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groE Mutants of *Escherichia coli* Are Defective in *umuDC*-Dependent UV Mutagenesis

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Overexpression of the SOS-inducible *umuDC* operon of *Escherichia coli* results in the inability of these cells to grow at 30°C. Mutations in several heat shock genes suppress this cold sensitivity. Suppression of *umuD*$^+$*C*$^+$-dependent cold sensitivity appears to occur by two different mechanisms. We show that mutations in *lon* and *dnaK* heat shock genes suppress cold sensitivity in a *lexA*-dependent manner. In contrast, mutations in *groES*, *groEL*, and *rpoH* heat shock genes suppress cold sensitivity regardless of the transcriptional regulation of the *umuDC* genes. We have also found that mutations in *groES* and *groEL* genes are defective in *umuDC*-dependent UV mutagenesis. This defect can be suppressed by increased expression of the *umuDC* operon. The mechanism by which *groE* mutations affect *umuDC* gene product function may be related to the stability of the UmuC protein, since the half-life of this protein is shortened because of mutations at the *groE* locus.

The GroES and GroEL proteins are 2 of 20 *Escherichia coli* heat shock proteins (25). The GroEL protein is homologous to members of the eucaryotic Hsp60 protein family, indicating that this protein has been highly conserved throughout evolution (29). GroES, GroEL, and other heat shock proteins are thought to be involved in increasing the cellular tolerance to damage due to heat as well as other forms of stress, but it is becoming obvious that many of these proteins are required for normal cellular functioning as well (25, 33).

In *E. coli*, the GroE proteins appear to be essential for growth. A mutant which is deleted for the *rpoH* gene, which encodes the sigma factor necessary for transcription from heat shock promoter sequences, is unable to grow at temperatures above 20°C (42). Suppressors of the Δ*rpoH* mutation which allow growth at higher temperatures cause increased expression of the *groES* and *groEL* genes (24). More recently, Fayet et al. have shown that *E. coli* cells cannot tolerate a deletion of the *groE* operon (13). Mutations in *groES* and *groEL* genes have pleiotropic phenotypes including defects in bacteriophage morphogenesis, temperature sensitivity, and alterations in cellular proteolysis (14, 38).

In *E. coli* and in higher organisms, the GroE proteins are thought to be involved in the macromolecular assembly of protein complexes. The assembly of the head proteins of λ and T-even phages and the tail proteins of other bacteriophages requires the products of the *groE* operon (15, 16, 39). In the chloroplasts of plants, the GroEL homolog Hsp60 is required for the correct posttranslational assembly of ribulose-1,5-bisphosphate carboxylase-oxygenase (20). Protein complexes which are assembled in mitochondria of the yeast *Saccharomyces cerevisiae* have been shown to require Hsp60 for functioning (8). By analogy, we might expect that the GroES and GroEL proteins would play a similar role in *E. coli*. In fact, Goloubinoff et al. have shown that assembly of a procaryotic protein complex (*Aspergillus nidulans* ribulose-1,5-bisphosphate carboxylase-oxygenase) also requires the GroES and GroEL proteins in *E. coli* (18). *E. coli* GroEL protein has been shown to physically interact with pre-β-lactamase and chloramphenicol acetyltransferase synthesized in vitro (3), although it is not known whether GroES and/or GroEL proteins affect the activity of these proteins. The expression molecular chaperone has been proposed to describe the role that the GroE proteins play in macromolecular assembly by preventing incorrect or promoting correct protein-protein interactions (3, 8, 18, 20).

We have recently become aware of another role for the *E. coli groE* gene products through our work in mechanisms of mutagenesis. In particular, we are interested in the molecular basis of UV and chemical mutagenesis, a process which requires the functions of the SOS-regulated *umuDC* operon (for a review, see reference 41). After treatment with a DNA-damaging agent, such as UV light, the RecA protein becomes activated and mediates the cleavage of the SOS repressor protein, LexA. This cleavage inactivates LexA and allows expression of the genes of the SOS regulon, including the *umuDC* operon. The UmuD protein is subsequently activated for its role in mutagenesis by a RecA-mediated cleavage that is mechanistically similar to the RecA-mediated cleavage of LexA (6, 32, 37). The products of the *umuDC* genes are thought to play a role in helping DNA replication to proceed beyond a noncoding or miscoding lesion (4).

Overexpression of the products of the *umuDC* operon causes *E. coli* cells to become cold sensitive (27). Cells that overproduce UmuD and UmuC proteins as a consequence of the presence of both a *lexA51*(Def) mutation and a multicopy plasmid carrying the *umuD*$^+$*C*$^+$ operon grow well at 42°C. However, when the temperature is shifted to 30°C, DNA synthesis stops immediately, and ultimately the cells die (27). The molecular nature of this cold sensitivity has not yet been determined, but it seems possible that the UmuD and UmuC proteins may interact with the cellular machinery involved in DNA replication. In this paper we describe our recent analyses of this phenomenon, which have led to the finding that GroES and GroEL proteins of *E. coli* are normally required for UV mutagenesis.

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype[a]	Reference or source
Strains		
AB1157	*argE3*	11
KM1190	*lexA51*(Def) *sulA11*	12
GW1000	*lexA*$^+$	21
GW2730	*lexA71*::Tn*5*(Def) *sulA11*	22
W3110	*lacI*^{q}	S. Elledge
GW7501	As AB1157, but *lon-146*::Tn*10*	28
GW7502	As AB1157, but *dnaK756 thr*::Tn*10*	C. Georgopoulos
GW7503	As AB1157, but *groEL100 purA*::Tn*10*	B. Bachman
GW7504	As AB1157, but *groES30 purA*::Tn*10*	B. Bachman
GW7505	As KM1190, but *lon-146*::Tn*10*	28
GW7506	As KM1190, but *dnaK756 thr*::Tn*10*	C. Georgopoulos
GW7507	As KM1190, but *groEL100 purA*::Tn*10*	B. Bachman
GW7508	As KM1190, but *groES30 purA*::Tn*10*	B. Bachman
GW7509	As GW2730, but *lon-146*::Tn*10*	28
GW7510	As GW2730, but *dnaK756 thr*::Tn*10*	C. Georgopoulos
GW7511	As GW2730, but *groEL100 purA*::Tn*10*	B. Bachman
GW7512	As GW2730, but *groES30 purA*::Tn*10*	B. Bachman
GW7513	As GW2730, but *rpoH165 mal*::Tn*10*	10
Plasmids		
pSE117	*umuD*$^+$*C*; pBR322	27
pKK223-3	P_{trc}; pBR322	Pharmacia
pGW3700	*umuD*$^+$*C*$^+$; pKK223-3	This work
pGW3701	*umuD*$^+$*C*; pKK223-3	This work
pLD1291	*umuDC*$^+$; pBR322	L. Dodson
pACT7	Encodes IPTG-inducible T7 RNA polymerase; pACYC184	S. Karnic
pGW2030	PT7 *umuC*$^+$; pBR322	J. Battista, unpublished results

[a] Mutations at heat shock loci were transduced by P1 phage (30) into various background strains. Tetracycline-resistant transductants were selected because of the linked Tn*10* marker and were screened for temperature sensitivity and/or λ resistance.

MATERIALS AND METHODS

Bacterial strains and plasmids. Table 1 lists the bacterial strains and plasmids described in the text. *E. coli* cells were made competent by $CaCl_2$ treatment and transformed with plasmid DNA (26). Strains transformed with pGW3700 or pGW3701 also contained an F plasmid carrying the *lacI*^{q} gene [F′ *proAB lacI*^{q}*Z* ΔM15 Tn*10*(Tet^r) or F′ *proAB lacI*^{q}*Z* ::Tn*5*(Kan^r)].

Enzymes and chemicals. Restriction and DNA modification enzymes were purchased from New England BioLabs, Inc., and International Biotechnology, Inc. Isopropyl-β-D-galactopyranoside (IPTG) was purchased from Sigma Chemical Co. [^{35}S]methionine was purchased from Amersham Corp.

Media. LB broth and LB agar plates were used for transformation, transduction, and cloning experiments (30). Minimal media used for UV survival and mutagenesis experiments and cell labeling experiments contained M9 buffer and salts (30) with added nutrients (4 μg of thiamine per ml, appropriate amino acid supplements, and 0.4% glucose) and antibiotics. For drug resistance selection or maintenance of plasmids and transposons, ampicillin (100 μg/ml), kanamycin (30 μg/ml), tetracycline (10 μg/ml), and chloramphenicol (30 μg/ml) were added separately or in various combinations to the media. IPTG (1 mM) was added to plates or liquid media.

Quantitative transformation. One milliliter of *E. coli* cells grown in LB broth at 30°C to an optical density at 600 nm (OD_{600}) of 0.5 was made competent. The resulting suspension was split in half; one portion was treated with 1 pmol of pBR322 DNA, and the other portion was treated with an equal molar amount of pSE117 DNA. Either all or a portion of the transformation mixture was spread on LB agar plates containing the appropriate antibiotics. Plates were incubated at 30°C, and colonies were counted after 36 h.

Construction of pGW3700 and pGW3701. Plasmid pDS101 contains the wild-type *umuDC* operon (32). This plasmid can be used to generate single-stranded DNA which is useful for mutagenesis of the *umuDC* locus. We used site-directed oligo mutagenesis to generate an *Eco*RI site upstream of the *umuD* gene centered at position −18 relative to the transcription start site (34). Generation of this restriction site allowed the *umuDC* genes without a promoter or LexA-binding site to be cloned downstream of the P_{trc} promoter sequence. We sequenced the mutagenized region of the resulting plasmid by the dideoxynucleoside triphosphate-chain termination technique (35) and determined that the only mutation in the *umuD* gene was at the new *Eco*RI site. Double-stranded DNA of the mutagenized plasmid was used to generate a 4-kilobase *Eco*RI fragment which was cloned into the plasmid which carried the P_{trc} promoter sequence just upstream of a unique *Eco*RI site (pKK223-3). The resulting plasmid, pGW3700, allowed inducible expression of the *umuDC* genes by the addition of the inducer IPTG.

We also constructed a plasmid, pGW3701, which is analogous to pGW3700 but is *umuD*$^+$ and *umuC*. Plasmid DNA of pGW3700 was cut at the unique *Mlu*I site, which generates a protruding 5′ end within the *umuC* gene. This site was blunt ended with the Klenow fragment of DNA polymerase I. The DNA was ligated with T4 DNA ligase and transformed into cells (26). The colonies were screened for the presence of a plasmid which had lost the *Mlu*I site. This mutation caused a frame shift in the *umu*C gene; pGW3701 complements a *umuD44* mutation, but not a *umuC36* mutation, for UV mutagenesis.

UV mutagenesis. UV mutagenesis was determined in various strains as described by Elledge and Walker (11). Cells were grown to mid-log phase in LB broth, pelleted, and suspended in 0.85% saline. The suspension was placed in a glass petri dish and irradiated at a UV fluence of 1 J/m^2 per s. Cells were diluted in saline and spread on M9 agar plates which contained histidine, threonine, leucine, and proline at 100 μg/ml. To measure UV survival, arginine was added to 100 μg/ml; to measure mutagenesis of the *argE* locus, a trace amount of arginine was added (1 μg/ml). Plates were incubated at various temperatures, and colonies were counted after 48 h. Very few additional colonies appeared after this time. The effect of *groE* mutations on UV mutagenesis and suppression of cold sensitivity was not dependent on the *purA*::Tn*10* mutation present in these strains.

Pulse-labeling with [^{35}S]methionine and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. To measure the half-life of UmuC protein in intact cells, we used a system

which allowed for induced synthesis of UmuC protein in the absence of synthesis of other proteins. Plasmid pGW2030 encodes the *umuC* gene under the control of a T7 phage promoter, and pACT7 encodes T7 RNA polymerase under the control of the IPTG-inducible promoter P_{tac} (J. Battista and G. Walker, unpublished results).

W3110(pGW2030)(pACT7) *groE*$^+$ and *groEL100* strains were grown overnight in M9 medium containing antibiotics. Cells were diluted into 5 ml of fresh medium and allowed to grow to an OD_{600} of 0.5. At this time, IPTG was added (1 mM) to induce synthesis of T7 RNA polymerase. After 30 min, transcription initiated by *E. coli* RNA polymerase was inhibited by the addition of rifampin (400 μg/ml dissolved in dimethylsulfonate). Twenty minutes later, the cells were pulse-labeled for 5 min with 0.2 μCi of [^{35}S]methionine per ml (>1,000 Ci/mmol). Cold methionine was added to a concentration of 1 mg/ml. Samples were taken at various times and added to an equal volume of cold 10% trichloroacetic acid. Precipitated proteins were collected by centrifugation. The pellet was washed in acetone, collected again, and washed in 0.1 M Tris hydrochloride, pH 8.0. The pellet was then suspended in 50 μl of sample buffer and boiled for 3 min. Sample buffer contained 0.25% sodium dodecyl sulfate, 2% β-mercaptoethanol, 0.05 M Tris hydrochloride, pH 6.8, 0.002 M EDTA, 10% glycerol, and bromophenol blue.

The amount of radioactivity was determined in each sample, and an equal amount of radioactivity (between 10,000 and 20,000 cpm) was loaded onto a 14% sodium dodecyl sulfate-polyacrylamide gel. Each lane was cut into 2-mm slices, the slices were solubilized overnight at 65°C in 5 ml of Fluorosol (National Diagnostics), and each sample was counted for 5 min in a Beckman LS 7000 scintillation counter. To determine half-life, peak fractions were plotted versus time on a semilog graph. Half-life estimates were averages of three experiments.

RESULTS

Mutations in heat shock genes suppress *umuD*$^+$*C*$^+$-dependent cold sensitivity. We have previously observed that the cold sensitivity of a *lexA51*(Def) strain carrying *umuD*$^+$*C*$^+$ on a multicopy-number plasmid can be suppressed by mutations in either of two heat shock loci, *lon* and *rpoH* (27). The *lon* locus encodes an ATP-dependent protease activity that is induced by heat shock (7, 9), and the *rpoH* locus encodes the alternative sigma subunit of RNA polymerase that enables it to recognize the promoters of heat shock genes (19). Since mutations in both *lon* and *rpoH* have been shown to cause defects in proteolysis (1, 17, 38), we considered the possibility that these two mutations were suppressing cold sensitivity by altering the half-life of certain proteins such as LexA51(Def), UmuD, or UmuC.

We investigated whether the LexA51(Def) protein could be stabilized by the *lon* or the *rpoH* mutations. The *lexA51*(Def) allele was isolated as a second site suppressor mutation of the *lexA3*(Ind$^-$) mutation (31). Krueger et al. showed that the LexA51(Def) protein retains some repressor function in vivo, since *lexA*::Tn*5* mutants express higher levels of LexA-repressed genes than do *lexA51*(Def) mutants (22). If the half-life of the LexA51(Def) protein were lengthened due to a proteolysis defect in a *lon* or an *rpoH* strain, expression of the *umuDC* genes would be repressed and thus lead to suppression of cold sensitivity.

To test this hypothesis, we examined the ability of *lon* and *rpoH* mutations to suppress cold sensitivity in a strain carrying the *lexA51*(Def) mutation or a strain carrying a Tn*5* insertion very early in the *lexA* gene [*lexA71*::Tn*5*(Def)] which appears to abolish *lexA* activity (22). Suppression of cold sensitivity was measured by using a quantitative transformation assay, in which the transformation efficiency of the *umuD*$^+$*C*$^+$ plasmid (pSE117) was compared with the transformation efficiency of the parent plasmid (pBR322). Previous work had shown that a *lon* mutation allows efficient transformation of the *lexA51*(Def) strain with the *umuD*$^+$*C*$^+$ plasmid at 30°C (27). In contrast, the *lexA71*::Tn*5*(Def) *lon* strain was not efficiently transformed by the *umuD*$^+$*C*$^+$ plasmid (Table 2). This result supports the notion that the *lon* mutation suppresses *umuD*$^+$*C*$^+$-dependent cold sensitivity by stabilizing the LexA51(Def) protein and reducing expression of the *umuDC* genes. This hypothesis is further supported by the observations that expression of a *umuC*::*lac* gene fusion is decreased in a *lexA51*(Def) *lon* strain and that the half-life of the LexA51(Def) protein appears to be lengthened in a *lon* background (C. Dykstra and G. Walker, unpublished results).

TABLE 2. Effect of mutations in heat shock genes on the efficiency of transformation of *lexA*(Def) strains by a *umuD*$^+$*C*$^+$ plasmid

Mutation in heat shock gene	Transformants obtained with pSE117(*umuD*$^+$*C*$^+$)/transformants obtained with pBR322 in:	
	lexA51(Def) (KM1190)	*lexA71*::Tn*5* (GW2730)
None	≤0.0035	≤0.004
lon-146::Tn*10*	0.25	≤0.003
rpoH165	0.80[a]	0.78
dnaK756	0.05	≤0.005
groEL100	0.63	0.78
groES30	0.50	0.86

[a] Transformation of the *rpoH165* strain with pSE117 was carried out in a GW1000 *lexA51*(Def) background since this strain also carries the amber suppressor mutation required for growth of the *rpoH165* strain at 30°C (23).

In contrast to our results with the *lexA71*::Tn*5*(Def) *lon* strain, the *lexA71*::Tn*5*(Def) *rpoH* strain was efficiently transformed with the *umuD*$^+$*C*$^+$ plasmid at 30°C. This observation indicated to us that there was a second possible mechanism of suppression of cold sensitivity which can occur in cells that totally lack the LexA protein but was difficult to interpret because the *rpoH* gene regulates the expression of several heat shock genes. Furthermore, mutations in many heat shock genes affect cellular proteolysis (38), so it is difficult to know at what level the *rpoH* gene product is involved in the suppression of *umuD*$^+$*C*$^+$-dependent cold sensitivity.

Previous experiments indicated that mutations in various heat shock genes could suppress *umuD*$^+$*C*$^+$-dependent cold sensitivity as well (C. Dykstra and G. Walker, unpublished results). These results led us to test whether mutations in other heat shock genes suppressed *umuD*$^+$*C*$^+$-dependent cold sensitivity in a *lexA*-independent manner. We again used the quantitative transformation assay to determine whether mutations in *dnaK*, *groES*, and *groEL* genes could suppress *umuD*$^+$*C*$^+$-dependent cold sensitivity in the *lexA51*(Def) strain and the *lexA71*::Tn*5*(Def) strain. The *lexA51*(Def) strain carrying a mutation in the *dnaK* gene could be transformed with the *umuD*$^+$*C*$^+$ plasmid, but the *lexA71*::Tn*5*(Def) *dnaK* strain could not be efficiently transformed with this plasmid. In contrast, we have found that strains carrying mutations in *groES* and *groEL* genes were

efficiently transformed with the *umuD*$^+$*C*$^+$ plasmid whether the strain also carried the *lexA51*(Def) mutation or the *lexA71*::Tn*5*(Def) mutation (Table 2).

Our results suggest that suppression of *umuD*$^+$*C*$^+$-dependent cold sensitivity by mutations in heat shock genes can occur by at least two different mechanisms. Since a *dnaK* mutation resembled a *lon* mutation in suppressing cold sensitivity in the *lexA51*(Def) background but not in the *lexA71*::Tn*5*(Def) background, it seems most likely that the *dnaK* mutation was acting by stabilizing the LexA51 protein and reducing expression of the *umuDC* genes. In contrast, *groES* and *groEL* mutants resemble the *rpoH* mutant in suppressing *umuD*$^+$*C*$^+$-dependent cold sensitivity, even in a background that completely lacks LexA protein, and thus appear to be exerting their effects by a second mechanism that does not depend on the nature of the *lexA*(Def) allele.

Expression of the *umuDC* genes from a *lexA*-independent promoter causes cold sensitivity. We decided to test our hypothesis that there are two possible mechanisms for suppressing *umuD*$^+$*C*$^+$-dependent cold sensitivity by examining the effect of mutations in heat shock genes when *umuDC* is overexpressed from a *lexA*-independent promoter. We therefore constructed a vector which allows expression of the *umuDC* operon from the IPTG-inducible promoter, P_{trc} (5) (see Materials and Methods). The resulting plasmid, pGW3700, allows inducible expression of the *umuDC* genes by the addition of IPTG. To reduce the amount of transcription from the P_{trc} promoter in the absence of IPTG, strains carrying the P_{trc}-*umuDC* plasmid also carried an F′ plasmid which encodes the *lacI*^{q} gene. However, even in the presence of the *lacI*^{q} repressor gene, a *umuD44* strain or a *umuC36* strain is complemented for UV mutagenesis by the P_{trc}-*umuDC* plasmid in the absence of IPTG, indicating that repression of transcription from P_{trc} is not complete (data not shown).

In order to show that the P_{trc}-*umuDC* plasmid could cause *umuD*$^+$*C*$^+$-dependent cold sensitivity, we transformed this plasmid into *lexA*$^+$ and *lexA51*(Def) backgrounds and tested whether growth of these strains at 30°C was affected by the addition of IPTG to LB agar plates. At 42°C, IPTG did not inhibit growth in either background. However, when these strains were plated at 30°C, the colonies formed after 24 h in the presence of IPTG were very small compared with those formed in the absence of IPTG (Fig. 1A and B). The effect of IPTG on the growth of strains carrying the P_{trc}-*umuDC* plasmid at 30°C appeared to be due to slow growth since approximately 25 to 40% of the number of colonies on the plate without IPTG grew in the presence of IPTG after the plates were incubated for another 12 h (Table 3).

We also tested the effects of induction of *umuDC* from the P_{trc} promoter in liquid medium. Addition of IPTG to a growing culture carrying the P_{trc}-*umuDC* plasmid at 30°C did not have a significant effect on growth in the *lexA*$^+$ strain or the *lexA51*(Def) strain. This result is surprising because the *lexA51*(Def) strain carrying a plasmid with the *umuDC* genes expressed from the natural promoter is cold sensitive in liquid culture (27). We cannot explain this result except to say that this discrepancy may be due to differences in expression of the *umuDC* genes under these conditions.

Taken together, these observations indicate that expression of *umuD*$^+$*C*$^+$ from the P_{trc} promoter causes a cold-sensitive inhibition of growth but that the magnitude of the inhibition is less severe than that observed when a multicopy *umuDC* plasmid is introduced into a *lexA*(Def) strain. We suspect that this difference is due to the relative amounts of *umuDC* gene products in the various strains, but we have not yet been able to test this directly.

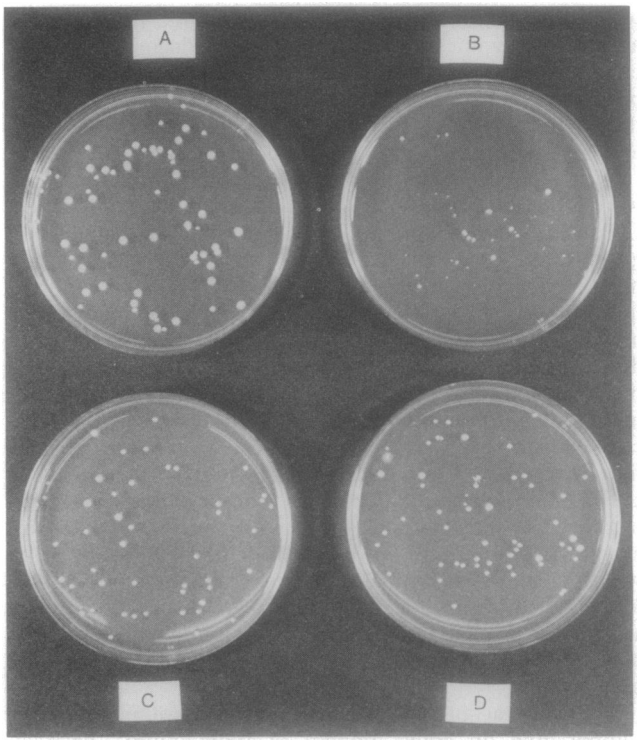

FIG. 1. Effect of IPTG on the growth of wild-type and *groEL100* strains carrying pGW3700 at 30°C. Cells were grown in LB broth containing ampicillin. The wild type was grown at 42°C, and the *groE* derivative was grown at 37°C. At an OD_{600} of 1.0, cells were diluted and plated on LB agar plates containing ampicillin with or without 1 mM IPTG. Plates were incubated at 30°C for 24 h. (A) AB1157 (pGW3700) without IPTG. (B) AB1157 (pGW3700) with IPTG. (C) AB1157 *groEL100* (pGW3700) without IPTG. (D) AB1157 *groEL100* (pGW3700) with IPTG.

The vector used to express *umuDC* from the P_{trc} promoter contains translational signals just upstream of the *umuDC* translational signals (5); this may interfere with efficient translation of the *umuDC* mRNA. Transcription of the *umuDC* genes from the P_{trc} promoter is inducible with IPTG, as indicated by primer extension experiments, in both the *lexA*$^+$ and *lexA51*(Def) backgrounds (data not shown). However, since the *lexA*$^+$ and the *lexA51*(Def) strains were similarly impaired in colony formation at 30°C in the pres-

TABLE 3. Cold sensitivity due to overexpression of *umuDC* from the P_{trc} promoter at 30°C[a]

Strain[b]	No. of colonies with IPTG/no. of colonies without IPTG
KM1190 *lexA51*(Def)	0.26
AB1157 *lexA*$^+$	0.40
AB1157 *lon-146*::Tn*10*	0.33
AB1157 *dnaK756*	0.33
AB1157 *groEL100*	0.83
AB1157 *groES30*	1.0

[a] Cells were grown in LB broth at 37°C, diluted, and plated on LB agar containing ampicillin with or without 1 mM IPTG. Plates were incubated at 30°C, and colonies were counted after 36 h.

[b] Each strain carried pGW3700(P_{trc}-*umuDC*).

ence of IPTG, we believe that other gene products under *lexA* control are not required for the cold-sensitive phenotype.

Mutations in *groES* and *groEL* suppress cold sensitivity when the *umuDC* genes are expressed from a *lexA*-independent promoter. Since the P_{trc}-*umuDC* vector can cause cold sensitivity, we tested whether mutations in heat shock genes could suppress this phenotype. Mutations in *lon* and *dnaK* were not able to suppress the P_{trc}-*umuDC*-dependent cold sensitivity (Table 3). These strains grew well in the absence of IPTG at 30°C, but in the presence of inducer the colonies were very small and reduced in number. However, strains carrying the *groES* or the *groEL* mutation as well as the P_{trc}-*umuDC* vector were plated at 30°C and appeared to grow just as well in the absence or presence of IPTG (Table 3, Fig. 1C and D).

Similarly, we found that a mutation in *rpoH* could suppress *umuDC*-dependent cold sensitivity when the *umuDC* genes were expressed from the P_{trc} promoter. We measured the effects of the P_{trc}-*umuDC* plasmid on a strain which carries the amber suppressor mutation required for growth of the *rpoH* strain at 30°C (23). This strain (GW1000) is Δ*lac*, so the P_{trc} promoter is constitutively expressed. Transformation of the P_{trc}-*umuDC* plasmid into the *rpoH*$^+$ derivative resulted in very small colonies at 30°C, and transformation of the *rpoH* derivative resulted in normal-size colonies (data not shown).

These results support our hypothesis that there are at least two mechanisms which allow suppression of *umuD*$^+$*C*$^+$-dependent cold sensitivity. Mutations in *lon* and *dnaK* genes appeared to act by having a specific effect on the ability of the LexA51 protein to control transcription of the *umuDC* genes. In contrast, mutations in *groES*, *groEL*, and *rpoH* genes exert their effects by a mechanism that is not dependent on the nature of the transcriptional control of the *umuDC* genes.

***groE* mutants are defective in UV mutagenesis.** In order to further investigate the mechanism by which *groES*, *groEL*, and *rpoH* mutations allow suppression of *umuDC*-dependent cold sensitivity, we considered the possibility that strains with these heat shock mutations might be defective in the in vivo activity of the *umuDC* genes, UV mutagenesis. We measured the reversion frequency of the *argE3* mutation as a function of UV dose in *groE*$^+$ and *groE* backgrounds at 37°C (Fig. 2). We found that mutations in *groES* and *groEL* genes caused a severe defect in the frequency of UV mutagenesis in an otherwise wild-type background. The *groE* mutations did not appear to affect UV survival of the strains in these experiments. The defect in mutagenesis observed in the *groE* strains is not limited to reversion of the *argE3* mutation. These strains are defective in reversion of the *his-4* mutation as well (data not shown).

We then ruled out the possibility that the *groE* mutations had any affect on the induction of transcription of the *umuDC* genes after UV irradiation by measuring the activity of a *umuC*::Mu d(Ap *lac*) transcriptional fusion. Induction of β-galactosidase from this fusion after UV irradiation was similar in the *groE*$^+$ and *groE* backgrounds (data not shown). Therefore, the *groE* mutations appear to exert their effects on UV mutagenesis posttranscriptionally.

Mutations in *groE* are defective in the morphogenesis of λ at all temperatures (14). However, the *groES* and *groEL* mutations used in these experiments restrict growth of the strains at high temperatures (40). We tested whether the defect in UV mutagenesis in the *groE* strains is dependent on temperature. We found that the amount of UV mutagenesis

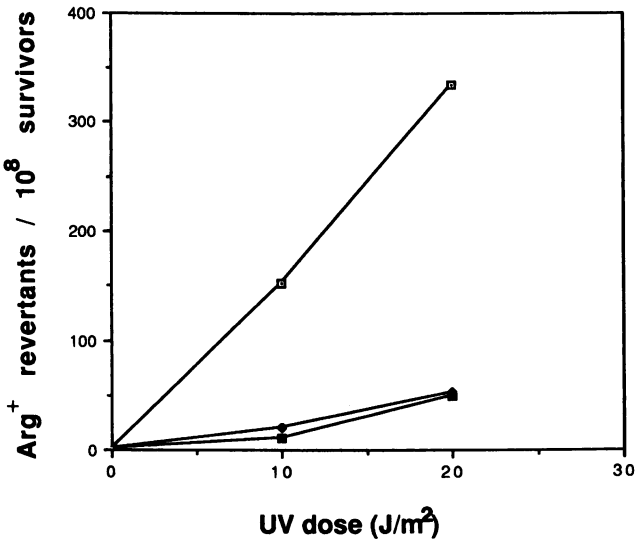

FIG. 2. UV mutagenesis in wild-type and *groE* cells. Cultures were grown at 37°C in LB broth to an OD_{600} of 0.5, centrifuged, and suspended in 0.85% saline. Cells were transferred to a glass petri dish, UV irradiated, diluted, and plated as described in Materials and Methods. Symbols: ⊡, AB1157; ◆, AB1157 *groEL100*; ■, AB1157 *groES30*.

in the *groE* strains at 30°C was very similar to the amount at 37°C. At 42°C, we saw an additional reduction in UV mutagenesis in the *groE* strains; however, these strains grow very poorly at this temperature, and the results are difficult to interpret. Therefore, like the defect in λ morphogenesis, UV mutagenesis in *groE* strains is defective even at temperatures which do not appear to affect the growth of the cells.

We then tested several alleles of *groES* and *groEL* and found that many were defective in UV mutagenesis (Table 4). We also found that all of the alleles shown in Table 4 were able to suppress *umuD*$^+$*C*$^+$-dependent cold sensitivity. This suggests that the mechanism by which mutations in *groE* suppress *umuD*$^+$*C*$^+$-dependent cold sensitivity may be related to the mechanism by which *groE* mutations cause a defect in UV mutagenesis. It is also interesting that all of the *groE* mutations originally selected for being defective in λ morphogenesis are defective for *umuDC*-related phenotypes.

TABLE 4. UV mutagenesis in various *groE* strains[a]

Strain	UV mutagenesis (% of mutagenesis in the wild type)
groE$^+$	100
groEL100	15
groEL35	12
groEL46	24
groEL59	25
groES30	13
groES7	22
groES24	23
groES97	32

[a] Various mutations in the *groE* operon were obtained, and the *purA*::Tn*10* marker was transduced into these strains by P1 phage. In order to measure the effects of these mutations on UV mutagenesis, the *groE* alleles were transduced into AB1157 (by linkage to the *purA*::Tn*10* marker). *arg*$^+$ reversion after UV mutagenesis at 20 J/m^2 was measured in various *groE* strains at 37°C. The results are the averages of three experiments.

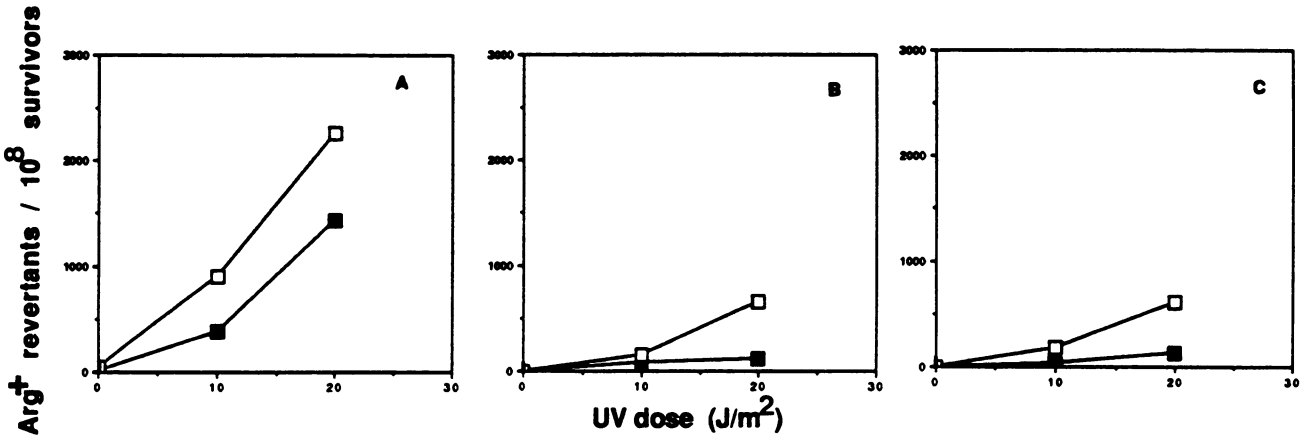

FIG. 3. UV mutagenesis in the presence of pGW3700. Cultures were grown at 37°C in LB broth plus ampicillin to an OD_{600} of 0.5 to 0.7. Cells were centrifuged, suspended in 0.85% saline, and transferred to a glass petri dish. Cells were UV irradiated, diluted in 0.85% saline, and plated on minimal agar plates containing ampicillin. Plates were incubated at 37°C. UV survival and mutagenesis were determined as described in Materials and Methods with or without IPTG on minimal agar plates. (A) AB1157(pGW3700). (B) AB1157 *groEL100*(pGW3700). (C) AB1157 *groES30*(pGW3700). Symbols: ■, No IPTG; □, IPTG.

The results presented above suggest that *groE* mutations have a specific effect on the products of the *umuDC* genes which reduces the amount of UV mutagenesis. To verify this hypothesis, we measured mutagenesis induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or by methylsulfonic acid ethyl ester, whose mutagenesis is largely independent of the *umuDC* genes (36). As suspected, mutagenesis by either of these agents was not affected by mutations at the *groE* locus (data not shown).

Multicopy *umuDC* suppresses nonmutability of *groE* mutants. Our results suggest that mutations in *groE* affect the activity of *umuDC* posttranscriptionally. If the *groE* gene products interact directly with the *umuDC* gene products, *groE* mutations might reduce the activity of UmuD and/or UmuC. Alternatively, *groE* mutants are known to have effects on cellular proteolysis (38), so *groE* mutations might alter the stability of the *umuDC* gene products. If either of these models were correct, then increasing expression of the *umuDC* genes in a *groE* strain might suppress the nonmutability of these strains.

We therefore measured UV mutagenesis in $groE^+$ and *groE* strains, in which expression of the *umuDC* genes could be induced from the P_{trc} promoter. The addition of IPTG increased UV mutagenesis in the $groE^+$ strain, indicating that *umuDC* gene expression is limiting (Fig. 3A). In the *groE* derivatives, IPTG also increased the frequency of UV mutagenesis (Fig. 3B and C). To some extent, the additional copies of the *umuDC* genes as well as increased transcription of these genes suppressed the defect in UV mutagenesis caused by mutations in the *groE* genes.

Marsh and Walker have shown that cold sensitivity due to *umuDC* overexpression required both *umuD* and *umuC* genes or only the *umuC* gene, but overexpression of *umuD* only did not result in cold-sensitive growth (27). We tested whether increasing expression of either *umuD* or *umuC* genes was sufficient to restore UV mutagenesis in the *groE* strains. We measured UV mutagenesis in $groE^+$ and *groE* strains carrying either the plasmid which encodes only the *umuD* gene (in the presence of IPTG) or the plasmid which encodes only the *umuC* gene (Table 5). Our results indicate that an increase in *umuC* gene expression is required to partially alleviate the defect in UV mutagenesis of the *groE* strains. The interpretation of these results is complicated by the fact that the *umuD* plasmid caused an increase in UV mutagenesis in the wild-type strain and in the *groE* derivatives. Therefore, we cannot rule out the possibility that additional copies of both the *umuD* and the *umuC* genes are required to correct this defect.

UmuC protein stability is altered in *groE* mutants. The results described above support the notion that at least part of the effect of *groE* mutations on mutagenesis may be to alter the stability of the UmuC protein. We therefore investigated this possibility by measuring the half-life of the UmuC protein in $groE^+$ and *groE* backgrounds. We specifically pulse-labeled the UmuC protein in intact cells (see Materials and Methods) with [^{35}S]methionine. The radioactivity was chased with excess unlabeled methionine, and samples were taken at various times to determine the half-life. We have estimated the half-life of UmuC protein to be approximately 17 min under these conditions in $groE^+$ cells (Fig. 4). In the *groEL* derivative, the half-life of UmuC protein is significantly shorter, approximately 6 min. The half-life of UmuC protein in the *groES* strain was approximately 6 min as well (data not shown).

Our results suggest that *groE* mutants are defective in UV mutagenesis because of a limiting amount of UmuC protein. Since we were unable to completely suppress the nonmutability of the *groE* strains with a plasmid containing the *umuDC* genes (Fig. 3), we cannot rule out the possibility that the *groE* genes play an additional role in UV mutagenesis. However, in these experiments we cannot be sure whether the UmuC protein is expressed at the same levels under different conditions.

Temperature has been shown to affect the level of proteolysis (17); however, we have found that the half-life of the UmuC protein at 37°C is very similar to its half-life at 30°C. We have measured the stability of the UmuD protein and found that this protein is very stable ($t_{1/2}$ >60 min) in the $groE^+$ and the *groE* derivatives (data not shown).

The *rpoH* mutation does not affect UV mutagenesis. The *rpoH* mutation suppressed cold sensitivity when the *umuDC* genes were overexpressed in the *lexA71*::Tn*5*(Def) host or when the *umuDC* genes were expressed from the P_{trc} promoter. However, we have found that the *rpoH* mutation has no detectable effect on UV mutagenesis (data not shown). The mechanism by which the *rpoH* mutation allows suppres-

TABLE 5. Effects of *umuDC* plasmids on UV mutagenesis in *groE* strains

Strain	UV mutagenesis[a] (% of UV mutagenesis in the wild type) with:		
	No plasmid	*umuD*$^+$ (pGW3701)	*umuC*$^+$ (pLD1291)
AB1157	100[b]	100[c]	100[d]
AB1157 *groEL100*	14	23	66
AB1157 *groES30*	10	4	57

[a] UV mutagenesis frequency was determined by measuring *arg*$^+$ reversion after a UV dose of 10 J/m^2 at 37°C. IPTG was included in plates in which mutagenesis with pGW3701 was performed. Results are the averages of at least two experiments.

[b] UV mutagenesis frequency of the wild type was 220 *arg*$^+$ strains per 10^8 survivors.

[c] UV mutagenesis frequency of the wild type was 410 *arg*$^+$ strains per 10^8 survivors.

[d] UV mutagenesis frequency of the wild type was 210 *arg*$^+$ strains per 10^8 survivors.

sion of cold sensitivity due to *umuDC* overexpression may be different from that of a *groE* mutation. Alternatively, *rpoH* may affect the *umuDC* gene products indirectly by affecting the levels of the GroE proteins.

DISCUSSION

In the process of studying the *umuD*$^+$*C*$^+$-dependent cold sensitivity in *E. coli*, we have found an unexpected contribution of the GroES and GroEL proteins in UV mutagenesis. This insight grew out of our observations that *umuD*$^+$*C*$^+$-dependent cold sensitivity can be suppressed by mutations in various heat shock genes. When we further investigated this suppression, we found that there are at least two mechanisms by which *umuD*$^+$*C*$^+$-dependent cold sensitivity is suppressed by mutations in heat shock genes. The observations that mutations in the *lon* gene or the *dnaK* gene suppressed cold sensitivity in a *lexA51*(Def) host but not in a *lexA71*::Tn*5*(Def) host suggest that these mutations act to suppress cold sensitivity by increasing the stability of the LexA51(Def) repressor, thereby reducing transcription of the *umuDC* genes. In contrast, mutations in *groES*, *groEL*, and *rpoH* genes suppressed cold sensitivity by a mechanism(s) that is independent of the nature of the *lexA* allele.

The finding that *groES* and *groEL* mutations efficiently suppress *umuD*$^+$*C*$^+$-dependent cold sensitivity led us to examine whether these mutations also affected the major biological process associated with the *umuDC* genes, i.e., UV mutagenesis. We found that strains carrying *groES* or *groEL* mutations were severely deficient in their capacities to be mutated by UV irradiation. Interestingly, this deficiency can be suppressed by multiple copies of the *umuDC* genes supplied on a high-copy-number plasmid. Mutations in *groE* did not affect expression of a *umuC*::Mu d(Ap *lac*) transcriptional fusion, so these mutations must exert their effects on mutagenesis posttranscriptionally.

One possible mechanism by which mutations in the *groES* and *groEL* genes cause a defect in *umuDC* activity without affecting transcription of these genes might involve the stability of the *umuDC* gene products. The GroES and GroEL proteins are not known to possess proteolytic activity themselves; however, mutations in these genes have been shown to alter cellular proteolysis (38). Indeed, we have been able to show that mutations in the *groES* and *groEL* genes caused the UmuC protein to become more unstable while the stability of the UmuD protein appeared to be unaltered. These results suggest that the wild-type GroE proteins are able to physically protect UmuC from proteolytic digestion. Alternatively, the *groES* and *groEL* mutations may affect UmuC protein stability indirectly by regulating the activity of a protease.

Since the GroEL protein and the Hsp60 proteins of eucaryotic systems have been implicated in assembly of macromolecular complexes, a reasonable model for the role of GroES and GroEL in UV mutagenesis would be to allow UmuC protein to correctly associate in a complex with other proteins involved in UV mutagenesis. In fact, Bridges and Woodgate have proposed that the *umuDC* gene products interact with the DNA replication machinery when DNA synthesis is stalled because of lesions caused by UV irradiation or chemicals (4). We have also observed that UmuD and UmuC share amino acid sequences with three T4 phage gene products, gp45, gp44, and gp62. These proteins are DNA accessory proteins which act together to make T4 DNA polymerase more processive (2). By analogy, we suggest that UmuD and UmuC act in a complex with other proteins, perhaps stimulated by the GroE proteins, to carry out mutagenesis.

The mechanism by which overexpression of the *umuDC* genes results in cold-sensitive growth remains undetermined. At present we have not been able to determine the relationship between temperature and *umuDC* activity. However, UV mutagenesis is not affected by temperature, and we have found no change in the stability of the UmuC protein at 30 and 37°C. We cannot explain this apparent paradox except to suggest that the conditions which contribute to the cold-sensitive phenotype because of overexpression of the *umuDC* genes may be separate from those involved in UV mutagenesis.

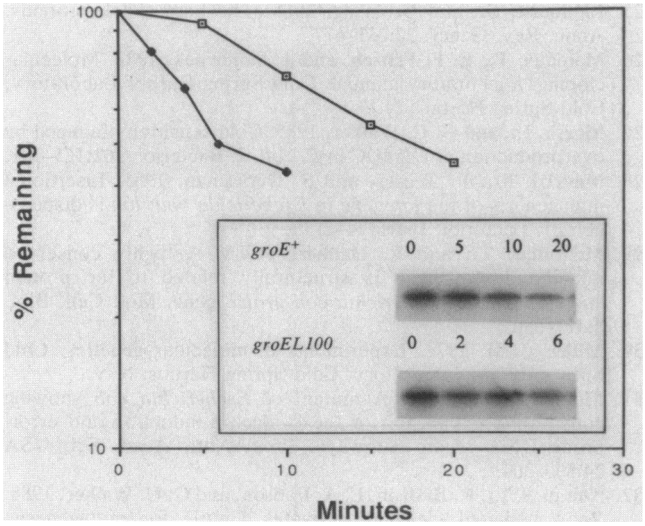

FIG. 4. Half-life of the UmuC protein. Cells were grown at 37°C in M9 medium containing thiamine, ampicillin, and kanamycin. At an OD_{600} of 0.5, IPTG was added. Thirty minutes later, rifampin was added and the cultures continued to incubate for 20 min at 37°C. The UmuC protein was labeled as described in Materials and Methods. A semilog-arithmic plot of the decay of the UmuC protein after pulse-chase is shown. Symbols: ⊡, W3110(pACT7)(pGW2030); ■, W3110 *groEL100*(pACT7)(pGW2030). The inset shows an example of an autoradiagram of products of the pulse-chase of the UmuC protein in *groE*$^+$ cells (top) and in *groEL100* cells (bottom). Time is given as minutes after addition of unlabeled methionine. This is the average of three experiments, and the error did not exceed 50%.

We also found that an *rpoH* mutation can suppress cold sensitivity independently of the LexA repressor, but the *rpoH* mutant did not show a defect in UV mutagenesis. The *rpoH* mutation may suppress cold sensitivity by yet another mechanism. Alternatively, since the product of the *rpoH* gene, σ^{32}, is required for transcription of the *groE* operon, the levels of GroES and GroEL proteins in the *rpoH* mutant may be limiting. Low levels of the wild-type GroE proteins in the *rpoH* strain might lead to suppression of cold sensitivity, but the levels of these proteins in this mutant may be sufficient for UV mutagenesis.

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