_{1/2}$). Each $t_{1/2}$ value is the average of two separate experiments.

Immunoprecipitation. UmuC was labelled as described above with three exceptions. First, strain GW7514(pGW2030)(pACT7) was used because only UmuC is labelled in this strain (6). Second, maximal labelling of UmuC requires rifampin treatment to be extended to 45 min. Last, 5 μCi of [^{35}S]methionine per ml was used and cells were labelled for 10 min with no chase.

The procedure for preparation of extracts and treatment for immunoprecipitation was based on that previously described (13). Cells were pelleted, washed once in 1 ml of saline, and resuspended in 1 ml of lysis buffer (50 mM Tris [pH 8.0], 50 mM glucose, 5 mM EDTA, 100 μg of lysozyme per ml). The cell suspension was freeze-thawed twice, and the insoluble material was removed by centrifugation. The supernatant was used as cellular extract. All subsequent incubations were done at 0°C.

Before GroEL antibodies were added, the entire extract (0.8 ml) was treated with 10 μl of rabbit serum, which was free of anti-GroEL antibodies (data not shown), and 0.5 mg of protein A (protein A-positive *Staphylococcus aureus*; Boehringer Mannheim) per ml. After 1 h, the precipitated material was removed. This precipitate was used as control material for nonspecific interactions. The supernatant was incubated with anti-GroEL antibodies (affinity purified from immune rabbit serum on a GroEL protein affinity column [13]) for 1 h, and protein A was added. After 1 h, the precipitated material was collected, washed three times in phosphate-buffered saline (13) and resuspended in 50 μl of sample buffer, and the sample was applied to a SDS–14% polyacrylamide gel. The gel was stained with Coomassie blue, enhanced with sodium salicylate, dried, and autoradiographed (6).

Cold sensitivity. To test the cold sensitivity phenotype associated with overexpression of *umuDC*, strain GW2730 was transformed with various plasmids by making the cells competent by treatment with $CaCl_2$. Transformed cells were spread on LB agar plates and incubated at 30°C. After 24 h, the plates were scored for growth of colonies. Part of the transformation mixture was also plated at 42°C to control for competence of the cells.

RESULTS

UV mutagenesis does not require *groE* when *umuD'* is expressed instead of *umuD*. To begin to address the role of *groE* in UV mutagenesis, we measured UV mutagenesis in *groE*$^+$ and *groE* strains which were expressing either the *umuDC* or *umuD'C* genes. (The *umuD'* gene was derived from the *umuD* gene by deletion of the codons for the amino-terminal domain of UmuD and thus directly encodes UmuD' [21].) The *groEL100* and *groES30* strains are se-

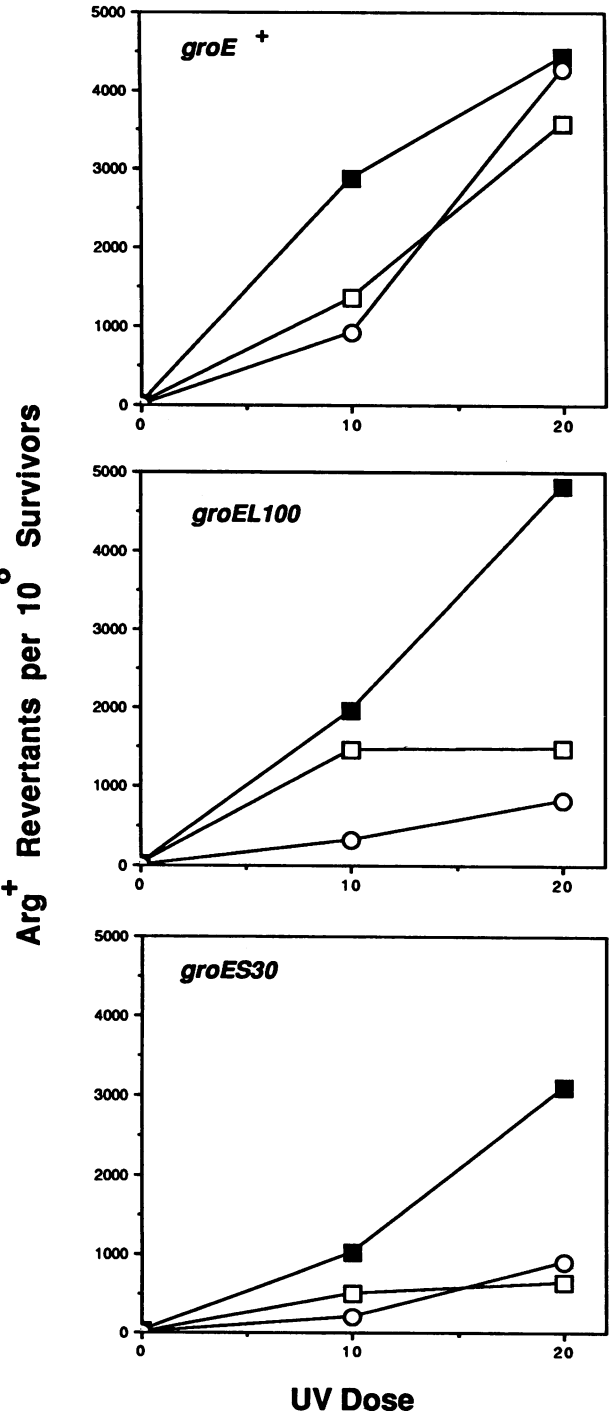

FIG. 1. Frequency of UV mutagenesis in wild-type and *groE* cells. UV mutagenesis of AB1157 expressing either *umuDC* or *umuD'C* was assayed by reversion of the *argE3* mutation in AB1157 in *groE*$^+$ and *groE* strains, as described in Materials and Methods. Symbols: ○, AB1157 and *groE* derivatives without a plasmid; □, AB1157(pSE115) (*umuD*$^+$*C*$^+$); ■, AB1157(pGW3750) (*umuD'C*$^+$).

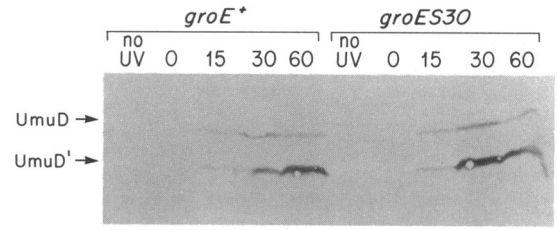

FIG. 2. In vivo cleavage of UmuD to UmuD' in *groE*$^+$ and *groES30* strains. AB1157(pGW2101) was grown as described in Materials and Methods. After UV irradiation, the cultures were allowed to grow in the dark. Five-milliliter samples were collected at 0, 15, 30, and 60 min after UV irradiation. The cells were collected by centrifugation, and the pellet was resuspended in 50 μl of sample buffer. The entire sample was run on a SDS–20% polyacrylamide gel, and the gel was subjected to Western blot analysis with anti-UmuD' antibodies.

verely deficient in UV mutagenesis (Fig. 1). Previously we had observed that expression of *umuDC* from a high-copy-number plasmid (pBR322) would partially suppress the nonmutability of the *groE* strains (6). In order to minimize suppression because of increased gene dosage, we assayed UV mutagenesis in strains in which the *umuDC* or *umuD'C* genes were expressed from a relatively low-copy-number plasmid (pSC101, with approximately 8 copies per chromosome) (14). Under these conditions, the nonmutability phenotype of *groEL100* and *groES30* derivatives of AB1157 is only slightly suppressed by a plasmid encoding *umuDC*, pSE115 (Fig. 1).

In contrast, UV mutagenesis of *groE* strains carrying a similar plasmid expressing *umuD'C* is significantly restored. The *groEL100* strain carrying the *umuD'C* plasmid reaches wild-type levels of UV mutagenesis. The *umuD'C* plasmid restored UV mutagenesis of the *groES30* strain to approximately 67 and 71% of the *groEL100* and *groE*$^+$ strains, respectively. We consistently observe that the *groES30* strain is not fully suppressed by expression of *umuD'C*. One possible explanation for this result would be that *groES30* strains are less viable than *groEL100* strains and this interferes with the assay. In support of this explanation, we observe that the density of *groES* cultures is always less than that of the *groEL100* or *groE*$^+$ cultures (data not shown). However, we cannot rule out the possibility that *groEL* and *groES* do not play the same role in UV mutagenesis.

***groES* strains do not affect UmuD cleavage to UmuD'.** The UmuD cleavage results indicated that moderate overexpression of *umuD'C* suppresses the nonmutable phenotype of the *groE* mutants more effectively than moderate overexpression of *umuDC*. A simple model to account for this result would be that *groE*$^+$ function affects the RecA-mediated cleavage of UmuD in vivo. However, we found that the *groES30* mutation (Fig. 2) and the *groEL100* mutation (data not shown) do not appear to affect the level of in vivo cleavage of UmuD to UmuD' after UV irradiation.

Since we could not attribute the effects of *groE* mutations on UV mutagenesis to an effect on UmuD cleavage, we next considered the possibility that the requirement for *groE*$^+$ function is reduced when UmuD' is expressed because UmuC is stabilized by UmuD'. This hypothesis was suggested by our observations that *groE* mutations affect the stability of UmuC when it is expressed without UmuD or UmuD' (6) and also by the observation of Woodgate et al. that UmuC associates with both UmuD and UmuD' (28).

Expression of UmuD' stabilizes the UmuC protein. We therefore carried out pulse-chase experiments to test whether UmuD and UmuD' altered the stability of UmuC in a *groE*$^+$ or *groE* background. Figure 3 shows the results of a pulse-chase experiment. Figure 3A and B show repre-

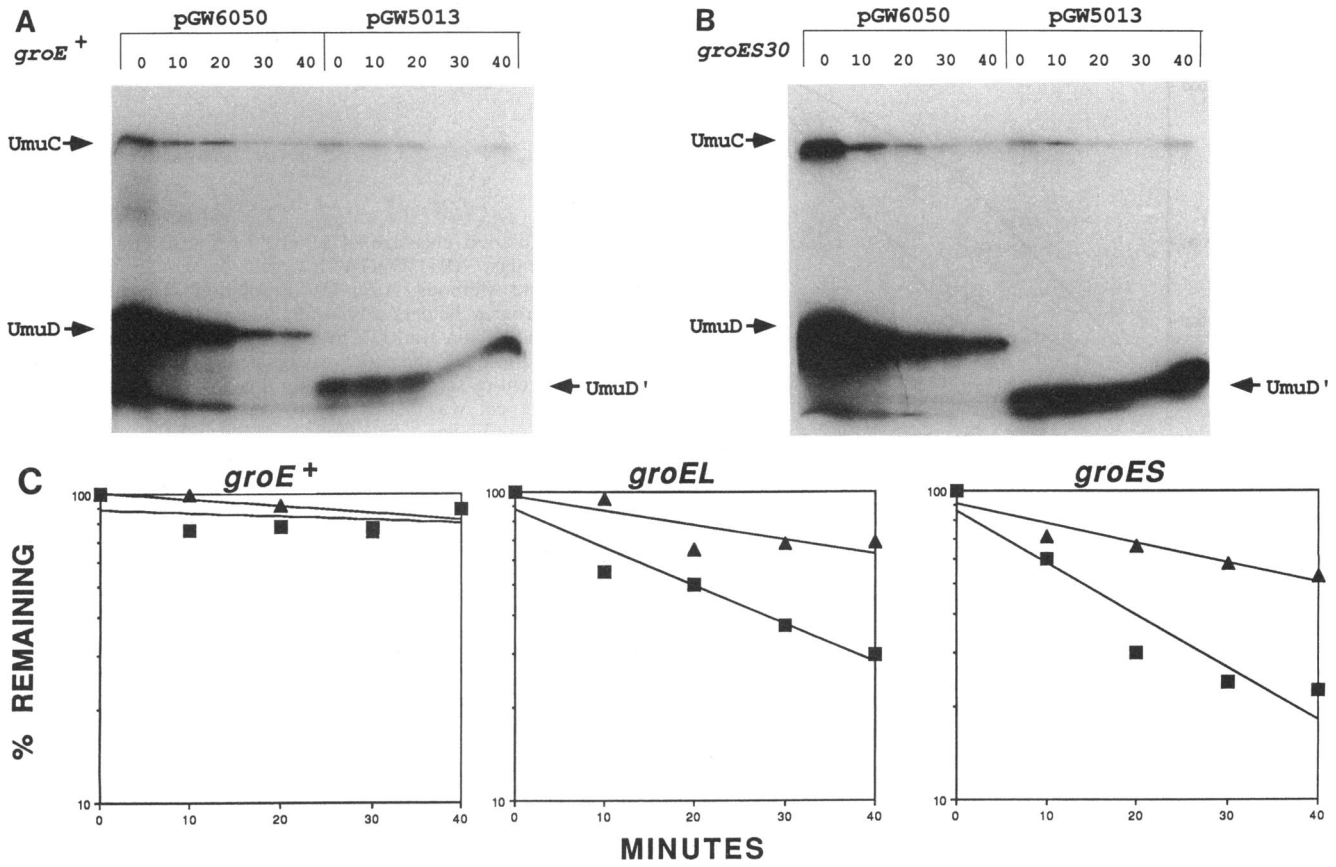

FIG. 3. $t_{1/2}$ of UmuC when coexpressed with UmuD or UmuD'. GW7514(pGW6050)(pACT7) and GW7514(pGW5013)(pACT7) were pulse-labelled as described in Materials and Methods. UmuC and UmuD were specifically labelled in cells harboring pGW6050, and UmuC and UmuD' were labelled in cells harboring pGW5013. (A) Labelled proteins from the *groE*$^+$ derivative and (B) labelled proteins from the *groES30* derivative. The numbers over the lanes indicate the time (in minutes) after the pulse. (C) Semilog graphs indicating the percent remaining of UmuC in the time after pulse-labelling in *groE*$^+$, *groEL100*, and *groES30* strains. Symbols: ▲, GW7514(pGW5013)(pACT7); ■, GW7514(pGW6050)(pACT7).

sentative examples of the gels from such an experiment. UmuC was coexpressed with either UmuD (pGW6050) or UmuD' (pGW5013). We quantitated the disappearance of UmuC in *groE*$^+$, *groES30*, and *groEL100* backgrounds by the analysis of gels from two separate experiments with PhosphorImager (Molecular Dynamics). The values from PhosphorImager were expressed as percentages of radioactivity left 10, 20, 30, and 40 min after the pulse. This data is shown on the semilog graphs in Fig. 3C. These analyses yielded estimates of the $t_{1/2}$ of UmuC in the various strains.

Our previous results have shown that the $t_{1/2}$ of UmuC, in the absence of overexpression of UmuD or UmuD', is 17 min in a *groE*$^+$ strain but only 6 min in a *groEL100* or *groES30* strain (6). When UmuC is overexpressed in a *groE*$^+$ strain with UmuD or UmuD', UmuC is very stable ($t_{1/2}$ > 100 min). When these proteins are overexpressed in the *groEL100* strain, UmuC is partially stabilized by coexpression with either UmuD or UmuD' (overexpression with UmuD, $t_{1/2}$ is approximately 20 min; overexpression with UmuD', $t_{1/2}$ is approximately 50 min). Although coexpression of both UmuD and UmuD' led to a stabilization of UmuC, coexpression of UmuD' had a greater stabilizing effect. In the *groES30* strain, the effect of UmuD' expression on the stability of UmuC is more striking (overexpression of UmuC with UmuD, $t_{1/2}$ is approximately 12 min; overexpression of UmuC with UmuD', $t_{1/2}$ is approximately 40 min). Taken together, these results indicate that the stability of UmuC in a *groE* strain can be affected by the overexpression of UmuD and that UmuC is stabilized even further when UmuD' is overexpressed.

UmuC is expressed from its own promoter at a very low level (27). In order to specifically measure the stability of UmuC, we utilized plasmid constructs in which *umuD*, *umuD'*, and *umuC* were under the control of the T7 promoter, PT7. However, the overexpression of UmuC (and UmuD or UmuD') may lead to the formation of aggregates that sequester the proteins from proteolysis so that our results may be limited to measurement of only a subpopulation of UmuC molecules. In addition, we have observed that expression of *umuD'C* from pGW5013 is always somewhat less than expression of *umuDC* from pGW6050. This would appear to be due to differing sequences at the 5' end of the *umuD'* gene that alter the efficiency of translation. Since we are measuring the decay of UmuC that has already been synthesized, we feel that the difference in expression in unlikely to account for the difference in UmuC stability that we observed.

Coimmunoprecipitation of UmuC with anti-GroEL antibodies. The effect of *groE* mutations on the stability of UmuC could be a direct effect of the GroE proteins forming a

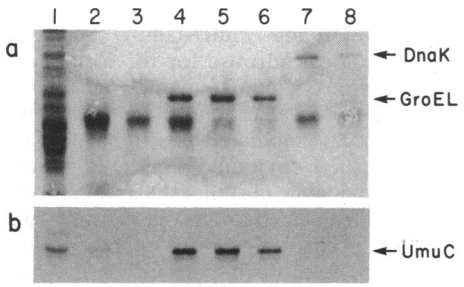

FIG. 4. Coimmunoprecipitation of UmuC with GroEL. GW7514 (pGW2030)(pACT7) was grown and labelled and cellular extracts were made as described in Materials and Methods. GroEL was immunoprecipitated from the extracts as described in Materials and Methods with anti-GroEL antibodies. Before immunoprecipitation, the entire extract was first treated with serum free of anti-GroEL antibodies and with protein A for 1 h (pretreated extract). Insoluble material after immunoprecipitation was suspended in 50 μl of sample buffer, boiled for 3 min, and loaded onto a gel as described in Materials and Methods. (a) Coomassie blue-stained gel of immunoprecipitates. (b) Autoradiograph of the same gel. Lane 1, 20 μl of untreated extract; lane 2, insoluble material left after pretreatment; lane 3, control immunoprecipitate from pretreated extract treated again with rabbit serum free of anti-GroEL antibodies; lanes 4, 5, and 6, immunoprecipitate from pretreated extract treated with 10, 5, and 2 μl, respectively, of anti-GroEL; lanes 7 and 8, same as in lanes 4, 5, and 6 but pretreated extract treated with 10 and 5 μl, respectively, of anti-DnaK.

complex with UmuC or the *groE* mutations could act indirectly by stimulating a cryptic protease. Therefore, we tested whether we could detect the association of GroEL and UmuC in vivo.

Coimmunoprecipitation of UmuC and GroEL with anti-GroEL antibodies (Fig. 4) indicates that these proteins do interact in vivo. Control experiments indicated that the interactions between GroEL and UmuC proteins were specific, because antibodies against another *E. coli* heat shock protein, DnaK, did not coprecipitate UmuC (Fig. 4). In addition, GroEL antibodies do not recognize UmuC by Western blot analysis. Anti-GroEL antibodies did not coprecipitate UmuD or UmuD', regardless of the presence of labelled UmuC in the extract, perhaps indicating that UmuC's interactions with GroEL do not allow UmuC to interact with UmuD or UmuD' (data not shown). We also tested whether anti-GroEL sera would coprecipitate UmuC from an extract made from a *groEL100* or *groES30* strain and found that UmuC was coprecipitated from these extracts as well, which indicates that these mutants still retain partial *groE*$^+$ function.

Cold sensitivity due to overexpression of *umuD'C* is not suppressed by mutations in *groE*. Our investigation into the role of the GroE proteins in UV mutagenesis began by studying complex phenotypes associated with overexpression of the *umuDC* genes. Marsh and Walker reported that expression of *umuDC* from a plasmid in a strain that encodes a defective LexA repressor protein (which results in constitutive overexpression of *umuDC*) causes cold-sensitive growth (19). In addition, the cold sensitivity phenotype is suppressed by mutations at the *groE* locus (6). The molecular explanation for the cold sensitivity phenotype is not clear, although DNA synthesis is immediately blocked after a shift of growing cells from 42 to 30°C (19).

We have shown that mutations at the *groE* locus alter the stability of UmuC and suppression of the cold sensitivity

TABLE 2. Cold sensitivity

Genotype[a]	Plasmid	Growth[b] after transformation at:	
		30°C	42°C
lexA(Def) *groE*$^+$	pSE117 (*umuDC*)	+/−	+++
lexA(Def) *groE*$^+$	pGW3751 (*umuD'C*)	+/−	+++
lexA(Def) *groEL100*	pSE117 (*umuDC*)	+++	+++
lexA(Def) *groEL100*	pGW3751 (*umuD'C*)	+/−	+++

[a] All strains are derivatives of GW2730 (16).
[b] +/−, growth of very small colonies after 36 to 48 h; +++, growth of normal-size colonies after 24 h.

phenotype by *groE* mutants may be due to lowering the levels of UmuC (6). Here we show that expression of *umuD'* instead of *umuD* stabilizes UmuC in a *groE* strain. One might expect then that the cold sensitivity caused by overexpression of *umuD'C* would not be suppressed by mutations in *groE*. We tested this hypothesis by transforming *groE*$^+$ and *groEL100* of a *lexA*(Def) strain with a plasmid which encodes *umuDC* and a similar plasmid which encodes *umuD'C*. As shown in Table 2, the *lexA*(Def) *groEL100* strain is transformable at 30°C with the plasmid expressing *umuDC*. However, the plasmid expressing *umuD'C* cannot be transformed into such a strain, indicating that the *groEL100* mutation does not suppress the cold sensitivity induced by overexpression of *umuD'C*.

DISCUSSION

We have shown that coexpression of *umuD'* with *umuC* can suppress the deficiencies of *groE* mutants in UV mutagenesis, and that the $t_{1/2}$ of UmuC appears altered by this coexpression as well. On the basis of these results, it is possible that *groE*$^+$ function is not absolutely required for assembly of the Umu mutagenesis proteins. Our investigation into the role of the GroE proteins in UV mutagenesis suggests that GroEL and GroES may act early in the pathway, before the association of UmuC with either UmuD or UmuD'. Liu and Tessman have reported that *groE* mutations may have a greater effect on mutagenesis than they do on repair. The interactions between UmuC and GroEL and GroES, in addition to affecting the stability of UmuC, may alter its activity to promote mutagenic repair (17, 18).

Since the *groEL100* and *groES30* mutations used in this study are partial loss-of-function alleles that were identified on the basis of defects they caused in bacteriophage growth, it is not clear whether there is a *groE*$^+$-independent component to UmuC folding and possibly UmuC complex formation or whether these alleles (and other similarly isolated alleles we have tested) are only partially defective in these functions. The fact that *groEL* and *groES* null mutants are not viable (10) precludes their use in such experiments. Our failure to observe an absolute requirement for *groE*$^+$ function in *umuDC*-dependent mutagenesis might be due to the fact that we have had to employ partial loss-of-function alleles of *groES* and *groEL*.

In Fig. 5 we propose a model for our observations that there is a reduced requirement for *groE*$^+$ function for UV mutagenesis in cells that directly express UmuD', along with UmuC, as opposed to cells that express the intact form of UmuD along with UmuC. Our observations that GroEL physically interacts with UmuC and that UmuC is always relatively less stable in *groEL100* and *groES30* mutants than

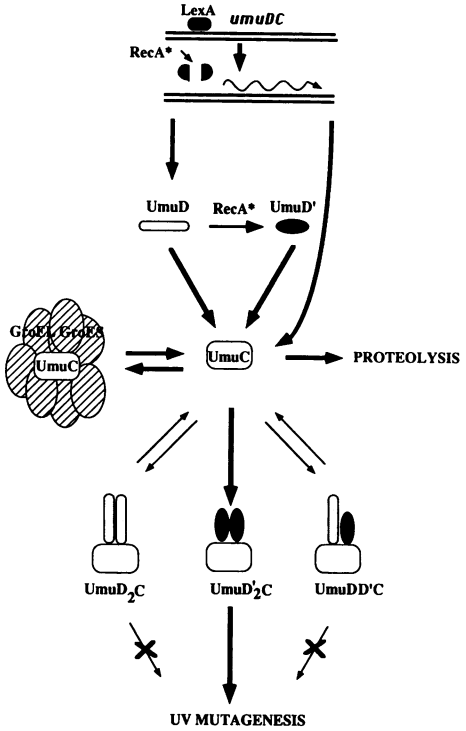

FIG. 5. Model of UV mutagenesis. After UV irradiation, the RecA protein is converted to its activated form (RecA*) and induces the cleavage of the LexA repressor. Transcription of the *umuDC* genes is increased, and UmuD and UmuC are synthesized. UmuD is activated by a RecA*-mediated cleavage which results in UmuD'. UmuC is unstable but stabilized by GroEL and GroES and forms a complex with dimers of UmuD and UmuD' or heterodimers with UmuD and UmuD'. While the UmuC-$UmuD_2$ complex (and perhaps the UmuC-UmuDD' complex) is labile, the UmuC-$UmuD'_2$ complex is quite stable. (The UmuC-$UmuD'_2$ complex is required for UV mutagenesis, but the UmuC-$UmuD_2$ complex and the UmuC-UmuDD' complex are thought to be inactive in UV mutagenesis.) The thick arrows indicate the major pathway of protein association, and the thin arrows indicate minor or reversible pathways. Arrows marked by an X indicate inactive pathways.

in a $groE^+$ strain suggest that the *groE* gene products interact with UmuC in a way that reduces its susceptibility to proteolysis. Thus, GroEL and GroES playing a role in facilitating UmuC folding would be consistent with these observations as well as with general suggestions for the action of molecular chaperones and with proposed interactions of *E. coli* GroEL and GroES with eukaryotic ribulose bisphosphate carboxylase (12).

However, even in a $groE^+$ background the interactions of UmuC with the GroE proteins are not particularly effective at protecting UmuC from proteolysis ($t_{1/2}$ = 17 min) in the absence of UmuD or UmuD'. We have observed that coexpression of UmuD along with UmuC (in a non-SOS-induced cell so that UmuD cleavage does not occur) greatly stabilizes UmuC ($t_{1/2}$ > 100 min). In fact, the effect of coexpression of UmuD on UmuC stability in *groE* mutants appears to be greater than the effect of $groE^+$ function on UmuC stability in the absence of UmuD coexpression. Thus, we propose that UmuD can stabilize UmuC in the absence of $groE^+$ function through the formation of a complex of UmuC with a dimer (28) of UmuD. However, we have previously presented evidence that such complexes containing intact UmuD are largely inactive in UV mutagenesis as are probably complexes containing UmuD-UmuD' heterodimers (1). Thus, the physiological purpose for the formation of these largely inactive UmuC · $UmuD_2$ complexes may be to sequester UmuC from degradation until enough active UmuD' is produced by RecA*-mediated posttranslational cleavage of UmuD to allow the formation of UmuD' homodimers. We have preliminary evidence (1a) that the association of UmuC with UmuD' is stronger than its association with intact UmuD. However, the observations reported here suggest that this difference is more important for the biochemical role of UmuD' and UmuC in UV mutagenesis rather than for the sequestration of UmuC from degradation.

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