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**Analyses of the Roles of the UmuDC Proteins of *E. coli* in SOS Mutagenesis and Cell Cycle Regulation**

by

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**Bachelor of Arts in Biochemistry  
Barnard College, 1990**

Submitted to the Department of Biology in Partial Fulfillment of the Requirements for the Degree of

**DOCTOR OF PHILOSOPHY IN BIOLOGY  
AT THE  
MASSACHUSETTS INSTITUTE OF TECHNOLOGY**

**FEBRUARY 1997**

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MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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## ABSTRACT

Regulated mechanisms that inhibit DNA synthesis and cell cycle progression in response to DNA damage have been shown to be essential for DNA damage tolerance in eukaryotes. Analogous mechanisms have not been as well defined in prokaryotes. Evidence presented in this thesis suggests that the UmuD and UmuC proteins of *Escherichia coli* participate in a mechanism to inhibit growth and DNA synthesis upon exposure to DNA damaging treatments, thereby increasing cell survival.

This hypothesis grew out of the study of *umuDC*-mediated growth inhibition at 30°C (cold sensitivity). The *umuDC* operon is a member of the SOS regulon, a group of twenty or more genes whose expression is coordinately induced by DNA damaging treatments. The UmuD' and UmuC proteins have been well characterized for their activity in SOS mutagenesis, the mutagenesis resulting from exposure to DNA damaging agents such as UV irradiation. Constitutive expression of the *umuDC* operon from a multicopy plasmid confers cold sensitivity for growth which is associated with an inhibition of DNA synthesis. The genetic requirements for observing *umuDC*-mediated cold sensitivity are distinct from those for SOS mutagenesis supporting that these are distinct functions of the *umuDC* gene products. High levels of intact UmuD, the form inactive in SOS mutagenesis, confer growth inhibition at 30°C in combination with UmuC.

Further analyses revealed that the *umuDC* gene products, expressed at physiologically relevant levels, inhibit the transition from stationary phase to exponential growth of UV irradiated stationary phase cells and increase UV resistance, possibly by allowing time for DNA repair processes to be completed. This is most likely the result of the inhibition by the *umuDC* gene products of a Fis-dependent activity. Suppression of the UV sensitivity of a  $\Delta$ *umuDC* strain by a *fis* mutation demonstrates that the inhibition of a *fis*-dependent activity is central to the UV resistance conferred by the *umuDC* gene products.

Intact UmuD and UmuC increase cell survival after UV irradiation. This previously uncharacterized activity of UmuD and UmuC is correlated with the inhibition of DNA synthesis after UV irradiation. The *umuC125* mutation, which specifically interferes with the ability of UmuC to confer growth inhibition at 30°C without interfering with SOS mutagenesis, also abolishes the ability of UmuC to regulate DNA synthesis after UV irradiation. This suggests that this novel activity of intact UmuD and UmuC is functionally related to *umuDC*-mediated growth inhibition at 30°C.

Thesis supervisor: Graham C. Walker  
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## Acknowledgments

The majority of the work presented in this thesis is the result of a close and very productive collaboration with Tim Opperman. I would like to thank Tim for his collegiality which has made it a pleasure to work alongside him essentially every day for two years. I am very cognizant of the fact that his patience and good humor were essential to our working in as close partnership as we have.

I am grateful to my advisor Graham Walker for his guidance through the course of my graduate studies. I am especially grateful for his patience and support which allowed me to explore and develop a project that was not at all what I had originally planned on studying. I would also like to thank Alan Grossman, Tania Baker, Tom RajBhandary and Roberto Kolter for serving on my thesis committee and for their helpful advice.

I would like to thank all the members of the Walker laboratory, past and present, for making the lab a very pleasant and enjoyable place to work. Special thanks are due to Juan González and Greg York for their friendship and support. I will always remember fondly the many stimulating scientific and nonscientific discussions that we have had. I would also like to thank Andrea Marra and Lynne Reuber for their essential roles in making my early years in the Walker lab as agreeable as they were. Last, but definitely not least, I am very grateful to Marianne White for her invaluable help and friendship over the years.

I have truly enjoyed my years in graduate school thanks in large part to the close friends that I have made among my fellow graduate students. Special mention is due to Tracy Smith, Eric Schmidt, Brian and Brenda Kennedy, Juli Klemm, Mike Brodsky, John Crispino and Tory Herman. Their fellowship has made my experience in graduate school a very positive one for me. Finally, I would like to thank my parents and my sister for their support.



## **Chapter 1**

### **Introduction**

Part of this introduction was published in *Current Opinion in Genetics and Development*, **3**: 719-725.

DNA damage caused by agents such as ultraviolet (UV) light or various chemicals can result in interference with DNA replication. DNA damage repair and tolerance mechanisms are thus critical for the survival of any organism. The mechanisms which cope with DNA damage without the introduction of mutations include excision repair, postreplication repair (also known as daughter strand gap repair), and photoreactivation of pyrimidine dimers (1, 32). Replicative bypass of DNA lesions represents an additional DNA damage tolerance mechanism that, in contrast to the processes listed above, can lead to the introduction of mutations in *Escherichia coli*, a process known as SOS mutagenesis (32). Whereas DNA damage repair and tolerance mechanisms such as these have been studied in great detail in prokaryotes, the analysis of the regulation of the inhibition of DNA synthesis upon exposure to DNA damaging treatments and its subsequent recovery has received less attention (1). Such regulation has been postulated to play an important role in mediating resistance to agents such as UV light by temporarily repressing DNA replication and thereby allowing time for repair processes to take place (23).

A well characterized link between DNA damage and the bacterial cell cycle is provided by the regulation of the *sulA* and *sfic* genes of *E. coli* by *recA* (18, 33). *sulA* and *sfic*, which are not essential for DNA damage tolerance, are inhibitors of septation whose expression is induced by *recA* in response to DNA damage (18, 33). In addition, a *recA*-dependent mechanism has been implicated in the inhibition of early sporulation gene expression in response to the inhibition of DNA synthesis in *Bacillus subtilis* (38). In contrast to the situation in prokaryotes, the inhibition of DNA synthesis and cell cycle progression in response to DNA damage has been the subject of more detailed analyses in eukaryotes (32). For example, the *RAD9* and *POL2* genes in *Saccharomyces cerevisiae* (32, 59) and p53 in mammalian cells (26), all of which play important roles in DNA damage tolerance, have been shown to constitute checkpoints that arrest the cell cycle following a DNA damaging treatment. The data presented in this thesis has led to the hypothesis that the *umuDC* gene products in *E. coli* participate in an analogous mechanism in prokaryotes that inhibits growth and DNA synthesis after UV irradiation and thereby contributes to increased survival.

## **I. The *umuDC* gene products of *Escherichia coli***

The *umuDC* gene products have been well-characterized for their role in SOS mutagenesis (32). There is fairly widespread agreement that most mutagenesis induced by DNA damaging agents such as UV light arises as a consequence of translesion synthesis in which a polymerase inserts nucleotides across from a misinformational or noninformational lesion. Although translesion synthesis apparently occurs in mammalian cells (79), the mutagenic consequences of translesion synthesis have been investigated most extensively in *E. coli*. In the case of this organism, the processing of damaged DNA that gives rise to mutations, known as SOS mutagenesis, requires the products of the *umuD*, *umuC*, and *recA* genes and is intimately connected with the induction of the SOS response [for reviews, see (32, 87, 93)].

### **Model for SOS regulation**

The *umuDC* operon is repressed by LexA and is regulated as part of the *recA*<sup>+</sup>*lexA*<sup>+</sup>-dependent SOS regulon (32) (Fig. 1). The RecA protein serves as an internal sensor of DNA damage in *E. coli*. In response to a DNA damaging treatment, the RecA protein becomes reversibly activated to RecA\* by binding, in the presence of nucleoside triphosphate, to single-stranded DNA gaps generated by the cell's attempts to replicate a damaged template (70). The RecA\* nucleoprotein filament mediates cleavage of the LexA repressor by facilitating an otherwise latent ability of this protein to autodigest (51). This inactivation of LexA leads to the induction of the more than twenty genes and operons that make up the SOS regulon.

### **Genetic requirements for SOS mutagenesis**

#### ***umuDC***

Screening for mutations that abolished UV and 4-nitroquinoline-1-oxide mutagenesis in *E. coli* led to the identification of the *umuD* and *umuC* genes (40, 80), which are organized in an operon (32). A  $\Delta$ *umuDC* strain is viable and only moderately sensitive to the lethal effects of

UV light, as are strains carrying previously described *umuDC* alleles (90). The *umuD* and *umuC* genes are the only LexA-regulated genes that must be derepressed for SOS mutagenesis to be observed (78). UmuD protein levels rise from about 180 molecules per uninduced cell to about 2400 molecules in a derepressed cell, while UmuC protein levels rise from an undetectable amount to about 200 molecules in a fully derepressed cell (91).

The predicted amino acid sequence of the *umuD* gene product exhibits similarity to the carboxyl terminal domain of LexA and  $\lambda$  repressor (65) and, like those proteins, UmuD undergoes a specific RecA<sup>\*</sup>-mediated cleavage at the Cys 24 - Gly 25 bond (15, 32, 74). Genetic studies demonstrated that this cleavage activates UmuD for its role in UV and chemical mutagenesis and that the 12 kDa carboxyl-terminal fragment of UmuD, called UmuD', is necessary and sufficient for this role (60). Three of the original chromosomal *umuD* mutations that abolished UV mutability (40, 80) resulted in non-cleavable UmuD proteins (42).

UmuD cleavage is significantly less efficient than LexA cleavage both *in vitro* and *in vivo* (15, 74). As this suggests, maximal SOS mutagenesis activity is not observed immediately after UV irradiation. Defais *et al.* (19) showed that maximal SOS mutagenic activity is observed approximately thirty minutes after UV irradiation and decays with a half-life of approximately thirty minutes.

*E. coli* thus uses both transcriptional derepression and posttranslational activation to control the levels and activity of UmuD and UmuC. The analyses of a set of noncleavable UmuD mutants (8) suggests that intact UmuD can function as a dominant inhibitor of UmuD'-dependent mutagenesis and that this occurs through the preferential formation of UmuD-UmuD' heterodimers. This has regulatory implications for both the induction and shutdown of the capability for SOS mutagenesis (8, 32) since it suggests that higher amounts of UmuD must be cleaved for the UmuD' homodimer to form at sufficient levels to allow SOS mutagenesis. As UmuD cleavage requires RecA to be in its activated state, this assures that SOS mutagenesis only occurs when DNA damage is sensed by the cell. Preferential UmuD-UmuD' heterodimer formation also provides an explanation for the observation that cells shut off their SOS

mutagenesis capability prior to the other SOS responses in the process of recovering from a DNA-damaging treatment.

The UmuD and UmuC proteins are rapidly degraded by Lon, an ATP-dependent serine protease (29). In contrast, UmuD' is a poor substrate for Lon and is instead degraded by the ClpXP protease when in a heterodimeric complex with UmuD, suggesting that preferential heterodimer formation between UmuD and UmuD' targets UmuD' for degradation (29). This provides another mechanism by which the cell can keep the levels of the mutagenically active UmuD'C complex to a minimum.

### ***umuDC* homologs**

*umuDC*-like sequences have been found in a variety of enteric bacteria (61, 72), as well as on several naturally occurring broad host-range plasmids (32, 93). Another related family appears to be constituted of *umuC*-like genes with no identified *umuD*-like partner (46, 47, 57, 62). *dinP* (also called *dinB*) in *E. coli* has recently been identified to be a member of this class of *umuC*-like genes (62). It is similarly regulated as a member of the SOS regulon. The function of *dinP* has not been clearly elucidated although it has been suggested to play a role in untargeted UV mutagenesis in bacteriophage  $\lambda$  (13). Sequence analysis of *REV1*, one of a set of *Saccharomyces cerevisiae* genes in which mutations confer reduced or non mutability upon UV irradiation, revealed an open reading frame with limited similarity to UmuC (47), suggesting that REV1 may represent a eukaryotic homolog of a bacterial protein required for mutagenesis. Finally, the identification of UmuC-like sequences in the archeon *Sulfolobus solfataricus*, *Caenorhabditis elegans* and among human EST products (46) suggests that this family of genes is widely distributed in nature with a, perhaps, as yet uncharacterized function that ensures their evolutionary maintenance.

The *mucAB* operon is a *umuDC* homolog encoded by pKM101, a derivative of the naturally occurring plasmid R46, which is used to enhance the susceptibility of the Ames *Salmonella typhimurium* tester strains to mutagenesis by a wide variety of agents (87). MucA

and UmuD are 41% identical at the amino acid level while MucB and UmuC are 55% identical (65). However, MucA cannot restore UV mutability to *umuD*<sup>-</sup>*umuC*<sup>+</sup> cells nor can MucB restore mutability to *umuD*<sup>+</sup>*umuC*<sup>-</sup> bacteria (65). Of the *umuDC*-like operons analyzed at the molecular level, *mucAB* is the most efficient at promoting mutagenesis (71), possibly due to an enhanced activation of MucA resulting from a faster rate of RecA-mediated cleavage (36). Conversely, inefficient processing of UmuD-like proteins in a number of other enteric bacteria (72, 93) may account for their weak UV-induced mutability despite the presence of *umuDC*-like sequences. The presence of *umuDC*-like sequences in several bacterial species that are nonmutable by UV irradiation (72) has led to speculation that the *umuD* and *umuC* gene products may have additional functions beyond their well-characterized role in SOS mutagenesis (93).

### *recA*

Two requirements for RecA\* in SOS mutagenesis have been clearly elucidated as discussed above, namely mediating the autodigestion of LexA repressor and UmuD. A third role for RecA was suggested by the failure of UmuD' to suppress the nonmutability of a  $\Delta recA$  strain lacking functional LexA repressor (60). Thus, RecA is required for SOS mutagenesis even in cells constitutively expressing *umuD'* and *umuC*. This finding was supported by the subsequent analysis of the properties of a *recA1730* mutant (3), which is specifically defective in this third role. Several hypotheses have been proposed for the activity of RecA in SOS mutagenesis. A suggestion that RecA functioned in SOS mutagenesis by inhibiting the  $\epsilon$  editing subunit of DNA polymerase III was contradicted by the observation that the deletion of the gene encoding the  $\epsilon$  subunit did not relieve the requirement for the third role of RecA in bypass of UV-induced lesions of bacteriophage  $\phi X174$  (76). Other possibilities for the third role of RecA are discussed below. The requirement for the RecA\* nucleoprotein filament for three distinct roles in SOS mutagenesis assures that mutagenic translesion synthesis occurs only when DNA has been damaged sufficiently to maintain RecA in its activated state.

### **DNA polymerase III**

DNA polymerase III holoenzyme, or some derivative, appears to be the polymerase required for SOS repair. The UV mutability of a *ΔpolA* mutant indicates that DNA polymerase I is not required for UV-induced mutagenesis (7). Similarly, although purified DNA polymerase II from SOS-induced cells demonstrates a limited ability to bypass abasic sites *in vitro* (9), its function is not required for lesion bypass or mutagenesis of  $\phi$ X174 *in vivo* (45). In contrast, Hagensee, *et al.* (35) found that there was no increase in mutagenesis upon exposure to UV light at the restrictive temperature for a strain carrying a temperature-sensitive mutation in *dnaE*, which encodes the  $\alpha$  subunit of DNA polymerase III, and that this effect could be alleviated by the introduction of a cloned *dnaE*<sup>+</sup> gene. However, the interpretation of these experiments is complicated by the requirement of the *pcbA1* mutation, which is thought to be a mutation in DNA gyrase, for replication in *dnaE*(Ts) mutants at the restrictive temperature (32, 35). In support of the participation of DNA polymerase III in SOS mutagenesis, *in vitro* experiments with a template with a synthetic abasic site suggest that UmuD' and UmuC, in the presence of RecA, modify the translesion synthesis capacity of DNA polymerase III (68). Thus, SOS repair seems to require the presence of some form of DNA polymerase III, although the additional participation of DNA polymerase I and DNA polymerase II cannot be ruled out at the present time.

### ***groEL* and *groES***

The efficiency of *umuDC*-dependent SOS mutagenesis in *E. coli* and in phage S13 is significantly reduced by mutations affecting the *groES* and *groEL* heat shock genes (21, 52), which encode the Hsp60 molecular chaperone machine. However, *mucAB*-dependent SOS mutagenesis is independent of *groEL* and *groES* (see Appendix A). Since *groES* and *groEL* mutations decrease the half-life of the UmuC protein and since UmuC co-immunoprecipitates with GroEL, it seems possible that these molecular chaperones play a role in mediating the stability and/or the folding of UmuC into a form that interacts with UmuD and UmuD' (22).

Since *mucAB*-dependent SOS mutagenesis is *groE*-independent, MucB, the homolog of UmuC, does not appear to require the GroES and GroEL molecular chaperones for folding or stability.

The Hsp70 complex, composed of DnaK, DnaJ and GrpE in *E. coli*, has also been implicated to promote the proper folding of UmuC *in vitro* (66). Sequential incubation of purified inactive UmuC with the DnaK-DnaJ-GrpE complex and then the GroEL-GroES complex was required to restore lesion bypass activity to UmuC. However, the *in vivo* significance for SOS mutagenesis of this activity of DnaK is unclear since only certain alleles of *dnaK* show any reduction in SOS mutagenesis (66). Further characterization will be required to clarify whether the Hsp70 complex is required in parallel with the Hsp60 complex for UmuC folding and stability *in vivo*.

### **Targeting of UV and chemical mutagenesis**

The findings that the majority of mutations that arise after treatment of *E. coli* with UV light or chemical mutagens are targeted (55) and that the nature of the lesion influences the nucleotide inserted (32) are consistent with a mechanism involving translesion synthesis being responsible for these mutations. The mutagenic potential of particular types of DNA damage has been assessed by the use of vectors that carry a defined, uniquely-located lesion. For example, a single abasic site is a severe block to replication which is bypassed more frequently in an SOS-induced cell, with a preference for dAMP insertion opposite the lesion but with other nucleotides being inserted as well (48). Similar analyses using vectors carrying a single *cis-syn* cyclobutane thymine dimer, *trans-syn* cyclobutane thymine dimer, or thymine-thymine pyrimidine-pyrimidone (6-4) photoproduct have led to the conclusions that: i) translesion synthesis is more frequent in SOS-induced cells, ii) there is an increased error frequency for translesion synthesis occurring under SOS-induced conditions, and iii) each of these three UV-induced lesions is misinformational rather than noninformational (4, 5, 49).

### **Model for SOS mutagenesis**



A model for the mechanism of UV mutagenesis has been proposed which is based on experiments using ultraviolet light and delayed photoreversal in bacteria defective for SOS mutagenesis. The Bridges and Woodgate model postulates that mutations arise from a two-step mechanism of misincorporation opposite a damaged base followed by chain elongation beyond the misincorporation (10, 12). This model provides a framework to interpret a set of experiments in which partial UV mutability was restored to nonmutable strains of *E. coli*, such as *umuD* or *umuC* mutants, if the bacteria were incubated after UV irradiation in the dark and subsequently exposed to photoreactivating light. Cyclobutane pyrimidine dimers, a major form of DNA damage induced by exposure to UV light, can be repaired by monomerization in a light-dependent reaction by the enzyme DNA photolyase in *E. coli* (32), a process known as photoreactivation. By using UV irradiation plus delayed photoreactivation, the frequency of UV mutagenesis was restored to approximately 20% of the wild-type level (10). The Bridges and Woodgate model suggests that the first step of misincorporation of a base opposite the lesion is independent of *recA*, *umuD*, and *umuC*, but further elongation is blocked by the lesion. Elongation can be restored in their experiments by direct photoreversal of the damage. Alternatively, RecA, UmuC and UmuD' are required for the bypass step to allow translesion synthesis (6, 10, 12) (Fig. 2).

Consistent with the Bridges and Woodgate model, analyses of the replication of a vector carrying a single N-2-acetylaminofluorene (AAF) adduct suggest that induction of the SOS response, and specifically the induction of the *umuDC* operon, results in an increased frequency of translesion synthesis events (43). The introduction of a sequence heterology at the site of the AAF adduct allowed the authors to distinguish between translesion synthesis and replication which did not proceed through the lesion. The induction of the SOS response increased the frequency of translesion synthesis from less than 1% to approximately 13%. This increase in translesion synthesis frequency was not observed in  $\Delta umuDC$  strains, regardless of whether the SOS regulon was induced. Although the increased frequency of translesion synthesis was accompanied by an increased frequency of AAF-induced frameshift mutations, no change in the

frameshift error rate per translesion synthesis event was observed upon SOS induction. Thus, the authors conclude that SOS mutagenesis results solely from the increased frequency of translesion synthesis and not from any modification of the intrinsic error rate of the polymerase (43) (Fig. 2).

### **Biochemical functions of UmuD', UmuC, and RecA in SOS mutagenesis**

UmuD and UmuD' exist in solution as homodimers (50, 92) and the UmuD-UmuD' heterodimer forms preferentially to either homodimer (8). The regulatory implications of preferential heterodimer formation for the induction and shutoff of SOS mutagenesis capacity have been discussed above. The analyses of a set of biologically active monocysteine derivatives of UmuD indicate that residues 34, 37, 38 and 44 are relatively close to the UmuD homodimer interface (34, 50). The crystal structure of the UmuD' homodimer has recently been solved to 2.5 Angstrom resolution (64). In this structure, residues 52, 54, 87, 94 and 128 are central to forming the UmuD' dimer interface whereas residues 34, 37 and 38 are not. These results suggest that the UmuD homodimer and the UmuD' homodimer adopt significantly different quaternary structures which may have important implications for the biological activities of UmuD and UmuD'. Woodgate *et al.* (92) demonstrated that UmuC and UmuD/D' form a complex, with a likely stoichiometry of one UmuC with a homo- or heterodimer of UmuD/D'. In a *Saccharomyces cerevisiae* two hybrid system, only the UmuC/UmuD' interaction was detectable (39), suggesting that UmuC has a higher affinity for UmuD' than UmuD.

The model for SOS mutagenesis presented above predicts a direct interaction between UmuD', UmuC, RecA and DNA polymerase III. An initial indication that the *umuDC* gene products might interact directly with the replication apparatus came from the observation that constitutive expression of the *umuDC* operon from a multicopy plasmid in *E. coli* led to cold sensitivity for growth associated with a rapid decrease in DNA synthesis after a shift to 30°C (54). Additional evidence has come from the analysis of UV mutagenesis when specific subunits of DNA polymerase III holoenzyme are overproduced. Overproduction of  $\epsilon$ , the 3' → 5' proofreading exonuclease subunit, inhibits UV mutagenesis (16, 27, 28). This inhibition can be

partially overcome by the concomitant overexpression of the *umuDC* operon (28). The  $\beta$  subunit of DNA polymerase III, a head-to-tail dimer of which forms a ring-shaped structure that encircles duplex DNA and can slide along it (44), tethers DNA polymerase III holoenzyme to the DNA to make it processive. *In vitro*, a high concentration of the  $\beta$  subunit decreases replicative bypass of DNA lesions by DNA polymerase III (73). *In vivo*, over-production of the  $\beta$  subunit causes a drastic reduction of UV mutagenesis, but this inhibition can be partially alleviated by increased production of the MucAB proteins (84). Taken together, these observations raise the possibility that the UmuDC (or MucAB) proteins modify DNA polymerase III and alter its ability to operate on damaged templates (Fig. 2).

Attempts have been made to reconstitute the translesion synthesis reaction *in vitro* in order to analyze the biochemical mechanism of replicative bypass mediated by UmuD', UmuC and RecA. To date, translesion synthesis capacity requiring the UmuC protein has only been shown for UmuC purified after denaturing with 8M urea (92). Recently, a protocol for purifying soluble UmuD'C complex has been reported (14) although the activity of this complex in translesion synthesis has not yet been demonstrated. Using reconstituted UmuC, as well as purified DNA polymerase III holoenzyme, UmuD', and RecA, Rajagoplan *et al.* (68) have demonstrated limited replicative bypass of an abasic site *in vitro*. The total level of translesion synthesis increased from approximately 0.5% in the absence of UmuD', UmuC and RecA, to 5% in the presence of these proteins. The ladder-like pattern of bands demonstrating replication through the abasic site in the presence of UmuD', UmuC and RecA suggests that the modified DNA polymerase III holoenzyme is not very processive but rather stops or dissociates after bypassing the lesion. Consistent with previous results (76), the absence of the  $\epsilon$  or proofreading subunit from DNA polymerase III holoenzyme did not eliminate the requirement for UmuD', UmuC and RecA for translesion synthesis in this assay (68). In addition, the translesion synthesis capability of neither DNA polymerase I nor DNA polymerase II was significantly altered by the presence of UmuD', UmuC and RecA, indicating that the activity of UmuD', UmuC and RecA is most likely specific for DNA polymerase III. Finally, intact UmuD inhibited

translesion synthesis by DNA polymerase III in the presence of UmuD', UmuC and RecA in this assay (68), consistent with its postulated role as an inhibitor of mutagenesis (8).

Despite the development of *in vitro* assays for translesion synthesis, the specific biochemical activities of UmuD', UmuC, and RecA in SOS mutagenesis remain unclear. It has been suggested that RecA might function in lesion bypass by directing UmuD'C to the site of the lesion (30). The observations that both UmuD' and UmuC associate with RecA\* nucleoprotein filaments is consistent with such a mechanism (30, 31). The UmuD'C complex functions as an antagonist of RecA-mediated Hfr recombination (77) suggesting that the appearance of UmuD' and UmuC could serve as a signal to switch repair from a homologous recombination mode to an SOS mutagenesis mode.

### **Biological importance of SOS mutagenesis**

Three hypotheses have been proposed for the biological purpose of SOS mutagenesis. The first is the processing of lesions not repairable by other error-free DNA repair processes (11, 17, 82). For example, the removal by excision repair of one of two closely opposed lesions would create a substrate with a lesion opposite a single-stranded gap. Translesion synthesis would provide a damage tolerance mechanism for such lesions. The second hypothesis for the biological purpose of SOS mutagenesis is the processing of special classes of lesions (32, 56). For example, *umuC* mutants are more sensitive to angelicin, a monofunction psoralen, than to UV irradiation (56). Lesions conferred by agents such as angelicin or neocarcinostatin, which induces an abasic site with a closely opposed strand break (25, 32), may not be good substrates for excision repair or other repair systems. Finally, it has been suggested that the biological advantage offered by SOS mutagenesis is a capacity to increase genetic variation in times of stress (24). However, the ability of this hypothesis to explain the evolutionary maintenance of *umuDC*-like genes has been questioned since only a small fraction of mutations are likely to be advantageous (93).

### **Other *umuDC*-dependent phenotypes**

Several other *umuDC*-dependent phenotypes have been reported which may or may not be related to the roles of the *umuDC* gene products in SOS mutagenesis. The possibility that the *umuDC* gene products may have additional activities other than SOS mutagenesis has been previously suggested (93) as a possible explanation for the evolutionary maintenance of *umuDC*-like genes.

### ***EcoK* restriction alleviation**

SOS induction alleviates the restriction of unmodified plasmid and phage DNA by the *EcoK* restriction system in *E. coli* (37). This process of restriction alleviation requires the cleavage of UmuD to UmuD' and is inhibited in *umuDC* mutants. However, in a distinction from SOS mutagenesis, a high copy number plasmid expressing *umuD* alone is able to partially complement a *umuC* mutant for SOS-induced *EcoK* restriction alleviation.

### **DNA replication recovery after DNA damage**

The exposure of *E. coli* to a DNA damaging treatment such as UV light leads to the transient inhibition of DNA synthesis (32). The inhibition of elongation is independent of RecA and has been proposed to be a direct consequence of DNA lesions blocking progression of the replication fork (41). The recovery of DNA synthesis after UV irradiation, also called induced replisome reactivation or IRR, requires protein synthesis and *recA* activity (41).

Induced replisome reactivation (IRR) is independent of *umuC* in wild-type cells (41). However, in *recA718* and *recA727* mutants, IRR requires *umuDC* activity (83, 89). The RecA718 protein is proficient in recombination and confers only a modest degree of UV sensitivity (89). *recA718 umuC* double mutants are highly UV sensitive due to their inability to resume DNA synthesis after UV irradiation, suggesting that the UmuC protein is required to compensate for a defect in RecA718 that inhibits its activity in IRR. The RecA727 protein similarly confers *umuDC*-dependence for IRR but is proficient in recombination and LexA and

$\lambda$ cI cleavage (83). In addition, *recA727* mutants are non-mutable by UV light (83) suggesting that the interactions between the *umuDC* gene products and the RecA727 protein which allow IRR are not identical to those that are required for SOS mutagenesis.

### ***umuDC*-mediated cold sensitivity**

Constitutive expression of the *umuDC* operon from a multicopy plasmid due to a *lexA*(Def) mutation confers growth inhibition at 30°C (54). The *mucAB* operon (20) and the *umuDC* operon from *Salmonella typhimurium* (85) similarly confer cold sensitivity for growth when carried on high copy number plasmids in a *lexA*(Def) background. *umuDC*-dependent cold sensitivity in *E. coli* is associated with a rapid and reversible inhibition of DNA synthesis at the restrictive temperature (54). Analyses of the *umuC125* gene product, which is proficient in SOS mutagenesis but deficient in its ability to confer cold sensitivity for growth (53), suggested that the function of the UmuD and UmuC proteins in SOS mutagenesis was distinct from that which conferred cold sensitivity for growth. Further analyses of *umuDC*-mediated growth inhibition presented in this thesis have led to the hypothesis that the *umuDC* gene products in *Escherichia coli* increase UV survival by modulating the kinetics of the recovery of DNA synthesis after UV irradiation. The regulated inhibition of DNA synthesis by DNA damage has been characterized in *Saccharomyces cerevisiae* (59, 63), but relatively little is known about such a process in prokaryotes.

## **II. Inhibition of DNA synthesis and cell cycle progression in response to DNA damage in *Saccharomyces cerevisiae***

It has long been known that treatment of eukaryotic cells with DNA damaging agents leads to the arrest of cell cycle progression (32). However, the fact that the inhibition of DNA synthesis and cell cycle progression by DNA damage was an actively regulated cellular response was proven relatively recently through the analysis of *rad9* mutants of *S. cerevisiae* (88). *rad9*

mutants continued to divide after irradiation with  $\gamma$  rays and subsequently die (88). However, the imposition of a cell cycle delay by the treatment of cells with an inhibitor of microtubule assembly restored  $\gamma$  ray resistance to *rad9* mutants (88) suggesting that *RAD9* is not directly involved in DNA repair, but rather is required to arrest the cell cycle and allow potentially lethal DNA damage to be repaired.

Since the analysis of *RAD9*, a number of other genes have been identified that are required for the DNA damage checkpoint and the induction of DNA damage inducible genes in *S. cerevisiae* (26). *RAD9*, and subsequently *RAD17*, *RAD24* and *MEC3*, have been shown to be required for cell cycle arrest in G1 (26, 75) or G2 (26, 88) in response to treatment with ionizing or UV radiation. It is currently unknown whether any of these gene products act as the actual sensors of DNA damage to activate the G1 or G2 checkpoint (58). *POL2*, *DPB11* and *RFC5* are required for the S-phase checkpoint in *S. cerevisiae* (2, 59, 81). Since *POL2* and *DPB11* encode subunits of DNA polymerase  $\epsilon$  (2, 59) and *RFC5* encodes a subunit of the replication factor C complex that is required for processive DNA synthesis (81), these genes are good candidates for actual damage sensors that coordinate the cell cycle response to blocks in ongoing replication in S phase (59).

Cell cycle arrest, the inhibition of DNA replication upon DNA damage, and the induction of DNA damage inducible genes is dependent on *MEC1* and *RAD53* (also known as *SPK1*, *MEC2* or *SAD1*) in all phases of the cell cycle (26, 63). *MEC1* is a member of the *ATM* family of phosphatidylinositol (PI) kinases (63). The disease ataxia telangiectasia (AT) is caused by a mutation in the *ATM* gene (94). Cells from AT patients are defective in inhibiting replication in response to DNA damage and are defective in arresting the cell cycle at the G1 and G2 checkpoints (94). In addition, AT cells are hypersensitive to ionizing radiation (94). The requirement for *MEC1* for the G2 checkpoint, as well as the inhibition of DNA synthesis in S phase after DNA damage in *S. cerevisiae* (63), suggests that *ATM* and *MEC1* are functional homologs. *RAD53* has been shown to be a protein kinase that is phosphorylated by *MEC1* in response to DNA damage (69). Thus, in *S. cerevisiae*, there are two distinct mechanisms

pathways to detect DNA damage (26). The *RAD9*-family of genes responds to DNA damage in G1 or G2 and the *POL2*-family senses DNA damage in S phase (58). DNA damage detected by either of these two pathways activates a signal transduction pathway constituted by *MEC1* and *RAD53* (58).

The effectors of the DNA synthesis inhibition and cell cycle arrest that are regulated by *RAD53* in response to DNA damage in *S. cerevisiae* are not well defined (26). However, the transcriptional response to DNA damage has been shown to require the Dun1 kinase, whose activity is increased in a *RAD53*-dependent manner (26). A possible mechanism for cell cycle arrest and DNA synthesis inhibition in *S. cerevisiae* is suggested by the activity and regulation of the p21 gene in mammalian cells (67). The p21 gene is required for the DNA damage checkpoint in mammalian cells (26, 32). p21 is induced after DNA damage by p53 and inhibits both cyclin-CDK (cyclin-dependent protein kinase) complexes and PCNA (proliferating-cell nuclear antigen) (67, 86). Cyclin-CDKs are required to regulate progression of the cell cycle, and PCNA is required for processive DNA synthesis (32, 67, 86). Thus, in mammalian cells, p21 is central to both the cell cycle arrest and the inhibition of DNA synthesis that results from treatment with a DNA damaging agent (32).

### **Responses to DNA damaging treatments in *E. coli***

The *recA* gene in *E. coli* serves as a sensor which induces expression of the SOS regulon in response to a DNA damaging treatment (32, 70). One class of effectors induced by activated RecA\* are the *sulA* and *sfiC* genes (18) that inhibit septation after DNA damage. Several other genes regulated as part of the *recA*<sup>+</sup>*lexA*<sup>+</sup>-dependent SOS regulon have unknown function (32) and could serve to regulate the bacterial cell cycle or inhibit DNA synthesis after DNA damage.

The further characterization of *umuDC*-mediated cold sensitivity in *E. coli* presented in this thesis support the proposal that this phenomenon is due to a novel activity of intact UmuD and UmuC that is exaggerated by the overexpression of these proteins. I have found that high



levels of UmuD and UmuC inhibit specific aspects of cell growth and division at 30°C. These results led to the proposal that intact UmuD and UmuC have a previously uncharacterized role in the regulation of bacterial growth and cell division upon exposure to a DNA damaging treatment, which results in increased survival. The analyses of the activity of intact UmuD and UmuC under physiologically relevant conditions, i.e. in the absence of overexpression, suggest that the activity predicted by the characterization of *umuDC*-mediated cold sensitivity plays an important role in DNA damage tolerance in *E. coli*.

The data presented in Chapter 2 demonstrates that *umuDC*-mediated cold sensitivity is genetically distinct from SOS mutagenesis. The growth inhibition at 30°C is predominantly conferred by UmuC and intact UmuD, the form inactive in SOS mutagenesis. In addition, physiologically relevant levels of the *umuDC* gene products inhibit cell division at 30°C by a mechanism that is independent of *sulA* and *sfiC*.

Subsequent analyses of *umuDC*-mediated cold sensitivity, as detailed in Chapter 3, led to the observation that physiologically relevant levels of the *umuDC* gene products specifically interfere with the ability of cells to make the transition from stationary phase to exponential growth upon nutrient upshift of cells that have accumulated DNA damage while in stationary phase. This activity of the *umuDC* gene products appears to occur via the inhibition of a Fis-dependent function. These analyses suggest that the *umuDC* gene products have a novel role in increasing cell survival by delaying the growth of cells exposed to DNA damaging agents, thereby allowing time for DNA repair processes to be completed.

These results suggested the importance of a more detailed examination of the role(s) of the *umuDC* operon in survival after UV irradiation, as discussed in Chapter 4. Intact UmuD and UmuC were found to have a previously uncharacterized role in increasing survival after UV irradiation. Thus, the UV resistance conferred by the wild-type *umuDC* operon appears to be a consequence of both the well-characterized translesion synthesis activity of UmuD' and UmuC and a novel activity of intact UmuD and UmuC. Analyses of the resumption of DNA synthesis after UV irradiation suggest that intact UmuD and UmuC increase UV resistance by inhibiting

DNA replication. The UmuC125 protein, which is deficient in conferring cold sensitivity (53), is also deficient in the inhibition of DNA synthesis after UV irradiation. Thus, the activity of the *umuDC* gene products which results in growth inhibition at 30°C is correlated with the activity of the UmuD and UmuC proteins in increasing UV survival by modulating the kinetics of the recovery of DNA synthesis after UV irradiation.

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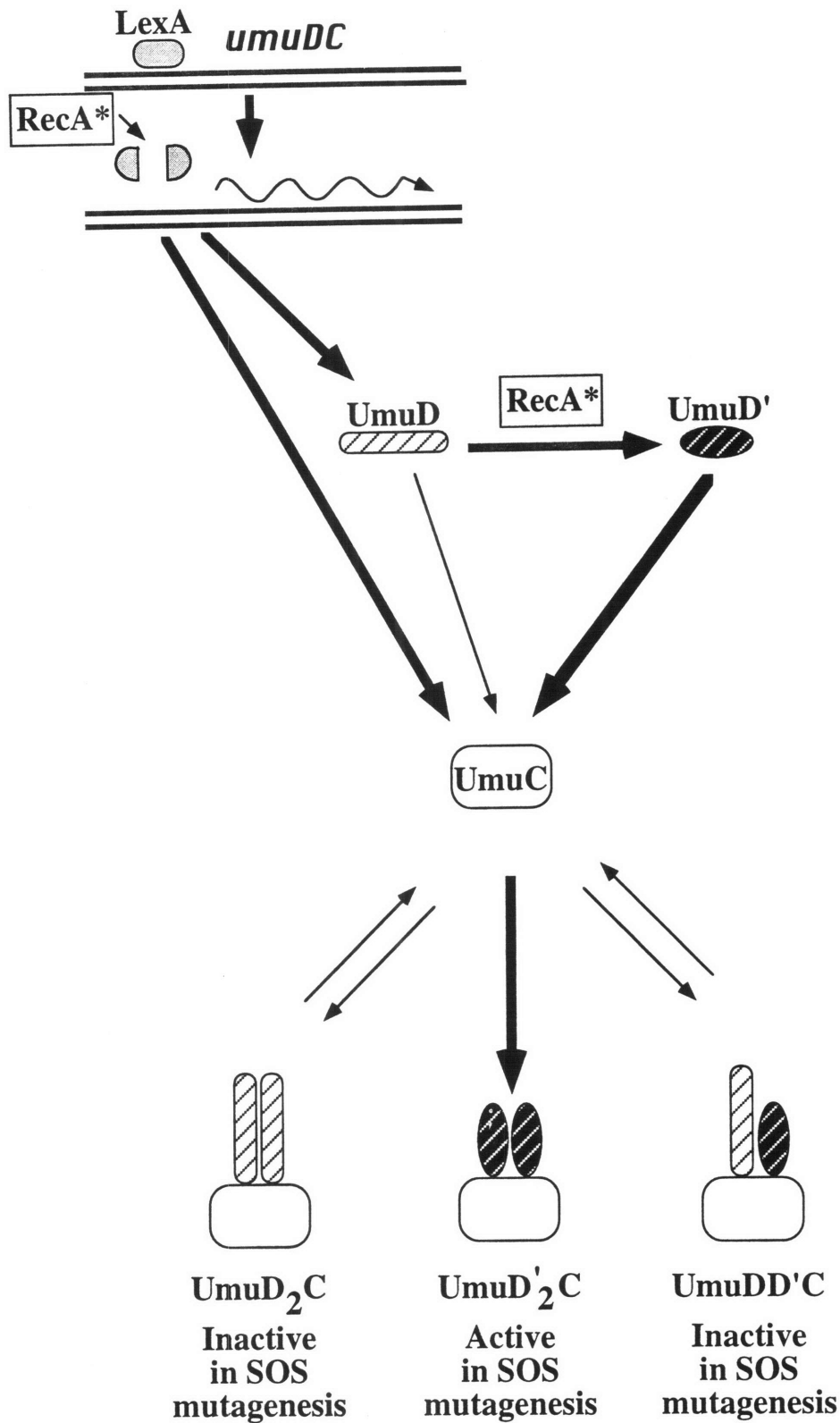
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**Figure 1.** Regulation of the *umuDC* operon by LexA and RecA. DNA damage results in the generation of a signal that activates RecA to RecA\*. RecA\* mediates a specific proteolytic cleavage of LexA which leads to the increased expression of the SOS regulon, including *recA* and the *umuDC* operon. RecA\* also mediates a specific proteolytic cleavage of UmuD to generate UmuD', the form that is active in SOS mutagenesis.



## **Chapter 2**

### **The Genetic Requirements for UmuDC-Mediated Cold Sensitivity Are Distinct from Those for SOS Mutagenesis**

Published in the *Journal of Bacteriology*, **178**: 4400-4411. This work was done in collaboration with Tim Opperman.

## Abstract

The *umuDC* operon of *Escherichia coli*, a member of the SOS regulon, is required for SOS mutagenesis. Following the post-translational processing of UmuD to UmuD' by RecA-mediated cleavage, UmuD' acts in concert with UmuC, RecA and DNA polymerase III to facilitate the process of translesion synthesis which results in the introduction of mutations. Constitutive expression of the *umuDC* operon causes an inhibition of growth at 30°C (cold sensitivity). The *umuDC*-dependent physiological phenomenon manifested as cold-sensitive growth is shown to differ from SOS mutagenesis in two respects. Intact UmuD, the form inactive in SOS mutagenesis, confers a significantly higher degree of cold sensitivity in combination with UmuC than does UmuD'. In addition, *umuDC*-mediated cold sensitivity, unlike SOS mutagenesis, does not require *recA* function. Since the RecA protein mediates the autodigestion of UmuD to UmuD', this supports the conclusion that intact UmuD is capable of conferring cold sensitivity in the presence of UmuC. The degree of inhibition of growth at 30°C correlates with the levels of UmuD and UmuC, which are the only two SOS-regulated proteins required to observe cold sensitivity. Analysis of the cellular morphology of strains that exhibit cold sensitivity for growth led to the finding that constitutive expression of the *umuDC* operon causes a novel form of *sulA*- and *sfiC*-independent filamentation at 30°C. This filamentation is observed in a strain constitutively expressing the single, chromosomal copy of *umuDC* and can be suppressed by overexpression of the *ftsQAZ* operon.

## Introduction

The *umuDC* operon in *Escherichia coli* was originally identified in a genetic screen for mutations that abolished mutagenesis resulting from exposure to ultraviolet light and various chemicals, a phenomenon also known as SOS mutagenesis (27, 44). The *umuDC* operon is part of the SOS regulon. It is repressed by LexA and its expression increases following SOS-induced cleavage of LexA by RecA\*, the activated form of RecA (18). RecA\* also mediates the cleavage of UmuD to UmuD', the 12 kDa carboxyl fragment of UmuD which is the active form in SOS mutagenesis (5, 34, 42). Since RecA\* does not function directly as a protease but rather facilitates a latent ability of LexA and UmuD to autodigest, the term "coprotease" is used to describe this activity of RecA. A third role for RecA in SOS mutagenesis was suggested by the inability of a plasmid expressing *umuD'C* to suppress the non-mutability of a  $\Delta recA$  strain lacking LexA repressor (34), and later supported by other analyses (2, 3, 14, 16, 18, 46). In *E. coli*, SOS mutagenesis appears to be a consequence of DNA synthesis across a lesion, a process known as translesion synthesis. Translesion synthesis on a template with an abasic site has been reconstituted *in vitro* in the presence of DNA polymerase III, RecA, UmuD' and UmuC (39).

Other *umuDC*-dependent phenotypes that are not as well characterized have been reported, although it is not clear how closely some of these are related to the roles of the *umuDC* gene products in SOS mutagenesis. Marsh and Walker (31) observed that the presence of a pBR322-derived plasmid carrying *umuDC* caused cold sensitivity for growth in backgrounds lacking LexA. The *umuDC* operon from *Salmonella typhimurium* as well as the *mucAB* operon, a *umuDC*-like operon found on the naturally occurring plasmid R46 and its derivative pKM101, similarly confer cold sensitivity for growth when carried on pBR322-derived plasmids (11, 47). An initial indication that the UmuD and UmuC proteins interacted with the replication apparatus came from the finding of Marsh and Walker (31) that the cold sensitivity was associated with a modest inhibition of DNA synthesis at the restrictive temperature. However, analysis of the *umuC125* mutation (30) suggests that the function of the UmuD and UmuC proteins in SOS mutagenesis may be distinct from that which confers cold sensitivity for growth. The

*umuC125* gene product is proficient in SOS mutagenesis but deficient in its ability to confer cold sensitivity. The *umuC125* mutation also sensitizes *lexA*<sup>+</sup> cells to killing by UV irradiation. A possible novel role for UmuD and UmuC, distinct from their role in SOS mutagenesis, is also suggested by the finding that the recovery from the inhibition of DNA replication following UV irradiation is dependent on the *umuDC* gene products under certain conditions (45, 49). This phenomenon, known as induced replisome reactivation (IRR) or replication restart, is normally *umuDC*-independent but becomes *umuC*-dependent in *recA718* or *recA727* mutants. In addition, SOS-induced restriction alleviation of type I restriction systems, such as EcoK, requires the *umuDC* gene products (22). Finally, the UmuD'C complex functions as an antagonist of RecA-mediated recombination (43). One possible explanation of this finding suggested by Sommer *et al.* (43) is that the UmuD'C complex switches the dominant form of repair from recombination to translesion synthesis.

In this study, the phenomenon of UmuDC-mediated cold sensitivity was further characterized in order to determine the genetic requirements for cold sensitivity and to see if they are similar to those for SOS mutagenesis. In addition, we examined the cells that exhibit UmuDC-mediated cold sensitivity by microscopy to see if there was a morphological phenotype associated with constitutive expression of the *umuDC* operon. The analysis of UmuDC-mediated cold sensitivity, although a multicopy phenomenon, may provide insight into a novel physiologically relevant activity of the UmuD and UmuC proteins, in a manner analogous to how genetic analyses of multicopy suppressors often provide insights into fundamental physiological phenomena.

## Materials and Methods

**Strains and plasmids.** The *E. coli* strains and plasmids used in this work are listed with their relevant features in Table 1. Genetic markers were transferred between strains using P1 transduction performed essentially as described by Miller (33). Transductants were selected for an antibiotic resistance gene closely linked to or inserted in the genetic marker of interest and then screened as follows to confirm cotransduction of the two markers. Immunoblot analyses using anti-UmuD/D' antibodies were performed on  $\Delta$ *umuDC* transductants to screen for strains lacking UmuD and on *recA430, srl::Tn10* (Tc<sup>r</sup>) transductants in a *lexA*(Def) background at 42°C to screen for strains that are deficient in coproteolysis of UmuD.  $\Delta$ (*recA-srlR*)306::Tn10 (Tc<sup>r</sup>) transductants were screened for UV sensitivity and *lexA300*(Def)::*spc* (Sp<sup>r</sup>) transductants were screened for UmuDC-mediated cold sensitivity.  $\Delta$ *ara714, leu::Tn10* (Tc<sup>r</sup>) transductants were screened on MacConkey plates containing 1% arabinose for the inability to utilize arabinose.

**Reagents and media.** High purity arabinose was purchased from Pfanstiehl Labs (Waukegan, IL). Ampicillin (Ap), tetracycline (Tc), kanamycin (Km), spectinomycin (Sp), chloramphenicol (Cm), 4,6-diamidino-2-phenylindole and poly-L-lysine were purchased from Sigma Chemical Co. (St. Louis, MO). The GeneAmp polymerase chain reaction kit was purchased from Perkin Elmer Cetus (Norwalk, CT). All restriction endonucleases and DNA ligase were purchased from New England Biolabs (Beverly, MA). The "Western Lights" kit for chemiluminescent immunoblot analyses was purchased from Tropix (Bedford, MA). LB liquid media and LB agar (40) were used in all experiments. The antibiotics were used at the following concentrations: ampicillin (100 µg/ml), kanamycin (25 µg/ml), spectinomycin (20 µg/ml), tetracycline (13 µg/ml), chloramphenicol (30 µg/ml).

**Plasmid constructions.** pBR322/kan contains a Km<sup>r</sup> gene and a deletion of the Tc<sup>r</sup> gene. The Km<sup>r</sup> cassette from mini-Tn5 Km (9) was isolated as a 2.0 kb *Hind* III fragment and introduced by ligation into the *Hind* III site of pBR322, which was then digested with *Ava* I to excise the



entire  $Tc^r$  gene. To construct pGW2101/kan, the  $Km^r$  cassette was isolated from mini-Tn5 Km as a 2.0 kb *Eco* RI fragment and introduced by ligation into the *Eco* RI site of pGW2101 (34).

pTO2 was constructed by cloning the *umuDC* operon into the *Eco* RI site of pBAD24 (20). This vector contains the  $P_{BAD}$  promoter and a ribosome binding site upstream of a multiple cloning cassette. The *umuDC* operon was amplified by the polymerase chain reaction (PCR) using the following primers: UmuD5', 5'-CGAATTCATATGTTGTTTTATCAAGCC-3'; Umu3', 5'-GGAATTCTTATTTGACCCTCAGTAAATC-3' (initiation and termination codons are underlined). PCR reactions were performed using the GeneAmp kit (Perkin Elmer Cetus, Norwalk, CT). Amplification products were digested with *Eco* RI and cloned in the *Eco* RI site of pBAD24. The nucleotide sequence of this construct was determined (United States Biochemical, Cleveland, OH) and found to be identical to the reported sequence of the *umuDC* operon. The  $Km^r$  cassette from mini-Tn5 Km (9) was isolated as a 2.0 kb *Sma* I fragment and cloned into the unique *Fsp* I site of pTO2, resulting in inactivation of the *bla* ( $Ap^r$ ) gene. The *umuDC* operon under the control of  $P_{BAD}$  in pTO3 was tested in an SOS mutagenesis assay performed as described (15) except that the UV irradiated cells were plated on selective media containing 0.2% arabinose.

**Quantitative transformation assays.** Preparation of competent cells using  $CaCl_2$  and the procedure for transformation of *E. coli* are described in Sambrook et al. (40). In the quantitative transformation assay, 0.3  $\mu$ g purified plasmid DNA was added to 100  $\mu$ l competent cells. After transformation, the cells were incubated in LB media at 42°C in an air shaker for 2 hr without antibiotic selection. Serial 10-fold dilutions of each transformation were made, 10  $\mu$ l aliquots were spotted on duplicate plates, incubated at 30°C or 42°C, and the colony forming units per ml at each temperature were determined. In some cases the cells from one ml of the transformation mix were concentrated, plated and incubated at 30°C. The ratio of colony forming units per ml at 30°C to 42°C was used as a measure of the degree of cold sensitivity.

**Growth curve analyses.** Growth curve analyses were performed as follows. Overnight cultures were grown in LB media with the appropriate antibiotics at 42°C and used to inoculate 25 ml cultures (1:125 dilution) in a 125 ml flask approximately 16 hr before the start of the growth curve experiment. The growth of this culture at 42°C was monitored to ensure that each culture would be in the same stage of growth at the start of the experiment. These cultures in turn were used to inoculate 100 ml LB (1:125 dilution) containing the appropriate antibiotics in a 500 ml flask and incubated with shaking (~215 rpm) at 42°C for 1 hr at which time the culture was split in equal halves, transferred to 250 ml flasks, and incubated with shaking at 42°C or 30°C. At various times, samples were removed from the cultures to measure OD<sub>600</sub>, to determine colony forming units (CFU) per ml, and for use in immunoblot and microscopic analyses described below.

**Immunoblot analyses.** Cells were harvested by centrifugation and the resulting cell pellet was resuspended in SDS-PAGE loading buffer (40) to a final concentration of 0.005 OD<sub>600</sub> units/μl. These samples were incubated in a boiling water bath for 5 min. A 10 μl aliquot of each sample (corresponding to 0.05 OD<sub>600</sub> units) was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described in Sambrook et al. (40). The proteins were transferred from the gel to PVDF membrane, with the subsequent antibody reactions and chemiluminescent detection performed as described in the "Western Lights" manual (Tropix, Bedford, MA). The anti-UmuD/D' antibody used in these experiments has been previously described (4).

**Microscopic analyses.** Sample preparation for microscopy and staining with 4,6-diamidino-2-phenylindole (DAPI) was performed as described by Hiraga et al. (23). The cell samples were spun down and resuspended in 0.85% saline at approximately 1 OD<sub>600</sub> unit per ml and 10 μl of these cell suspensions were examined. Samples were examined and photographed with an axioscope (Carl Zeiss, Inc., Thornwood, NY) equipped with an oil immersion lens. Photographs were taken with Kodak TMax black and white film (ASA 400).

## Results

**RecA is not required for *umuDC*-mediated cold sensitivity.** The *recA* gene product plays three pivotal roles in SOS mutagenesis. It is required for mediating the autodigestion of LexA repressor, thereby increasing expression of the SOS regulon including the *umuDC* operon, for mediating the autodigestion of UmuD to UmuD', which is the active form in SOS mutagenesis, and for a third as yet poorly understood role in the process of translesion synthesis (18). We therefore used a quantitative transformation assay to examine whether *recA* function was similarly important for the phenomenon of *umuDC*-mediated cold sensitivity. A set of plasmids was transformed into a set of *lexA(Def)* strains carrying different alleles of *recA*. The *lexA(Def)* mutation results in constitutive expression of the *umuDC* operon. The ratio of transformants per ml obtained at 30°C relative to that at 42°C was used as a measure of cold sensitivity. We have compared the results obtained using this assay with those from an assay in which the plating efficiency at 30°C and 42°C of cultures growing exponentially at 42°C is used as a measure of cold sensitivity. The results obtained from both of these assays were essentially identical. The data in Table 2 shows that *recA* function is not required for *umuDC*-mediated cold sensitivity. Both pSE115 (*umuDC*; pSC101) and pSE117 (*umuDC*; pBR322) cause significant cold sensitivity in a *lexA(Def) ΔrecA* background. The greater degree of cold sensitivity conferred by pSE117 correlates with the increased copy number of this plasmid relative to pSE115 (discussed in greater detail below). Constitutive expression of *umuD* alone (pLM206) does not confer cold sensitivity in any *lexA(Def)* background, as has been shown previously (31). Thus, pLM206 has been used as a control in many of the experiments described below. The ratio of transformants per ml at 30°C to 42°C is slightly less than one in a *lexA(Def) ΔrecA* background for the control plasmids pLM206 and pBR322, suggesting that this strain is slightly cold-sensitive on its own in the transformation assay. However, the additional significant degree of cold sensitivity conferred by the *umuDC* expressing plasmids (greater than 40,000 fold for the pBR322 derived plasmid) demonstrates that *umuDC*-mediated cold sensitivity, unlike SOS mutagenesis, does not require *recA* function.

The cold sensitivity for growth produced by *umuDC*-containing plasmids in *lexA*(Def) backgrounds is more severe in *recA430* and  $\Delta$ *recA* derivatives than it is in a *recA441* derivative (see Table 2). The RecA430 protein is deficient in coprotease activity while remaining proficient in recombination (16), whereas the RecA441 protein exhibits increased coprotease activity (32, 38). These data suggest that the coprotease activity of RecA can reduce the severity of *umuDC*-mediated cold sensitivity in a *lexA*(Def) background. This conclusion is further supported by the observation that a *lexA*(Def) *recA*<sup>+</sup> strain also exhibits a greater degree of cold sensitivity than does a *lexA*(Def) *recA441* strain. Thus, there appears to be an inverse correlation between the amount of RecA coprotease activity in a *lexA*(Def) cell and the degree of cold sensitivity conferred by *umuDC*-expressing plasmids. Since the RecA coprotease activity is required to mediate the autodigestion of UmuD, the *recA*-independence of *umuDC*-mediated cold sensitivity suggests that intact UmuD, the form inactive in SOS mutagenesis, can act in combination with UmuC (31) to confer cold sensitivity, and that UmuD' is less potent than intact UmuD with respect to this activity.

#### **Intact UmuD is proficient in mediating cold sensitivity in combination with UmuC.**

In order to test directly the hypothesis that intact UmuD is able to work in combination with UmuC to cause inhibition of growth at 30°C, we examined the ability of plasmids carrying *umuD* alleles that encode noncleavable versions of the UmuD protein, as well as *umuC*<sup>+</sup>, to confer cold sensitivity in a *lexA*(Def) background. The data in Table 3 demonstrate that mutations in *umuD* that block UmuD cleavage do not interfere with its ability to confer cold sensitivity. Similar degrees of cold sensitivity are observed with pGW2101, which encodes the wildtype *umuDC* operon, and pGW2111, pGW2112 and pGW2115, derivatives of pGW2101 which contain mutations in *umuD*. The mutations in *umuD* encoded by pGW2111 (GK25), pGW2112 (SA60) and pGW2115 (KA97) block RecA\*-mediated cleavage of UmuD to UmuD' and largely abolish the ability of these mutant UmuD proteins to function in SOS mutagenesis (34). The variation in

the degree of cold sensitivity conferred by the noncleavable mutants of UmuD may reflect the effect of the mutations on the conformation and/or stability of the protein.

**UmuD and UmuC confer a greater degree of cold sensitivity than do UmuD' and UmuC.** The inverse relationship between the degree of RecA coprotease activity in a *lexA(Def)* background and the severity of *umuDC*-mediated cold sensitivity, and the fact that intact UmuD is capable of conferring the phenotype, suggested that intact UmuD might be more active than UmuD' in acting in combination with UmuC to cause cold sensitivity. The results in Table 2 show that in all *lexA(Def)* backgrounds tested, a plasmid encoding *umuD'C* (pGW3751) confers a lesser degree of cold sensitivity than a corresponding plasmid encoding *umuDC* (pSE117). The cold sensitivity mediated by UmuD and UmuC is 500 fold to 4,000 fold more severe than that mediated by UmuD' and UmuC. This conclusion is strengthened by growth curve analyses performed to quantitate growth at 30°C and 42°C. In these analyses, saturated cultures grown at 42°C were diluted into fresh media and the subsequent growth of these cultures was followed at 30°C and 42°C. The data in Fig. 1 demonstrate that pSE117 (encoding *umuDC*) confers a greater inhibition of growth at 30°C than does pGW3751 (encoding *umuD'C*). No significant difference in growth of these strains is observed at 42°C. Immunoblot analyses confirmed that the presence of pGW3751 in a *lexA(Def)* background leads to the accumulation of high levels of UmuD' with no detectable UmuD whereas the presence of pSE117 leads to the accumulation of both UmuD and UmuD' (Fig. 3E and 3D respectively). Thus, intact UmuD and UmuC confer a more severe cold-sensitive phenotype than do UmuD' and UmuC, although high levels of UmuD' and UmuC do confer a moderate degree of cold sensitivity. This is in contrast to what is known for SOS mutagenesis where UmuD' rather than UmuD acts in concert with UmuC.

Constitutive expression of *umuC* alone under the control of the  $P_{umuDC}$  promoter does not appear to confer cold sensitivity for growth (data not shown) or cause cellular filamentation (see below). This construct was able to complement the defect of a *umuC122::Tn5* strain in SOS mutagenesis (data not shown). However, the level of UmuC expressed from this construct, as

measured by immunoblot, was modest despite the fact that the ATG of *umuC* was located at the same position as the ATG of *umuD* in the natural situation. Thus, it cannot be ruled out that high levels of UmuC alone could confer cold sensitivity for growth.

The greater level of cold sensitivity conferred by *umuDC*-expressing plasmids in *lexA*(Def) strains with reduced RecA coprotease activity ( $\Delta recA$  and *recA430*) does not appear to be due solely to the accumulation of UmuD in these strains, since a similar effect is also observed in these strains with pGW3751, a *umuD'*C-expressing plasmid (see Table 2). It seems likely that the coprotease activity of RecA performs an additional role in the partial alleviation of the effects of UmuDC-mediated cold sensitivity, either by negatively regulating the activity of an unknown protein(s) that enhances the effects of UmuDC-mediated cold sensitivity or by positively regulating the expression of an unknown protein(s) that partially alleviates these effects. These effects are not mediated directly through LexA since the strains used in these experiments are *lexA*(Def).

**Correlation of UmuD and UmuC levels with cold sensitivity for growth.** To determine whether UmuD and UmuC are the limiting factors required to confer cold sensitivity for growth, we tested the effects of increasing the copy number of the *umuDC* operon on inhibition of growth at 30°C. We examined this possibility using the quantitative transformation assay described above with *umuDC*-containing plasmids that are stably maintained in the cell at different copy numbers. As noted earlier, a graded increase in the degree of cold sensitivity is observed in all of the *lexA*(Def) strains as the copy number of *umuDC* is increased from 0 copies/cell (pBR322) to ~30 copies/cell (*umuDC*; pBR322 origin) (see Table 2). The increase in cold sensitivity compared to the vector pBR322 is between 3-15 fold for the pSC101-derived plasmid (~5 copies *umuDC*) and is at least 40,000 fold for pSE117, the pBR322-derived plasmid (~30 copies *umuDC*). Furthermore, a decrease in viability is observed even at 42°C when a *umuDC*-containing plasmid with an extremely high copy number, pGW2101 (pBR322  $\Delta rop$ ), is introduced into a *lexA*(Def) strain. Thus, an increase in the copy number of *umuDC* results in a

greater degree of cold sensitivity for growth, and at very high levels of *umuDC* an inhibition of growth is observed even at 42°C.

We analyzed the effect of the copy number of *umuDC* on the degree of cold sensitivity for growth in more detail using the growth curve analyses described earlier. In these experiments, we directly determined the inhibition of growth at 30°C, measured the levels of UmuD and UmuC in these strains, and examined cellular and nucleoid morphologies (see below). In isogenic *lexA*(Def) strains that differ only in the copy number of *umuDC*, a graded increase in the degree of cold sensitivity for growth at 30°C, as measured by OD<sub>600</sub> and CFU/ml, is observed as the copy number of *umuDC* is increased from 0 to ~30 copies/cell (see Fig. 2A and 2C). *lexA*(Def) strains containing pSE117 (~30 copies/cell) exhibit a decrease in viability during prolonged incubation at 30°C (Fig. 2C). No significant differences in the growth of these strains at 42°C is observed (Fig. 2B and 2D). *lexA*(Def) strains expressing *umuD* alone did not exhibit cold sensitivity for growth, which is consistent with previously reported results (31). These results confirm that the severity of cold sensitivity for growth increases in a *umuDC* dose dependent manner.

To verify that the greater degree of cold sensitivity in strains with increasing copies of *umuDC* is indeed correlated with higher levels of UmuD and UmuC, the levels of these proteins in cellular extracts derived from the cultures used in the growth curve experiments described above were analyzed in immunoblots using affinity purified antibodies against UmuD/D' (Fig. 3A-D). The immunoblot analyses demonstrate that increasing the copy number of *umuDC* from 0 copies to ~30 copies leads to an increase in the levels of UmuD/D' in cells growing at 42°C and 30°C (Fig. 3A-D). Similar results are obtained in immunoblot analyses using antibodies specific for UmuC (data not shown). These results confirm that the increased cold sensitivity for growth in these strains is correlated with increased levels of UmuD and UmuC.

**Cellular morphologies associated with UmuDC-mediated cold sensitivity.** The cellular morphologies of the different strains used in the growth curve analyses were examined in

an attempt to determine the physiological basis of UmuDC-mediated cold sensitivity. Filamentation and failure to form septa are often indicative of a block in the normal progression of the cell cycle (10). We examined the cellular morphology of these strains using a microscope equipped with Nomarski optics. Fluorescent microscopy following staining with DAPI, which specifically stains DNA (23), was used to examine nucleoid morphology. In *E. coli*, the chromosome is organized in a compact structure called the nucleoid that is visible in fluorescence microscopy after staining with DAPI (see Fig. 5). As described in the Materials and Methods section, all of the cell samples were equalized to approximately 1 OD<sub>600</sub> unit per ml before they were prepared for microscopic examination. The majority of cells of a *lexA(Def)* strain containing the  $\Delta$ *umuDC595* deletion appear as short rods at 30°C, approximately 3-4 times the length of the cells from the same strain grown at 42°C (Fig. 4E). Fluorescence microscopy revealed that these cells each contain 3-4 evenly spaced nucleoids (Fig. 5A). In contrast, the strain (GW8018) that constitutively expresses a single chromosomal copy of the *umuDC* operon at 30°C forms filaments that increase in length (up to ~20 cell lengths) with time of incubation at 30°C (Fig. 4A-C). These filaments appear to be non-septated and contain multiple nucleoids that are evenly spaced (Fig. 5B). This *umuDC*-dependent filamentation which occurs at 30°C is independent of *sulA* and *sfiC*, the previously described SOS-regulated inhibitors of cell division, since this strain carries the *sulA11* allele and is a derivative of a strain shown by D'Ari and Huisman (8) to carry a *sfiC2* mutation. Thus, a significant phenotype, filamentation, is associated with constitutive expression of a single copy of the *umuDC* operon.

Strains carrying higher copy number *umuDC* plasmids (pSE115 and pSE117) form somewhat longer filaments at 30°C of lengths between 15 and 45 cell lengths (Fig. 4 G and I). Whereas the strain containing pSE115 (pSC101-derived) does not form filaments at 42°C (Fig. 4F), a significant number of cells containing pSE117 (pBR322-derived) form filaments at 42°C (Fig. 4H). This suggests that at the highest levels of *umuDC* tested, cell growth is affected at 42°C, which is consistent with the finding that pGW2101, a very high copy number *umuDC* plasmid, reduces transformation efficiency in a *lexA(Def)* strain even at 42°C (Tables 2 and 3).



DAPI staining of *lexA(Def)* cells containing pSE115 and pSE117 revealed a number of distinct nucleoid morphologies. In the strain with pSE115 at 30°C, the majority of cells contain multiple, evenly spaced nucleoids (Fig. 5C). However, a significant fraction of these cells have large masses of DAPI-staining material that are probably due to several unsegregated nucleoids (Fig. 5C). In cells with pSE117 at 30°C, the proportion of cells containing apparently unsegregated nucleoids increases (Fig. 5D) while the number of cells containing evenly spaced nucleoids decreases. In addition, filamented cells are evident that are almost completely devoid of DAPI staining material or contain a single, small mass of DAPI-staining material. These results demonstrate that low levels of UmuD and UmuC lead to filamentation at 30°C that appears to result from an inhibition of normal septation. In these cells, nucleoid segregation does not appear to be affected. Higher levels of UmuD and UmuC appear to affect additional aspects of cell growth besides cell division. In these cells, nucleoid segregation appears to be perturbed as evidenced by the increase in the proportion of cells containing abnormal masses of DAPI-staining material.

**Overexpression of *ftsQAZ* suppresses UmuDC-mediated filamentation.** To explore the basis of the filamentation associated with overproduction of UmuD and UmuC, we examined the effect of increasing the levels of the FtsQ, FtsA, and FtsZ proteins. The levels and activity of the FtsZ protein control the frequency of cell division in *E. coli* (48). The plasmid pZAQ (48), which causes a 7-fold increase in FtsZ levels, was introduced into a *lexA(Def) recA441* strain carrying pSE115 (the pSC101-derived *umuDC* plasmid). The introduction of pZAQ did not suppress the cold-sensitive phenotype of this strain (data not shown). However, the filamentation caused by high levels of the UmuD and UmuC proteins was completely suppressed by increasing the levels of the FtsQ, FtsA, and FtsZ proteins (see Fig. 6).

**Constitutive expression of *umuDC* in the absence of expression of the SOS regulon confers cold sensitivity for growth.** Since the *lexA(Def)* strains described above constitutively

express all of the SOS-regulated proteins, it is possible that one of these proteins is required to act in concert with UmuD and UmuC to confer UmuDC-mediated cold sensitivity for growth. To test this possibility, growth curve analyses were performed in which the *umuDC* operon was expressed from an inducible, exogenous promoter in the presence and absence of expression of the SOS regulon.

The *umuDC* operon was cloned into the plasmid pBAD24 (20) under the control of the  $P_{BAD}$  promoter, which is tightly regulated by the concentration of arabinose in the media. The resulting plasmid is called pTO3. The levels of UmuD and UmuC in the *lexA*<sup>+</sup>  $\Delta$ *umuDC* strain GW8028 carrying pTO3 grown in media containing 0% to 0.2% arabinose were measured in immunoblot analyses using antibodies against UmuD/D' and UmuC. UmuD and UmuC are not detectable when there is no arabinose in the media, but the levels of these proteins increase as the concentration of arabinose is increased from 0.005% to 0.2% (data not shown). The maximal levels of expression are observed at 0.2% arabinose, an observation which is consistent with the results reported by Guzman *et al.* (20). pTO3 was also tested for its ability to function in SOS mutagenesis. pTO3 is able to restore SOS mutagenesis to a  $\Delta$ *umuDC* strain (GW8028) in the presence but not in the absence of arabinose to levels similar to those obtained with pSE117 (data not shown).

To test whether UmuD and UmuC are able to confer cold sensitivity for growth in the absence of expression of other *lexA*-regulated genes, we introduced pTO3 ( $P_{BAD}$ -*umuDC*) into GW8028 [*lexA*<sup>+</sup>, *recA*<sup>+</sup>,  $\Delta$ *umuDC*] and GW8029 [*lexA*(Def), *recA441*,  $\Delta$ *umuDC*] and analyzed growth of these strains at 42°C and 30°C in the presence and absence of arabinose. The results of these analyses are shown in Fig. 7. Cold sensitivity for growth is observed in both strains carrying pTO3 only in the presence of arabinose (Fig. 7 A and C). Little or no effect on growth is evident at 30°C in the absence of arabinose (Fig. 7 A and C) or at 42°C in the presence or absence of arabinose (Fig. 7 B and D). Interestingly, the *lexA*<sup>+</sup> *recA*<sup>+</sup> strain carrying pTO3 incubated at 30°C after growth in the presence of arabinose exhibits an increase in OD<sub>600</sub> without a concomitant increase in CFU/ml. This suggests that these cells continue to divide at

30°C but that the progeny cells are not viable. The possibility that the increase in optical density of these cultures is due to filamentation was ruled out by a microscopic examination of these cells (see below). The levels of UmuD were determined in these strains using immunoblot analyses. The results are shown in Fig. 3. UmuD is not detectable in cells grown in the absence of arabinose (Fig. 3 G and I), but high levels are detected in the presence of 0.2% arabinose at 42°C and 30°C (Fig. 3 H and J). These results demonstrate that constitutive expression of the *umuDC* operon in the absence of other SOS-regulated functions is sufficient to confer cold sensitivity for growth.

The cellular morphology of GW8028 [*lexA*<sup>+</sup>, *recA*<sup>+</sup>,  $\Delta$ *umuDC*] and GW8029 [*lexA*(Def), *recA441*,  $\Delta$ *umuDC*] containing pTO3 (P<sub>BAD</sub>-*umuDC*) incubated at 30°C and 42°C in the absence and presence of 0.2% arabinose was also examined. GW8028 (pTO3) cells grown in the presence of arabinose at 30°C are seen as short rods approximately 3-4 cell lengths long (Fig. 4J), despite the high levels of UmuD and UmuC in these cells (see Fig. 3H). However, the majority of GW8029(pTO3) cells grown in the presence of arabinose at 30°C form filaments of approximately 10-30 cell lengths (Fig. 4L). In contrast, GW8029(pTO3) cells incubated in the absence of arabinose at 30°C form short rods of 2-3 cell lengths (Fig. 4K). None of these strains form filaments at 42°C in the presence or absence of arabinose (data not shown). These results demonstrate that one or more *lexA*-regulated functions is required for UmuDC-dependent filamentation at 30°C. In addition, the observations demonstrate that the phenomena of UmuDC-dependent cold sensitivity for growth and UmuDC-dependent filamentation can be genetically separated.

## Discussion

We have carried out a further characterization of the phenomenon of UmuDC-mediated cold sensitivity in the hope of gaining insights into other possible physiological roles of the *umuDC* gene products in addition to their role in SOS mutagenesis. The possibility that UmuD and UmuC may perform a distinct role in the bacterial cell other than SOS mutagenesis is consistent with the phenotype of the *umuC125* mutation, which separates the activities of UmuC in UmuDC-mediated cold sensitivity and SOS mutagenesis (30). The studies reported here support and extend this hypothesis by demonstrating that the genetic requirements of UmuDC-mediated cold sensitivity are strikingly different from those of SOS mutagenesis. In addition, our studies have revealed a previously unreported class of SOS-induced filamentation that is *umuDC*-dependent (even with a single chromosomal copy), is *sulA*- and *sfiC*-independent, and is seen at 30°C but not at 42°C. Based on the observed effects of growth inhibition and of inhibition of the cell cycle produced by constitutive expression of *umuDC* at 30°C, we propose that UmuDC-mediated cold sensitivity is a physiological manifestation of a novel function of the UmuD and UmuC proteins.

UmuDC-mediated cold sensitivity is genetically distinct from SOS mutagenesis in that it does not require a functional *recA* allele and that the intact form of UmuD appears to be most active in producing this phenomenon.  $\Delta recA$  and *recA430* strains [*recA430* is proficient in recombination but deficient in coprotease activity (16)] exhibit equal levels of cold sensitivity. Therefore, neither the recombination nor the coprotease activity of RecA is necessary for UmuDC-mediated cold sensitivity. The *recA* independence of UmuDC-mediated cold sensitivity implies that the posttranslational processing of UmuD to UmuD', the form of the protein that is active in SOS mutagenesis (34), is not required. The observations that mutations in *umuD* that block RecA\*-mediated cleavage of UmuD to UmuD' do not interfere with UmuDC-mediated cold sensitivity and that plasmids encoding UmuD' and UmuC are 500 - 4,000 fold less active in conferring cold sensitivity than plasmids encoding UmuD and UmuC strongly suggest that the intact UmuD is the form of the protein that is most active in conferring cold sensitivity in

combination with UmuC. This is the first evidence of a distinct activity for the intact form of UmuD apart from its previously reported role in negatively modulating the activity of UmuD' in SOS mutagenesis (4). Although modest levels of UmuC alone do not confer cold sensitivity for growth, we have not yet ruled out the possibility that sufficiently high levels of UmuC alone might be capable of conferring the cold-sensitive phenotype.

UmuDC-mediated cold sensitivity is exacerbated in *lexA*(Def) strains by the presence of the *ΔrecA*, *recA*<sup>+</sup> and *recA430* mutations as compared to the *recA441* mutation. This cannot be fully explained by the fact that UmuD and UmuC are more potent in conferring cold sensitivity than UmuD' and UmuC since cold sensitivity for growth is exacerbated when *umuD'C* is constitutively expressed in *ΔrecA*, *recA*<sup>+</sup> and *recA430* strains compared to *recA441* strains. Thus, in a *lexA*(Def) strain, the activation state of RecA for its coprotease activity is inversely correlated with the level of UmuDC-mediated cold sensitivity. This suggests that the coprotease activity of RecA plays a role in the alleviation of UmuDC-mediated cold sensitivity other than the cleavage of UmuD to UmuD'. One possible explanation for this is that activated RecA is capable of binding to UmuD or UmuD', either alone or in combination with UmuC, and this alleviates UmuDC-mediated cold sensitivity by an unknown mechanism. Activated RecA has affinity for both UmuD and UmuD' and it has been suggested that its role may be to "target" UmuD and UmuD' to DNA (17). Presumably, the state of activation of RecA reflects its ability to bind to UmuD and UmuD'. Alternatively, it is possible that activated RecA is required to facilitate the cleavage of another protein that is involved in UmuDC-dependent cold sensitivity, or to induce the expression of genes that are required for alleviation of the effects of UmuDC overexpression. If the latter were to be the case, these genes must be under the negative control of a repressor protein other than LexA since the strains used in these experiments are *lexA*(Def). Several genes in the SOS regulon have been shown to be regulated by RecA, but not repressed by LexA, such as *sfiC* (8) or *dinY* (36). In addition, there are several examples of genes, such as *dnaA*, *dnaN*, *dnaQ*, *phr*, and *recQ* (28), that appear to be regulated in a *recA*- and *lexA*-dependent manner but have not been shown to be directly repressed by LexA. It has been

suggested that the expression of these genes is controlled by other unknown repressors that are inactivated by RecA\*-facilitated cleavage (36). It is possible that one or more of these genes, or an unknown gene that is similarly regulated, may play a role in the alleviation of UmuDC-mediated inhibition of growth at 30°C.

On the basis of our observations, we postulate that UmuDC-mediated cold sensitivity for growth is the result of a specific interaction between UmuD and UmuC complexes and an unidentified protein(s), resulting in a perturbation of the normal physiological state of the cell at 30°C. Since UmuDC-mediated cold sensitivity for growth has been correlated with a rapid, but reversible, partial inhibition of DNA synthesis at 30°C (31) and since it has been postulated that UmuD and UmuC interact with the DNA replication apparatus during SOS mutagenesis (39), an attractive candidate for this target protein is one or more of the subunits of DNA polymerase III holoenzyme. It is possible that such an interaction normally occurs in the cell under certain conditions and that the stable interaction between these proteins under abnormal circumstances causes the inhibition of growth observed at 30°C. This interaction is specific for UmuD and UmuC, since cold sensitivity is not observed with UmuD alone, and does not require other SOS-regulated proteins. One simple explanation for growth inhibition at 30°C may be that the rate of dissociation of this putative complex is slower at 30°C compared to 42°C, which would favor the formation of these complexes at 30°C. It is possible that cold shock proteins (26) are involved in the formation or stability of this complex. However, we have shown that growth is inhibited at 42°C, as well as at 30°C, by very high levels of UmuD and UmuC proteins. This suggests that the observed growth inhibition is due to an equilibrium phenomenon and is not solely due to temperature or temperature-dependent gene expression. Alternatively, the stability of this putative complex could be influenced by the abundance and activity of certain heat shock proteins (chaperones) at the two temperatures, particularly since UmuC has been shown to interact with the heat shock-controlled chaperones GroEL/ES both *in vivo* and *in vitro* (13) and with DnaK/J/GrpE *in vitro* (37). It is possible that these proteins may be involved in the disassembly of an inhibitory UmuDC-containing protein complex. However, mutations in the

*groEL* locus suppress UmuDC-mediated cold sensitivity (12), suggesting that GroEL/ES are not involved in disassembly of the putative, inhibitory protein complex but are required for the proper folding of UmuC into its active conformation as previously reported (13). Finally, it is possible that UmuD and UmuC are less stable at 42°C. We do not favor this hypothesis due to the fact that the steady state levels of these proteins do not decrease during growth at 42°C. Since UmuD and UmuC appear to be more active in conferring cold sensitivity than UmuD' and UmuC', it is likely that UmuD and UmuC have a higher affinity for the putative "target" protein(s) than do UmuD' and UmuC'.

In the course of these studies we have uncovered what appears to be a unique pathway for filamentation at 30°C that requires UmuD, UmuC and another SOS-regulated function(s), but is independent of the SOS-regulated inhibitors of cell division, *sulA* and *sfiC*. It has been previously shown that UV irradiation or inhibition of DNA synthesis leads to the formation of filamentous cells, probably resulting from an inhibition of cell division (7, 24). This filamentation proceeds by two independent pathways that are both *recA*-dependent. One of these pathways requires *sulA* and *sfiC* while the other pathway is independent of *sulA* and *ftsZ* and is resistant to rifampicin (6). The facts that the strains used in this study carry *sulA* and *sfiC* mutations and that the filamentation is dependent on *umuDC* expression (which would be rifampicin sensitive) suggest that the filamentation we observed occurs by a distinct pathway. In retrospect, it appears that this phenomenon has not been described previously because earlier studies of SOS-dependent filamentation used temperature sensitive alleles of *recA*, *lexA*, or DNA polymerase III subunits to induce the SOS response and were thus carried out at 42°C, not at 30°C (8, 19, 24). In the course of our investigation, we have observed that a *lexA*(Def) strain that constitutively expresses a single, chromosomal copy of the *umuDC* operon forms filaments but is not cold-sensitive for growth. Since this strain does not exhibit inhibition of growth at 30°C, it is unlikely that DNA synthesis is affected to a significant degree. This suggests that this novel, UmuDC-dependent filamentation pathway is independent of pathways involving DNA synthesis. Unlike cold sensitivity for growth, physiologically relevant levels of UmuD and UmuC

expressed from a single chromosomal copy of *umuDC* at 30°C are sufficient to inhibit cell division. Although the filamentation associated with constitutive expression of *umuDC* in a *lexA(Def)* strain at 30°C appears to proceed through a novel pathway, it can be suppressed by increased expression of the *ftsQAZ* operon, suggesting that UmuD, UmuC and another SOS-regulated protein(s) affect the activity or expression of these proteins. The FtsQ, FtsA, and FtsZ proteins are involved in the initiation of septation that leads to cell division (10). Decreases in the levels of any of these proteins results in delayed or aborted cell division (10) making the FtsQ, FtsA, and FtsZ proteins important targets for the regulation of cell division. Since SulA-mediated filamentation also involves inhibition of FtsZ activity (25, 29) , it appears that these distinct filamentation pathways converge at a common target protein.

Increased expression of *ftsQAZ* does not, however, suppress UmuDC-mediated cold sensitivity for growth. This supports the observation that cold sensitivity and filamentation are genetically separable and involve different "target" proteins. Furthermore, there does not appear to be a causal relationship between the filamentation observed in cells constitutively expressing UmuD and UmuC and cold sensitivity for growth, since strains expressing high levels of UmuD and UmuC from an exogenous promoter in the absence of the other SOS-regulated functions (*lexA*<sup>+</sup>) do not form filaments, but exhibit cold sensitivity for growth. In contrast, a *lexA(Def)* strain grown under the same conditions exhibits filamentation as well as cold sensitivity for growth. High levels of UmuD and UmuC cause cold sensitivity for growth possibly by inhibiting DNA synthesis (31) but cause filamentation through a distinct pathway in conjunction with another SOS-regulated protein.

The growth inhibition and filamentation caused by constitutive expression of *umuDC* at 30°C may be the result of a significant, yet previously undescribed activity of UmuD and UmuC. Our observations demonstrate that physiologically relevant levels of UmuD and UmuC can interfere with cell division in conjunction with another LexA-regulated protein(s) in a *sulA*- and *sfiC*-independent manner. In addition, the fact that higher levels of UmuD and UmuC appear to inhibit DNA synthesis and nucleoid segregation raises the possibility that these proteins play an



as yet undetected role in modulating DNA synthesis when they are present at physiological levels. This leads us to suggest that UmuD and UmuC may function in cell cycle regulation, more specifically, that they may serve to coordinate the progression of the cell cycle and DNA replication with DNA repair processes in an SOS-induced cell. It seems most likely that UmuD and UmuC perform this novel function during induction of the SOS response, since at least one other *lexA*-regulated protein, other than Sula and SfiC, is required for UmuDC-mediated inhibition of cell division and since cellular levels of UmuD and UmuC are highest after SOS induction (51) . This hypothesis is also supported by the observation that one of the phenotypes of the *umuC125* mutation, besides its inability to confer cold sensitivity, is increased sensitivity to UV light (30), which may reflect a failure of this mutant to couple the cell cycle to DNA repair.

Based on the lack of an obvious evolutionary selection for proteins that promote mutagenesis, and on the fact that there are several bacterial species that contain *umuDC* homologs but are not mutable by UV (41), it has been previously suggested that the UmuD and UmuC proteins may play a role(s) in the bacterial cell in addition to their role in SOS mutagenesis (52). Our data supports the hypothesis that there is indeed another role for the UmuD and UmuC proteins in the physiology of the bacterial cell. This is particularly interesting in light of the recent discovery of the *dinP* gene, an *E. coli* homolog of *umuC* (35). The function of the *dinP* gene is currently unknown, however, one interesting possibility is that the elevated levels of UmuD and UmuC that lead to cold sensitivity for growth may actually mimic the normal activity of DinP. A possible involvement of the UmuD and UmuC proteins in cell cycle control is highly intriguing, since prokaryotes lack a eukaryotic-like cell-cycle checkpoint system yet must face the same issues with respect to controlling DNA replication and cell division after DNA damage and other forms of stress.

**Acknowledgments.** We would like to thank L.M. Guzman and J. Beckwith for providing us with the plasmid pBAD24 prior to publication and C. Kaiser for the use of his microscope.

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**Table 1.** List of strains and plasmids.

Strain	Relevant Genotype	Source/Reference
GW1000	<i>recA441, sulA11, sfiC2</i>	(1)
RW82	$\Delta(\text{umuDC})595::\text{cat}$	(50)
JL2301	<i>lexA300(Def)::spc</i>	(21)
GW6752	$\Delta(\text{recA-srlR})306::\text{Tn10}$	(34)
GW6900	<i>recA430, srl::Tn10</i>	(34)
LMG194	$\Delta\text{ara714, leu}::\text{Tn10}$	(20)
GW2771	GW1000, <i>recA</i> <sup>+</sup>	J. Kreuger
GW8018	GW1000, <i>lexA300(Def)::spc</i>	GW1000 x P1(JL2301)
GW8023	GW2771, $\Delta(\text{umuDC})595::\text{cat}$	GW2771 x P1(RW82)
GW8024	GW8023, <i>lexA300(Def)::spc</i>	GW8023 x P1(JL2301)
GW8025	GW8018, $\Delta(\text{umuDC})595::\text{cat}$	GW8018 x P1(RW82)
GW8026	GW8018, $\Delta(\text{recA-srlR})306::\text{Tn10}$	GW8018 x P1(GW6752)
GW8027	GW8018, <i>recA430, srl::Tn10</i>	GW8018 x P1(GW6900)
GW8028	GW8023, $\Delta\text{ara714, leu}::\text{Tn10}$	GW8023 x P1(LMG194)
GW8029	GW8025, $\Delta\text{ara714, leu}::\text{Tn10}$	GW8025 x P1(LMG194)

Plasmid	Comments	Source/Reference
pBR322		New England Biolabs
pBR322/kan	Km <sup>r</sup> , Tc <sup>s</sup>	This work
pSE115	<i>umuDC</i> ; pSC101	(15)
pSE117	<i>umuDC</i> ; pBR322	(15)
pGW3751	<i>umuD'</i> C; pBR322	(13)
pLM206	<i>umuD</i> ; pBR322	(31)
pGW2101	<i>umuDC</i> ; pBR322 $\Delta\text{rop}$	(34)
pGW2101/kan	Km <sup>r</sup>	This work
pGW2111	pGW2101 derivative, UmuD GK25 (noncleavable)	(34)
pGW2112	pGW2101 derivative, UmuD SA60 (noncleavable)	(34)
pGW2115	pGW2101 derivative, UmuD KA97 (noncleavable)	(34)
pBAD24	P <sub>BAD</sub> expression vector	(20)
pTO2	P <sub>BAD</sub> - <i>umuDC</i>	This work
pTO3	pTO2 derivative, Km <sup>r</sup> , Ap <sup>s</sup>	This work
pZAQ	<i>ftsQAZ</i> ; pBR322	(48)

**Table 2.** Transformation efficiency of *umuDC*-expressing plasmids in *lexA*<sup>+</sup> and *lexA*(Def) strains.<sup>a</sup>

strain	plasmid	transformants per ml		
		30°C	42°C	30°C/42°C
GW2771 <i>lexA</i> <sup>+</sup> <i>recA</i> <sup>+</sup>	pBR322/kan	4.3x10 <sup>5</sup>	3.5x10 <sup>5</sup>	1.2
	<i>umuDC</i> ; pSC101	3.3x10 <sup>4</sup>	2.6x10 <sup>4</sup>	1.3
	<i>umuDC</i> ; pBR322	6.5x10 <sup>4</sup>	5.4x10 <sup>4</sup>	1.2
	<i>umuD'</i> C; pBR322	7.8x10 <sup>4</sup>	6.2x10 <sup>4</sup>	1.3
	<i>umuDC</i> ; pBR322 $\Delta$ <i>rop</i> , Km <sup>R</sup>	9.5x10 <sup>4</sup>	9.3x10 <sup>4</sup>	1.0
	<i>umuD</i> ; pBR322	1.6x10 <sup>5</sup>	1.2x10 <sup>5</sup>	1.3
GW8024 <i>lexA</i> (Def) <i>recA</i> <sup>+</sup>	pBR322/kan	2.4x10 <sup>5</sup>	2.5x10 <sup>5</sup>	0.95
	<i>umuDC</i> ; pSC101	2.2x10 <sup>3</sup>	3.4x10 <sup>4</sup>	0.066
	<i>umuDC</i> ; pBR322	3	1.3x10 <sup>5</sup>	0.000024
	<i>umuD'</i> C; pBR322	4.7x10 <sup>2</sup>	4.0x10 <sup>4</sup>	0.012
	<i>umuD</i> ; pBR322	1.0x10 <sup>5</sup>	1.1x10 <sup>5</sup>	0.94
GW8018 <i>lexA</i> (Def) <i>recA441</i>	pBR322/kan	1.7x10 <sup>6</sup>	1.8x10 <sup>6</sup>	0.96
	<i>umuDC</i> ; pSC101	1.0x10 <sup>4</sup>	3.4x10 <sup>4</sup>	0.29
	<i>umuDC</i> ; pBR322	15	3.4x10 <sup>5</sup>	0.000044
	<i>umuD'</i> C; pBR322	1.4x10 <sup>4</sup>	9.2x10 <sup>4</sup>	0.16
	<i>umuDC</i> ; pBR322 $\Delta$ <i>rop</i> , Km <sup>R</sup>	3	770	0.0039
	<i>umuD</i> ; pBR322	3.6x10 <sup>5</sup>	3.8x10 <sup>5</sup>	0.94
GW8026 <i>lexA</i> (Def) $\Delta$ <i>recA</i>	pBR322/kan	1.6x10 <sup>5</sup>	3.0x10 <sup>5</sup>	0.53
	<i>umuDC</i> ; pSC101	8.4x10 <sup>2</sup>	1.6x10 <sup>4</sup>	0.053
	<i>umuDC</i> ; pBR322	0	7.2x10 <sup>4</sup>	<0.000014
	<i>umuD'</i> C; pBR322	4	3.4x10 <sup>4</sup>	0.00012
	<i>umuD</i> ; pBR322	6.0x10 <sup>4</sup>	1.1x10 <sup>5</sup>	0.55
GW8027 <i>lexA</i> (Def) <i>recA430</i>	pBR322/kan	6.9x10 <sup>4</sup>	6.9x10 <sup>4</sup>	1.0
	<i>umuDC</i> ; pSC101	7.7x10 <sup>2</sup>	1.3x10 <sup>4</sup>	0.061
	<i>umuDC</i> ; pBR322	0	3.6x10 <sup>4</sup>	<0.000028
	<i>umuD'</i> C; pBR322	2	1.4x10 <sup>4</sup>	0.00014
	<i>umuD</i> ; pBR322	6.1x10 <sup>4</sup>	6.5x10 <sup>4</sup>	0.93

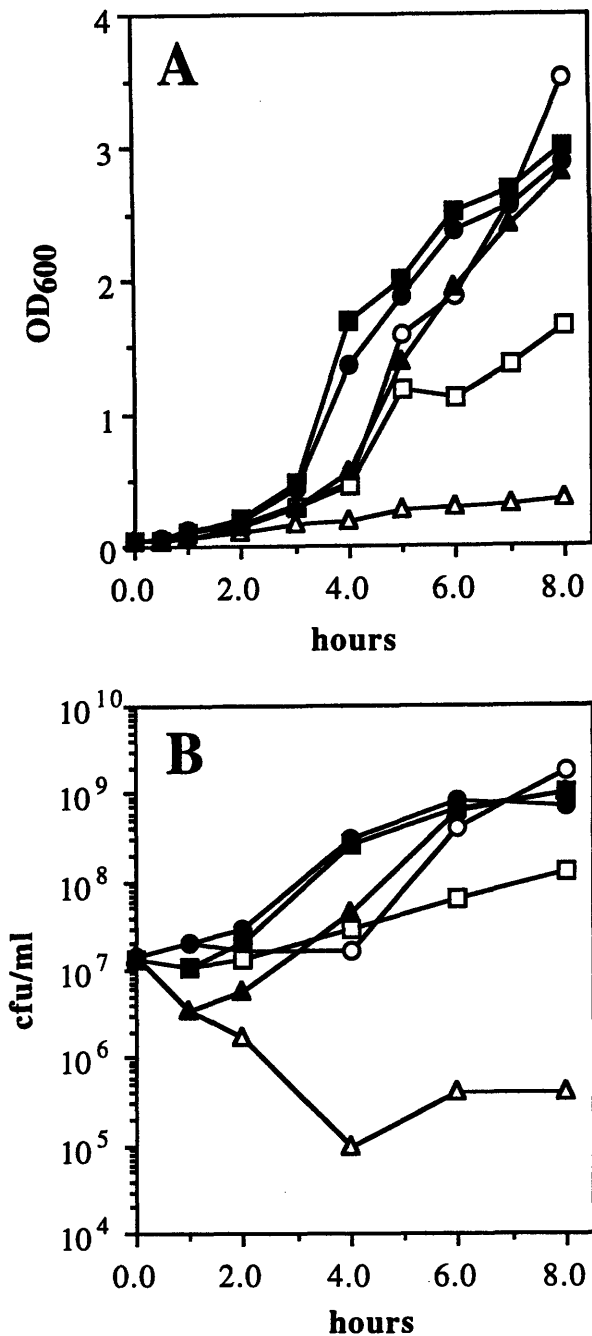
<sup>a</sup> The data in this table are a representative set of results. These experiments were repeated two to four times. The standard deviation of the average of the results from multiple experiments was never more than 5% of the numbers shown.



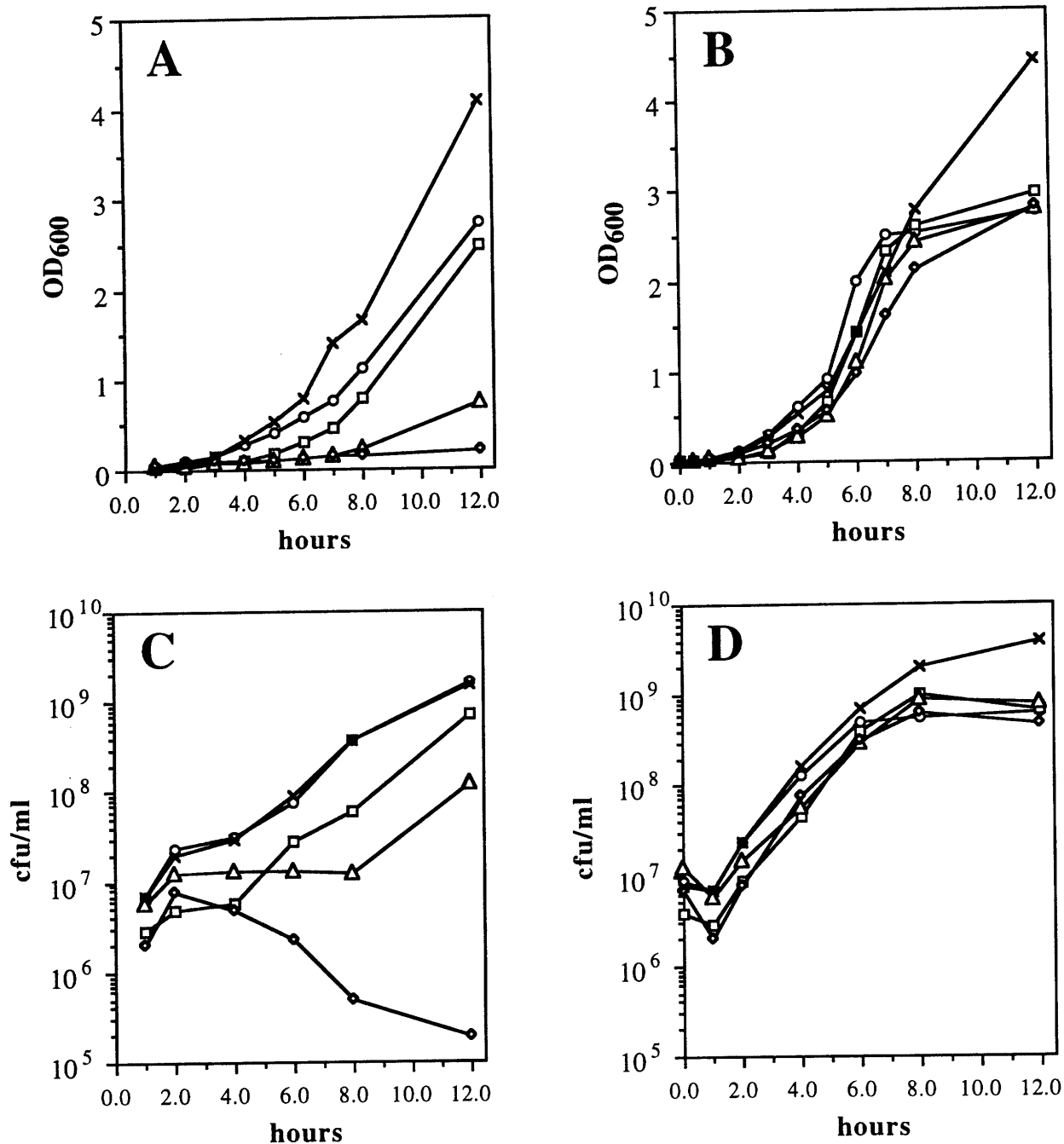
**Table 3.** Transformation efficiency of non-cleavable *umuD* alleles in *lexA*<sup>+</sup> and *lexA*(Def) strains.<sup>a</sup>

strain	plasmid	transformants per ml		
		30°C	42°C	30°C/42°C
GW2771 <i>lexA</i> <sup>+</sup> <i>recA</i> <sup>+</sup>	pBR322	3.6x10 <sup>4</sup>	3.9x10 <sup>4</sup>	0.92
	pGW2101 [ <i>umuDC</i> ]	4.6x10 <sup>4</sup>	3.5x10 <sup>4</sup>	1.3
	pGW2111 [ <i>umuD</i> (GK25) C]	2.0x10 <sup>5</sup>	1.6x10 <sup>5</sup>	1.2
	pGW2112 [ <i>umuD</i> (SA60) C]	1.4x10 <sup>5</sup>	1.1x10 <sup>5</sup>	1.3
	pGW2115 [ <i>umuD</i> (KA97) C]	3.0x10 <sup>5</sup>	2.4x10 <sup>5</sup>	1.2
GW8018 <i>lexA</i> (Def) <i>recA441</i>	pBR322	1.4x10 <sup>5</sup>	1.3x10 <sup>5</sup>	1.1
	pGW2101 [ <i>umuDC</i> ]	9	9.3x10 <sup>2</sup>	0.0097
	pGW2111 [ <i>umuD</i> (GK25) C]	44	3.0x10 <sup>5</sup>	0.00015
	pGW2112 [ <i>umuD</i> (SA60) C]	5	9.0x10 <sup>2</sup>	0.0056
	pGW2115 [ <i>umuD</i> (KA97) C]	7	3.4x10 <sup>4</sup>	0.00020

<sup>a</sup> The data in this table are a representative set of results. These experiments were repeated two to four times. The standard deviation of the average of the results from multiple experiments was never more than 5% of the numbers shown.



**Figure 1.** Inhibition of growth at 30°C mediated by *umuDC* is more severe than that mediated by *umuD'C*. After inoculation, cultures were grown at 42°C for one hour, and then divided in two with half shifted to 30°C and half remaining at 42°C. Samples were removed at the indicated times for analyses by optical density, colony forming units per ml, and immunoblot (see Fig. 3). Cells grown at 30°C are represented by open symbols and cells grown at 42°C are represented by closed symbols. GW8025 [*lexA(Def) ΔumuDC*] carrying: (○, ●) pBR322, (□, ■) pGW3751 [*umuD'C*; pBR322] and (△, ▲) pSE117 [*umuDC*; pBR322]. A.) Absorbance at 600 nm measured as a function of time, B.) colony forming units per ml measured as a function of time.

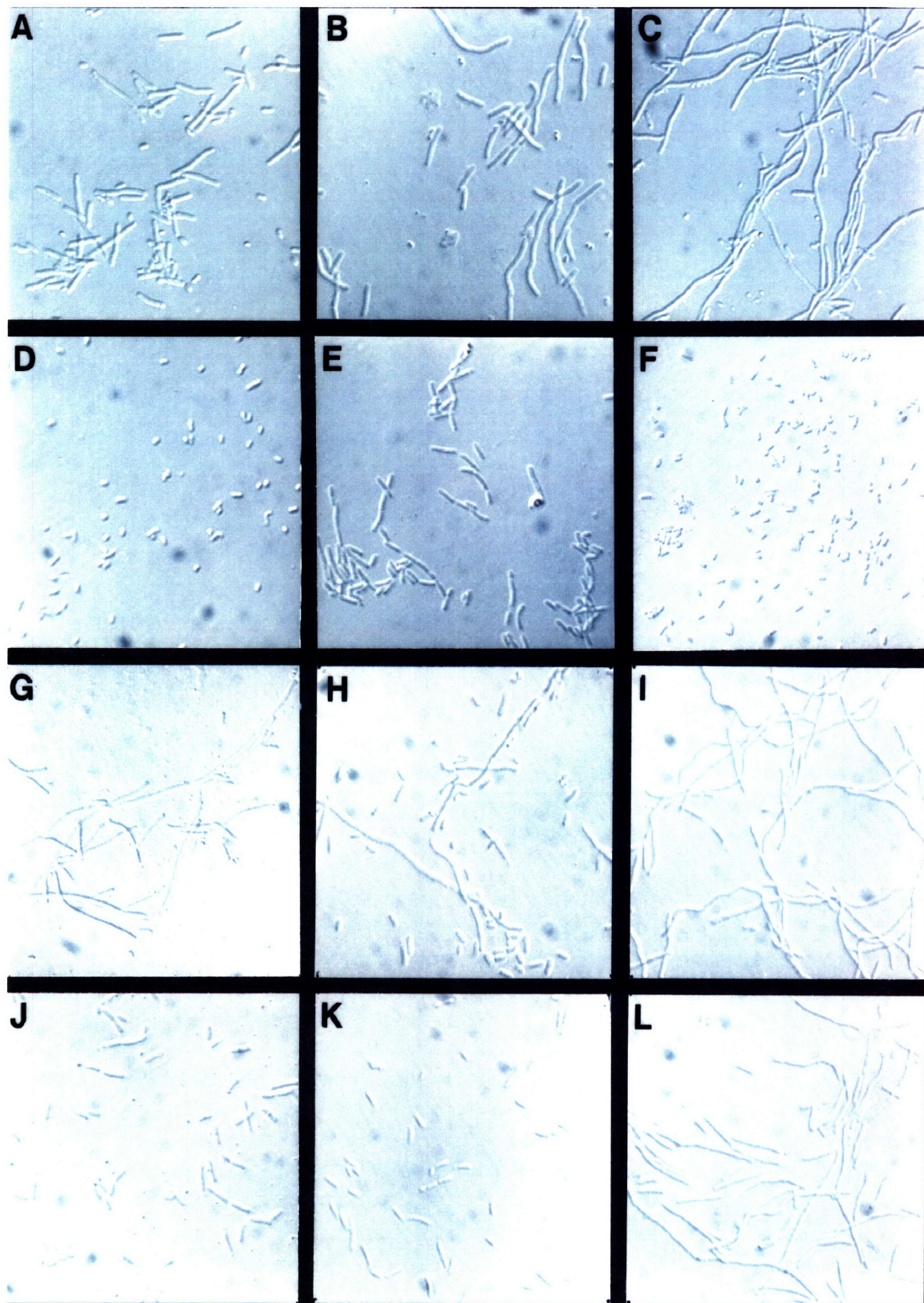


**Figure 2.** Correlation of *umuDC* dosage with cold sensitivity for growth. After inoculation, cultures were grown at 42°C for one hour, and then divided in two with half shifted to 30°C and half remaining at 42°C. Samples were removed at the indicated times for analyses by optical density, colony forming units per ml, immunoblot (see Fig. 3) and microscopy (see Figs. 4 and 5). All strains were *lexA*(Def) and: (○)  $\Delta umuDC$  pBR322, (□)  $umuD^+C^+$  pBR322, (△)  $\Delta umuDC$  pSE115 [*umuDC*; pSC101], (◇)  $\Delta umuDC$  pSE117 [*umuDC*; pBR322], (×)  $\Delta umuDC$  pLM206 [*umuD*; pBR322]. A.) Absorbance at 600 nm at 30°C measured as a function of time, B.) absorbance at 600 nm at 42°C measured as a function of time, C.) colony forming units per ml at 30°C measured as a function of time, D.) colony forming units per ml at 42°C measured as a function of time.

**Figure 3.** Immunoblot analyses of UmuD/D' protein levels. Samples from the growth curves shown in Figs. 1, 2 and 7 were removed at the indicated time, equalized for optical density, and analyzed by immunoblot using antibodies to UmuD/D'. A.) GW8025 [*lexA*(Def) *recA441*  $\Delta$ *umuDC*] pBR322, B.) GW8018 [*lexA*(Def) *recA441 umuD*<sup>+</sup>*C*<sup>+</sup>] pBR322, C.) GW8025 pSE115 [*umuDC*; pSC101], D.) GW8025 pSE117 [*umuDC*; pBR322], E.) GW8025 pGW3751 [*umuD*'*C*; pBR322], F.) GW8025 pLM206 [*umuD*; pBR322], G.) GW8028 [*lexA*<sup>+</sup> *recA*<sup>+</sup>  $\Delta$ *umuDC*  $\Delta$ *ara*] pTO3 [*P*<sub>BAD</sub>-*umuDC*] in the absence of arabinose, H.) GW8028 pTO3 in the presence of 0.2% arabinose, I.) GW8029 [*lexA*(Def)  $\Delta$ *umuDC*  $\Delta$ *ara*] pTO3 in the absence of arabinose, J.) GW8029 pTO3 in the presence of 0.2% arabinose.

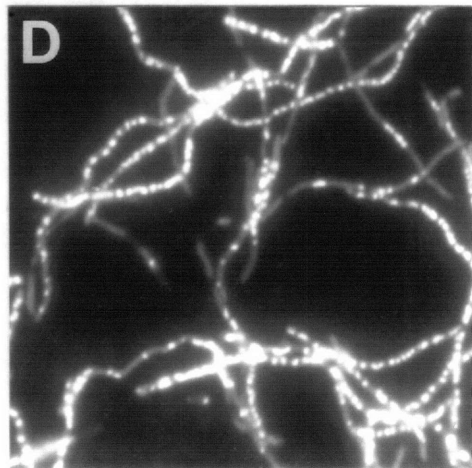
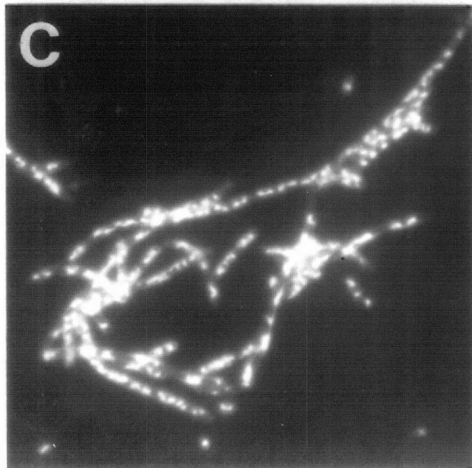
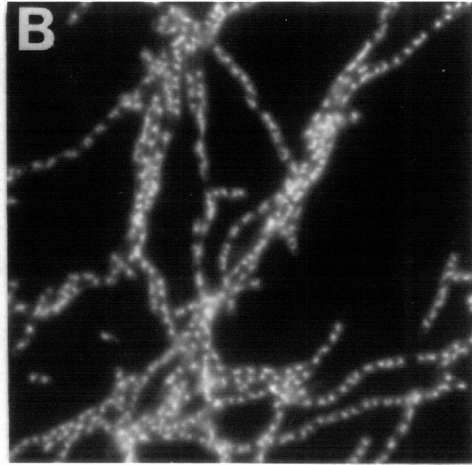
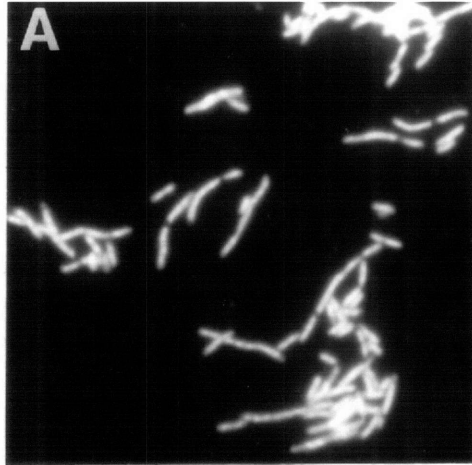


**Figure 4.** Cellular morphologies associated with UmuDC-mediated cold sensitivity. Samples were removed during the growth curve analyses shown in Figs. 2 and 7 at 6 hrs unless otherwise indicated, equalized for optical density and fixed on microscope slides. Microscopic analyses were done on a Zeiss Axioscope with Nomarski optics. A.) GW8018 [*lexA*(Def) *umuD*<sup>+</sup>*C*<sup>+</sup>] pBR322, 30°C, 4 hrs, B.) GW8018 pBR322, 30°C, 6 hrs, C.) GW8018 pBR322, 30°C, 8 hrs, D.) GW8018 pBR322, 42°C, E.) GW8025 [*lexA*(Def)  $\Delta$ *umuDC*] pBR322, 30°C, F.) GW8025 pSE115 [*umuDC*; pSC101], 42°C, G.) GW8025 pSE115, 30°C, H.) GW8025 pSE117 [*umuDC*; pBR322], 42°C, I.) GW8025 pSE117, 30°C, J.) GW8028 [*lexA*<sup>+</sup>  $\Delta$ *umuDC*  $\Delta$ *ara*] pTO3 [P<sub>BAD</sub>-*umuDC*] in the presence of 0.2% arabinose, 30°C, K.) GW8029 [*lexA*(Def)  $\Delta$ *umuDC*  $\Delta$ *ara*] pTO3 in the absence of arabinose, 30°C, L.) GW8029 pTO3 in the presence of arabinose, 30°C.

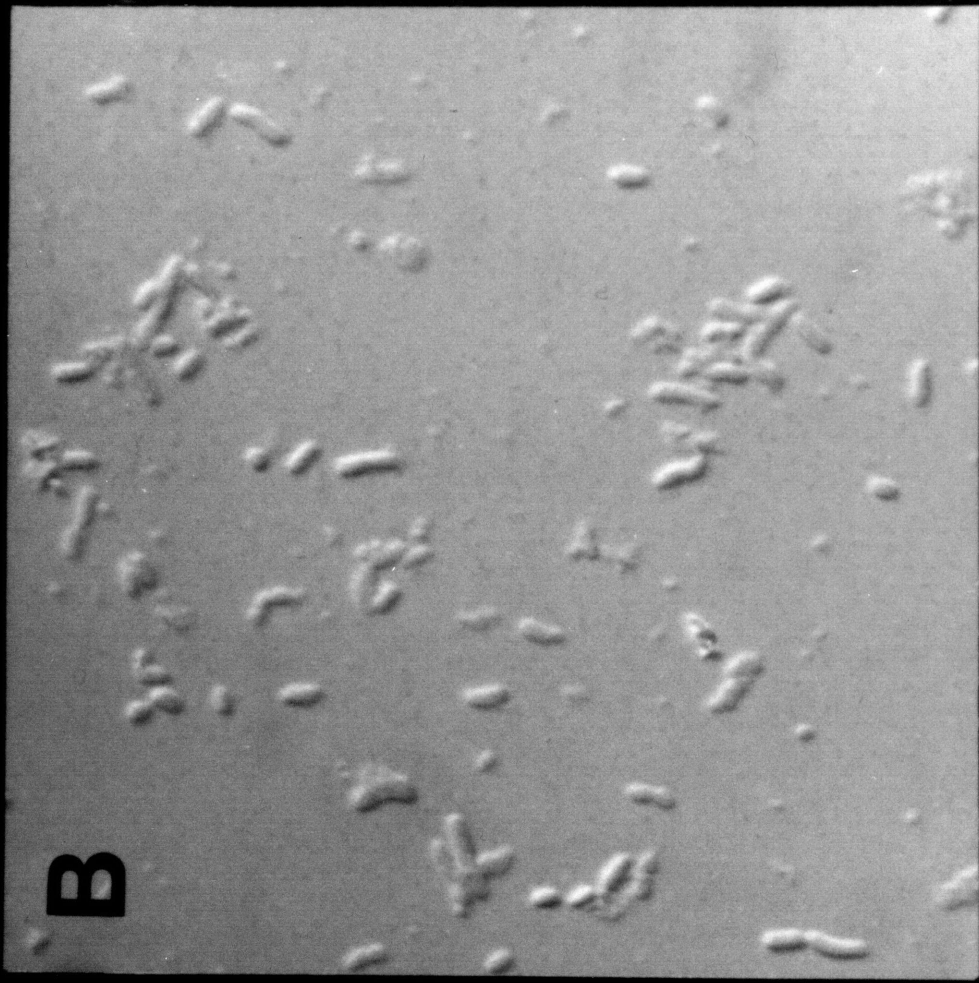
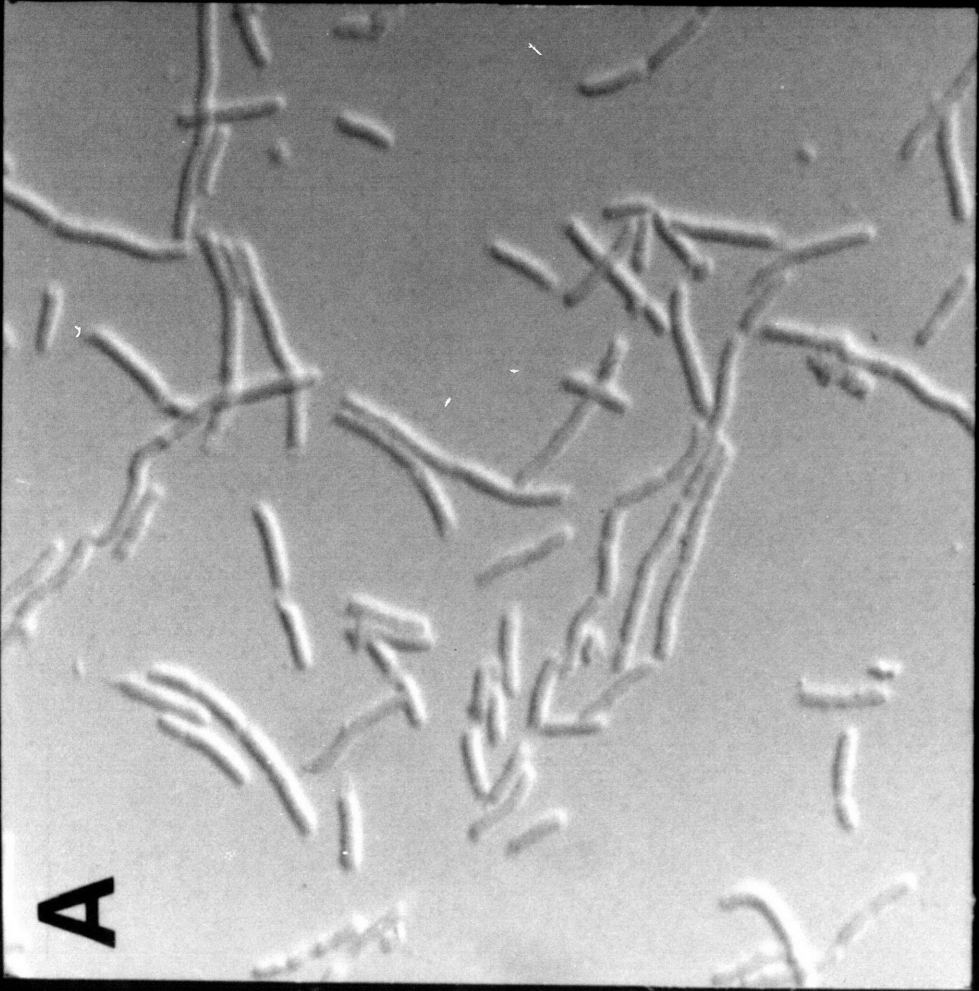


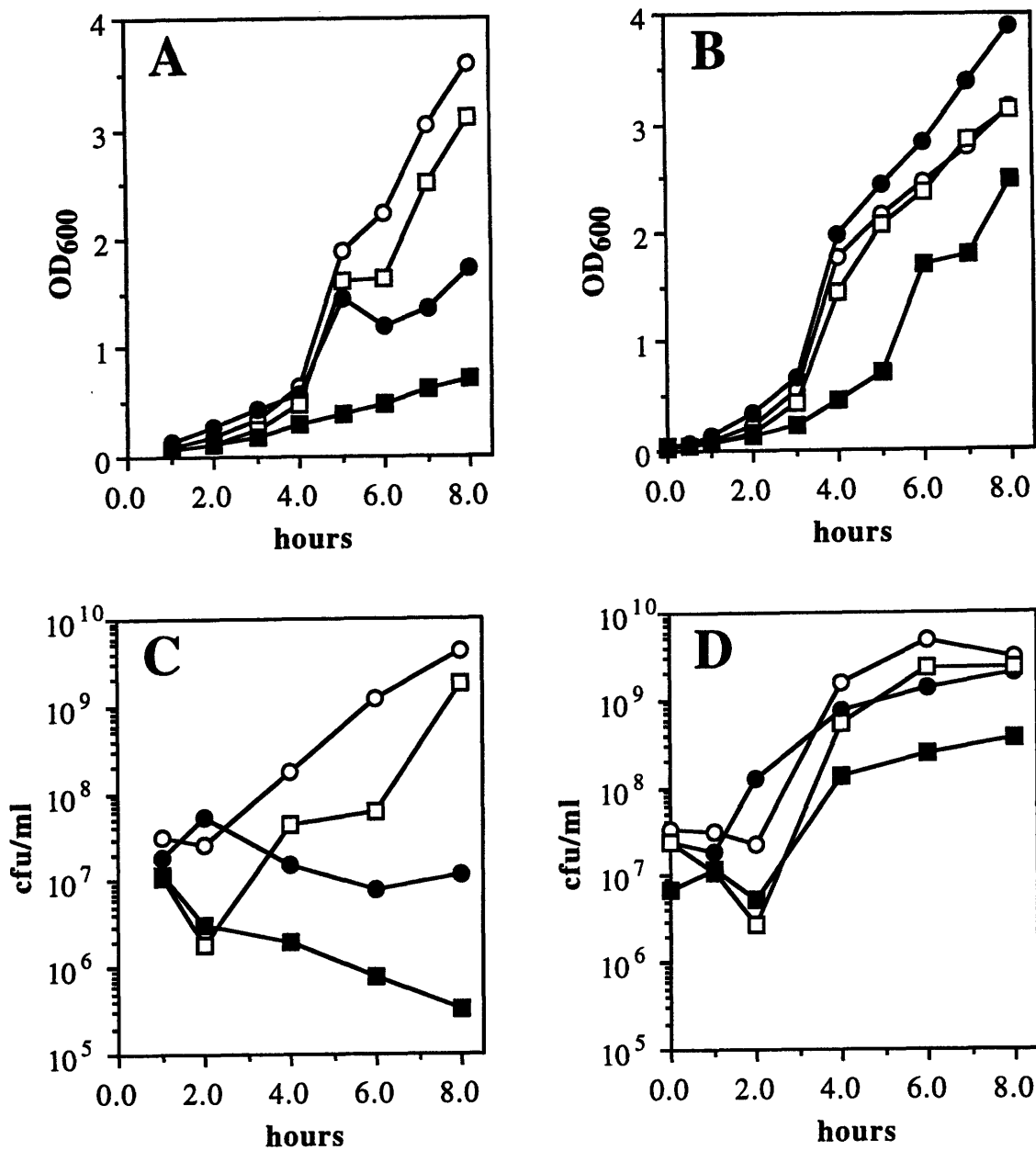
**Figure 5.** Nucleoid morphologies associated with UmuDC-mediated cold sensitivity. Samples were removed during the growth curve analyses shown in Figs. 2 and 7 at 6 hrs unless otherwise indicated, equalized for optical density and fixed on microscope slides. Following the addition of DAPI, fluorescent microscopic analyses were done on a Zeiss axioscope. A.) GW8025 [*lexA(Def) ΔumuDC*] pBR322, 30°C, B.) GW8018 [*lexA(Def) umuD<sup>+</sup>C<sup>+</sup>*] pBR322, 30°C, 8 hrs, C.) GW8025 pSE115 [*umuDC*; pSC101], 30°C, D.) GW8025 pSE117 [*umuDC*; pBR322], 30°C.





**Figure 6.** Overexpression of the *ftsQAZ* operon suppresses UmuDC-mediated filamentation. Samples were removed from cultures of GW8025 [*lexA*(Def)  $\Delta$ *umuDC*] carrying pSE115 (*umuDC*; pSC101) or pSE115 and pZAQ (*ftsQAZ*; pBR322) after 3.5 hrs of growth, equalized for optical density, and prepared for microscopic analyses as described in Materials and Methods. The cellular morphology of these cultures was analyzed using Nomarski optics on a Zeiss Axioscope. A.) GW8025 pSE115, 30°C, B.) GW8025 pSE115 pZAQ, 30°C.





**Figure 7.** Constitutive expression of *umuDC* confers cold sensitivity in the absence of any other SOS-regulated proteins. After inoculation, cultures were grown at 42°C for one hour, divided in half with half shifted to 30°C and half remaining at 42°C. Samples were removed at the indicated times for analyses by optical density, colony forming units per ml, immunoblot (see Fig. 3) and microscopy (see Fig. 4). Cultures grown in the absence of arabinose are indicated by open symbols and cultures grown in the presence of arabinose to induce expression of *umuDC* are indicated by closed symbols. All strains were  $\Delta umuDC \Delta ara$  and: (○, ●) *lexA*<sup>+</sup> pTO3 [P<sub>BAD</sub>-*umuDC*], (□, ■) *lexA*(Def) pTO3. A.) Absorbance at 600 nm at 30°C measured as a function of time, B.) absorbance at 600 nm at 42°C measured as a function of time, C.) colony forming units per ml at 30°C measured as a function of time, D.) colony forming units per ml at 42°C measured as a function of time.

## Chapter 3

The *umuD*<sup>+</sup>*C*<sup>+</sup> Gene Products of *E. coli* Increase DNA Damage Tolerance in Stationary Phase by Regulating the Transition to Exponential Growth

This work was done in collaboration with Tim Opperman.

## Abstract

Mechanisms which temporarily block cell cycle progression have been shown to play an important role in DNA damage tolerance in eukaryotes. The evidence presented in this paper suggests that the *umuDC* gene products in *Escherichia coli* may act in an analogous mechanism in prokaryotes. The *umuDC* gene products, whose expression is induced by DNA damaging treatments, have been extensively characterized for their role in SOS mutagenesis. The study of the growth inhibition at 30°C associated with constitutive expression of the *umuDC* gene products from a multicopy plasmid has suggested that this phenomenon is a reflection of a novel activity of the *umuDC* gene products. The inhibition of growth at 30°C by high levels of the *umuDC* gene products is specifically due to the inhibition of the transition from stationary phase to exponential growth at the restrictive temperature. Consistent with this finding, an immediate *umuDC*-dependent inhibition of DNA synthesis is observed after a shift to 30°C in lag phase cells, but not in exponentially growing cells. Physiologically relevant levels of the *umuDC* gene products modulate the transition from stationary phase to exponential growth in *E. coli* cells that have experienced DNA damage in stationary phase. This activity is correlated with an increase in survival after UV irradiation which suggests that it contributes to increased DNA damage tolerance. The *umuDC* gene products appear to counteract a function of Fis and thereby regulate the transition to exponential growth in cells that have experienced DNA damage in stationary phase.

## Introduction

Sequences with homology to *umuC* of *Escherichia coli* have been identified in eukaryotes [*Saccharomyces cerevisiae* (21), *Caenorhabditis elegans* (20) and possibly humans (20)], archaea [*Sulfolobus solfataricus* (20)], as well as both Gram negative bacteria [*Salmonella typhimurium* (30), several species of Enterobacteria (39)] and Gram positive bacteria [*Streptomyces coelicolor* (26), *Bacillus subtilis* (20)], demonstrating that this is an ancient family of genes which is widely distributed in nature. In *Escherichia coli*, the *umuC* gene product, together with the *umuD* gene product, is required for SOS mutagenesis, the mutagenesis resulting from exposure to UV light and various chemicals (19, 44). The possibility that the gene products of the *umuDC* operon may have additional roles, beyond their well-characterized role in SOS mutagenesis, has previously been suggested by the discovery that several bacterial species that are nonmutable by UV light carry *umuDC* homologs (39, 50). This observation argues against the possibility that the sole selective pressure to maintain these genes is the requirement for their activity in promoting mutagenesis. With respect to this viewpoint, it is interesting that the *E. coli* *dinP* gene encodes a *umuC* homolog that is not required for SOS mutagenesis (32) and that *Salmonella typhimurium* encodes two operons homologous to *umuDC*, *umuDC<sub>ST</sub>* and *samAB* (30, 42), only one of which (*umuDC<sub>ST</sub>*) contributes significantly to SOS mutagenesis in *S. typhimurium* (31).

The *umuDC* operon is a member of the SOS regulon whose expression is regulated by RecA and the transcriptional repressor LexA. Following a DNA damaging treatment, the cleavage of LexA is mediated by RecA\*, the activated form of RecA, to induce the expression of the SOS regulon (14). RecA\* activity is required for at least two additional steps in SOS mutagenesis. RecA\* facilitates the autodigestion of UmuD to UmuD', the 12 kDa carboxyl terminal fragment of UmuD, which in combination with UmuC is active in SOS mutagenesis (5, 29, 40). In addition, RecA plays another pivotal, but poorly characterized, role in SOS mutagenesis in addition to mediating the cleavage of LexA and UmuD (14). SOS mutagenesis

appears to arise as a consequence of errors introduced during the process of replicative bypass of a DNA lesion by DNA polymerase III modified by UmuD', UmuC and RecA (36).

Several physiologically observable effects of the *umuD* and *umuC* gene products besides their role in SOS mutagenesis have been reported (18, 23, 24, 43, 48). We have analyzed one of these phenomena, *umuDC*-mediated cold sensitivity, in greater detail (33) in order to gain possible insights into other functions of the *umuDC* gene products. Constitutive expression of *umuDC* from a multicopy plasmid in *E. coli* causes growth inhibition at 30°C but not at 42°C, which is associated with an inhibition of DNA replication at the restrictive temperature (24, 33). We have previously shown that *umuDC*-mediated cold sensitivity is genetically distinct from SOS mutagenesis (33). Intact UmuD, which is inactive in SOS mutagenesis (29), is the form of *umuD* that acts in combination with UmuC to confer cold sensitivity for growth (33). In addition, we have observed that constitutive expression of a single chromosomal copy of *umuDC* leads to the formation of nonseptated filaments at 30°C, indicating that physiologically relevant levels of the *umuDC* gene products inhibit cell division at that temperature (33). This *umuDC*-dependent filamentation is independent of *sulA* and *sfiC*, the known SOS-regulated inhibitors of cell division, and appears to proceed by a novel pathway that functions at 30°C and requires another LexA-regulated function (33). Taken together, the results of our previous analyses of the physiological consequences of constitutive *umuDC* expression suggested that intact UmuD, the form inactive in SOS mutagenesis, acts together with UmuC to inhibit cell division and cell growth (33).

Mechanisms which temporarily block DNA replication and cell cycle progression after exposure to DNA damaging agents have been shown to play an important role in mediating resistance to these agents in eukaryotes (10, 14). It has been suggested that similar, but as yet unidentified, mechanisms of controlling DNA replication and growth after DNA damage would increase survival in prokaryotes (7, 8). In this paper, we describe how our studies of the phenomenon of growth inhibition at 30°C caused by overexpression of the *umuDC* operon have led to our discovery that physiologically relevant levels of the *umuDC* gene products modulate



the transition from stationary phase to exponential growth in *E. coli* cells that have experienced DNA damage in stationary phase. This activity of the *umuDC* gene products is correlated with an increase in survival after UV irradiation. The mechanism of this regulatory effect of the *umuDC* gene products appears to involve the counteraction of an activity of Fis, a small DNA binding protein that has been implicated in the regulation of the transition from stationary phase to exponential growth (34). This is one of the first direct demonstrations of a specific regulated mechanism in prokaryotes that increases survival by inhibiting growth in response to DNA damage.

## Materials and Methods

**Strains and plasmids.** The *E. coli* strains and plasmids used in this work are listed with their relevant features in Table 1. Genetic markers were transferred between strains using P1(vir) transduction performed as described by Miller (25).

**Reagents and Media.** Ampicillin, kanamycin, spectinomycin, chloramphenicol and tetracycline were purchased from Sigma (St. Louis, MO). [methyl-<sup>3</sup>H] thymidine (83 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL). Thymidine and 2' deoxyadenosine were purchased from Sigma (St. Louis, MO). The Western Lights kit for chemiluminescent detection in immunoblot assays was purchased from Tropix (Bedford, MA). The anti-Fis antibody (1) was a kind gift from Reid C. Johnson. The anti-UmuD/D' antibody used in these studies has been previously described (2). Bacteria were grown in LB media or M9 media supplemented with casamino acids as indicated, and LB agar (38). Antibiotics were used at the following concentrations: ampicillin, 150 µg/ml; kanamycin, 25 µg/ml; spectinomycin, 20 µg/ml; chloramphenicol, 30 µg/ml; tetracycline, 12.5 µg/ml.

**Cold sensitivity assays and growth curve analyses.** Quantitative transformation assays, growth curve analyses and immunoblot analyses were performed as described (33). β-galactosidase activity assays were performed at various times during growth curve analyses at 42°C and 30°C of strains containing *lacZ*-gene fusions as described by Miller (25). For experiments involving UV irradiation, cells were grown in M9 media supplemented with 0.4% casamino acids. One ml samples of stationary phase cultures were irradiated with UV light on 60x15 mm petri dishes at the indicated dose. In experiments where growth after nutrient upshift was followed, UV-irradiated and unirradiated stationary phase cultures were immediately diluted 1:100 into fresh M9 media and grown in the dark at 37°C. At the indicated times, serial dilutions from each culture were plated and incubated in the dark to determine colony forming units per ml.

**DNA synthesis assays.** All DNA synthesis assays were performed in duplicate. Briefly, 16 hr-old overnight cultures were diluted 1:125 in LB and grown at 42°C. During lag phase and exponential growth, an aliquot of each culture growing at 42°C was shifted to 30°C. After 10 min and 2 hours, duplicate aliquots of the cultures at 42°C and 30°C were transferred to tubes at 42°C and 30°C, respectively, with continued aeration. DNA synthesis assays were performed essentially as described by Bukau and Walker (4). Incorporation of [<sup>3</sup>H methyl] thymidine into DNA at each time point was normalized to the OD<sub>600</sub> of the culture at the start of the assay.

## Results

**Constitutive expression of *umuD*<sup>+</sup>*C*<sup>+</sup> inhibits the transition from stationary phase to exponential growth at 30°C.** Following up on our previous observation that constitutive expression of *umuD*<sup>+</sup>*C*<sup>+</sup> from a multicopy plasmid leads to growth inhibition and filamentation at 30°C but not at 42°C (24, 33), we examined whether constitutive expression of *umuD*<sup>+</sup>*C*<sup>+</sup> inhibits bacterial growth at 30°C in both exponentially growing and lag phase cells. A culture of a strain that constitutively expresses *umuD*<sup>+</sup>*C*<sup>+</sup> from a multicopy plasmid due to the presence of a *lexA*(Def) mutation [GW8025 (pSE117)] as well as a corresponding strain that lacks *umuDC* [GW8025 pBR322kan] were diluted after 11 hours in stationary phase into fresh LB media and grown at 42°C. At various times during growth, a portion of each culture was shifted to 30°C and subsequent growth was monitored by following OD<sub>600</sub>. Maximal *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent growth inhibition at 30°C is observed when the strain that constitutively expresses *umuD*<sup>+</sup>*C*<sup>+</sup> is shifted to 30°C during lag phase, i.e. within one hour of dilution into fresh media (Fig. 1A). When this strain is shifted to 30°C during exponential growth, *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent growth inhibition is markedly reduced. In contrast, similar rates of growth were observed for the control strain lacking *umuDC* shifted to 30°C during either lag phase or exponential growth (data not shown). These results suggest that cells in lag phase at 30°C are susceptible to the inhibitory effects on growth of the overexpressed *umuD*<sup>+</sup>*C*<sup>+</sup> gene products, while exponentially growing cells are largely resistant to this inhibitory effect. Taken together, these analyses indicate that high levels of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products interfere with the growth-phase transition from lag phase to exponential growth at 30°C.

The possibility that cells growing exponentially are less susceptible to *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent growth inhibition at 30°C is due to the increased cell density of the culture was ruled out by the results of the experiment shown in Fig. 1B. Growth inhibition was monitored after a culture of [GW8025 (pSE117)] that was in stationary phase for 11 hours was diluted 1:20, 1:125, or 1:400 in fresh media, grown for one hour at 42°C, and shifted to 30°C during lag phase. The degree of growth inhibition at 30°C for all three cultures constitutively expressing *umuD*<sup>+</sup>*C*<sup>+</sup>

was essentially the same, and markedly distinct from the rapid growth at 30°C of the parent strain which carries a vector lacking *umuDC* [GW8025 (pBR322kan)]. This indicates that *umuD<sup>+</sup>C<sup>+</sup>*-dependent growth inhibition at 30°C is growth-phase dependent, but cell density-independent.

**The *umuD<sup>+</sup>C<sup>+</sup>* gene products inhibit DNA synthesis at 30°C.** It has been previously shown that *umuD<sup>+</sup>C<sup>+</sup>*-dependent growth inhibition at 30°C is associated with a rapid, reversible inhibition of DNA synthesis upon a shift to the restrictive temperature (24). To test the possibility that the rapid inhibition of DNA synthesis at 30°C is dependent on the growth phase of the culture, we compared the rate of DNA synthesis of a strain that constitutively expresses *umuD<sup>+</sup>C<sup>+</sup>* from a plasmid [GW8025 (pSE117)] to that of a control strain lacking *umuDC* [GW8025 (pBR322kan)] (Fig. 2). No *umuD<sup>+</sup>C<sup>+</sup>*-dependent difference in the rate of DNA synthesis is observed between cultures growing at 42°C either during lag phase (within one hour of dilution into fresh media) or during exponential growth (Fig. 2A-B).

When these cultures are shifted to 30°C and examined after ten minutes, a rapid *umuD<sup>+</sup>C<sup>+</sup>*-dependent decrease in the rate of DNA synthesis (approximately 3-fold) is specific for cells shifted during lag phase (Fig. 2C). No *umuD<sup>+</sup>C<sup>+</sup>*-dependent decrease in the rate of DNA synthesis is observed in the exponentially growing cultures ten minutes after the shift to 30°C (Fig. 2D). In fact, the exponentially growing *umuD<sup>+</sup>C<sup>+</sup>* culture [GW8025 (pSE117)] has a reproducibly slightly higher rate of DNA synthesis than the control strain which lacks *umuDC*. Thus, in a striking parallel to the results from the growth curve analyses, a rapid, approximately 3-fold inhibition of DNA synthesis at 30°C is conferred by the *umuD<sup>+</sup>C<sup>+</sup>* gene products and is only observed when the shift to the restrictive temperature is made during lag phase.

In contrast, prolonged incubation (2 hours) at the restrictive temperature of 30°C leads to a more general *umuD<sup>+</sup>C<sup>+</sup>*-mediated inhibition of DNA synthesis which is independent of the growth phase of the culture (Fig. 2E-F). When lag phase and exponentially growing cultures are shifted to 30°C and incubated for 2 hours, DNA synthesis is inhibited 5-fold and 2-fold, respectively, in the presence of the overexpressed *umuD<sup>+</sup>C<sup>+</sup>* gene products (Fig. 2E-F). From

these experiments, it is not possible to tell whether the *umuD*<sup>+</sup>*C*<sup>+</sup>-mediated inhibition of DNA synthesis observed in these cultures is due to a direct effect of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products on DNA synthesis itself, or whether they exert their effect on DNA synthesis indirectly by affecting some other aspect of growth control. In any case, it appears that the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products inhibit DNA synthesis by at least two different mechanisms: one which is growth-phase-dependent and another which is growth-phase-independent.

***umuD*<sup>+</sup>*C*<sup>+</sup>-dependent inhibition of growth at 30°C is exacerbated by prolonged starvation.**

The above results suggest that the overexpressed *umuD*<sup>+</sup>*C*<sup>+</sup> gene products inhibit the transition from stationary phase to exponential growth. We therefore tested whether the time-dependent physiological changes that occur during stationary phase influence the *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent inhibition of this growth phase transition. Cultures of *E. coli* strains constitutively expressing *umuD*<sup>+</sup>*C*<sup>+</sup> from a low copy number plasmid [GW8025 (pSE115)] and a higher copy number plasmid [GW8025 (pSE117)] as well as a control strain lacking *umuDC* [GW8025 (pBR322kan)] were grown in LB media at 42°C (Fig. 3A). At various times (8, 16, and 24 hrs) a portion of each culture was diluted 1:125 in fresh media to measure *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent inhibition of growth at 30°C (Fig. 3B-D). This experiment revealed that an increase in the time the cells had spent in stationary phase from approximately 3 to 19 hr leads to a concomitant increase in the degree of *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent inhibition of growth at 30°C. This is most clearly seen in the strain that expresses lower levels of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products because it contains a lower copy plasmid [GW8025 (pSE115)]. The growth at 30°C of this strain [GW8025 (pSE115)] and the control strain lacking *umuDC* are indistinguishable upon nutrient upshift after approximately 3 hrs in stationary phase (Fig. 3B). However, after approximately 11 hrs in stationary phase, a slight *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent growth inhibition at 30°C is seen upon nutrient upshift (Fig. 3C). By the time the cells have been in stationary phase for 19 hours, a marked *umuD*<sup>+</sup>*C*<sup>+</sup>-mediated growth inhibition at 30°C is seen upon nutrient upshift (Fig. 3D). This increase in *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent growth inhibition at 30°C was not due to a significant decrease

in cfu/ml during prolonged incubation in stationary phase (data not shown). Similarly, no significant changes in the levels of UmuD as determined by immunoblot analyses are correlated with the increase in growth inhibition at 30°C (data not shown). This suggests that prolonged incubation in stationary phase results in physiological changes that increase the susceptibility of cells to the *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent inhibition of the transition from stationary phase to exponential growth at 30°C.

**The *umuD*<sup>+</sup>*C*<sup>+</sup> gene products affect survival and growth of UV-irradiated stationary phase cultures upon nutrient upshift.** Our previous analyses of the physiological effects of constitutive expression of the *umuD*<sup>+</sup>*C*<sup>+</sup> operon (33) and the results of the experiments presented above suggest the possibility that the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products may play a physiologically relevant role in inhibiting the transition from stationary phase to exponential growth in cells that experience DNA damage while in stationary phase. This putative activity of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products might be of physiological importance to delay growth and allow repair of DNA damage that accumulated while the cells were not growing. This hypothesis predicts that the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products, expressed from the single chromosomal copy of the operon and regulated in a normal SOS-dependent manner, should inhibit the transition to exponential growth of cells exposed to DNA damaging agents in stationary phase, thereby increasing survival. To test this prediction, we examined the effect of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products on the growth kinetics and survival of UV irradiated stationary phase cultures after dilution into fresh media (nutrient upshift). Cultures that had been in stationary phase for 11 hrs were irradiated with UV light at a dose of 50 J/m<sup>2</sup> and immediately subcultured 1:100 into fresh media at 37°C in the dark. This dose results in an approximately 40% reduction in viability in GW2771 [*umuD*<sup>+</sup>*C*<sup>+</sup>] and an approximately 80% reduction in viability in GW8023 [ $\Delta$ *umuDC*] under these conditions. After nutrient upshift, the colony forming units of each culture were followed during outgrowth (Fig. 4). The growth, assayed by the change in CFU/ml, after nutrient upshift of the unirradiated *umuD*<sup>+</sup>*C*<sup>+</sup> (data not shown) and  $\Delta$ *umuDC* (Fig. 4, closed

circles) cultures is identical. In contrast, there is a clear difference in the growth kinetics of the *umuD<sup>+</sup>C<sup>+</sup>* and  $\Delta$ *umuDC* cultures after UV irradiation in stationary phase. The irradiated *umuD<sup>+</sup>C<sup>+</sup>* culture shows a pronounced increase in the lag phase upon nutrient upshift whereas the growth kinetics of the irradiated  $\Delta$ *umuDC* culture resembles that of the unirradiated cultures. This experiment shows that physiologically relevant levels of the *umuD<sup>+</sup>C<sup>+</sup>* gene products (expressed from the single chromosomal copy of the *umuD<sup>+</sup>C<sup>+</sup>* operon in a *lexA<sup>+</sup>recA<sup>+</sup>* background) can interfere with the growth transition from stationary phase to exponential growth after UV exposure. In addition, the increase in UV sensitivity in stationary phase conferred by the deletion of the *umuDC* operon could be partially due to the loss of this activity, which would then lead to unregulated growth after nutrient upshift prior to the repair of the DNA damage accumulated in stationary phase.

***umuD<sup>+</sup>C<sup>+</sup>*-mediated inhibition of growth at 30°C is independent of RpoS and cAMP control.** Since growth inhibition at 30°C conferred by constitutive expression of *umuD<sup>+</sup>C<sup>+</sup>* from a multicopy plasmid seems to reflect a physiologically relevant activity of the *umuD<sup>+</sup>C<sup>+</sup>* gene products, we used such cold sensitivity assays in an attempt to identify other gene products involved in inhibiting the transition from stationary phase to exponential growth. The finding that *umuD<sup>+</sup>C<sup>+</sup>*-dependent inhibition of growth at 30°C is exacerbated by the physiological changes that occur during prolonged incubation in stationary phase (Fig. 3) suggested that *rpoS* or *rpoS*-regulated proteins may be involved in this phenomenon. *rpoS* encodes the stationary-phase-specific sigma factor  $\sigma^S$  which is responsible for the coordinate regulation of thirty or more genes expressed in response to starvation (22) which enhance long-term survival under starvation conditions. We examined the role of *rpoS<sup>+</sup>* in *umuD<sup>+</sup>C<sup>+</sup>*-mediated cold sensitivity using the previously described quantitative transformation assay, which has proven to be the most sensitive measure of growth inhibition at 30°C (33). In this assay, either pBR322 or a derivative carrying the *umuD<sup>+</sup>C<sup>+</sup>* operon (pSE117) was transformed into *lexA(Def)* strains



which differed in their *rpoS* genotype. The data in Table 2 show that *rpoS* does not play a role in *umuD*<sup>+</sup>*C*<sup>+</sup>-mediated inhibition of growth at 30°C.

The data of Taddei, *et al.* (45) suggest that the SOS regulon is induced under starvation conditions in a cAMP-dependent and *rpoS*-independent manner. We tested the possibility that genes regulated by cAMP may be involved in *umuD*<sup>+</sup>*C*<sup>+</sup>-mediated inhibition of growth at 30°C by examining the effect of the  $\Delta$ *cya* mutation, which abolishes the ability to produce cAMP, on this phenomenon. We found that the  $\Delta$ *cya* mutation has no effect on *umuD*<sup>+</sup>*C*<sup>+</sup>-mediated growth inhibition at 30°C in a quantitative transformation assay (data not shown). Thus, *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent inhibition of growth at 30°C proceeds by a pathway that is independent of *rpoS*- and *cya*- regulated genes.

**Fis alleviates *umuD*<sup>+</sup>*C*<sup>+</sup>-mediated inhibition of growth at 30°C.** The growth-phase specificity of *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent inhibition of growth at 30°C suggests that the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products may interfere with the expression or function of gene products required during the growth-phase transition from stationary phase to exponential growth. The *fis* gene product undergoes a striking 500-fold induction in the first two cell divisions after nutrient upshift (1) and is the best-characterized protein to date known to play a role in the transition from stationary phase to exponential growth. We therefore examined whether Fis plays a role in *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent growth inhibition at 30°C by employing a quantitative transformation assay (33). The data in Table 3 show that there is a 400-fold increase in the cold sensitivity conferred by pSE117 (*umuD*<sup>+</sup>*C*<sup>+</sup>; pBR322) in a *lexA*(Def) *fis* strain relative to that in a *lexA*(Def) *fis*<sup>+</sup> strain. Reciprocally, overexpression of Fis from the plasmid pRJ400 partially suppresses *umuD*<sup>+</sup>*C*<sup>+</sup>-mediated inhibition of growth at 30°C (data not shown). These results suggest that high levels of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products inhibit growth at 30°C by antagonizing Fis or a Fis-regulated function.

To determine whether high levels of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products directly alter Fis protein levels or Fis activity at 30°C, we analyzed the levels of Fis protein during growth of a

culture constitutively expressing *umuD*<sup>+</sup>*C*<sup>+</sup> by immunoblot analyses with anti-Fis antibody (1). We also examined whether constitutive expression of *umuD*<sup>+</sup>*C*<sup>+</sup> affected the expression of two Fis-regulated *lacZ* fusions, *frg-733::TnphoA'-4* and *proP-104::TnphoA'-4* (51, 52). No change in Fis protein levels, the pattern of Fis protein induction or Fis regulation of either of these fusions was conferred by high levels of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products (data not shown). Therefore, the influence of Fis upon *umuD*<sup>+</sup>*C*<sup>+</sup>-mediated growth inhibition at 30°C occurs either through another activity of Fis or indirectly through a gene regulated by Fis.

***fis* mutations suppress UV sensitivity of  $\Delta$ *umuDC* strains.** The results presented above suggest that the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products inhibit the transition from stationary phase to exponential growth by counteracting a Fis-dependent function. Furthermore, the inhibition of growth after UV irradiation appears to be a physiologically relevant activity of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products. We examined whether the antagonism of a Fis-dependent activity by the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products leads to increased survival after UV irradiation of cultures in stationary phase. The increased UV sensitivity of  $\Delta$ *umuDC* strains (Fig. 5, GW8023) has been observed previously (19, 49) and has been attributed to the inability of these strains to carry out SOS mutagenesis (9, 47). However, we found that inactivation of *fis* suppresses the UV sensitivity conferred by the deletion of the *umuDC* operon in stationary phase cultures (Fig. 5A, GW8038). This suggests that the UV sensitivity of  $\Delta$ *umuDC* strains in stationary phase is due to an unregulated *fis*<sup>+</sup>-dependent activity, and that the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products increase UV resistance by antagonizing this activity. The *fis*  $\Delta$ *umuDC* strain remains non-mutable by UV light (data not shown) indicating that the increased survival of the *fis*  $\Delta$ *umuDC* strain is not mediated through the restoration of the ability to undergo UV mutagenesis. Furthermore, inactivation of *fis* alone does not have a striking effect on UV resistance (Fig. 5A, GW8037) and does not affect SOS mutagenesis (data not shown). These results are consistent with the model that the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products inhibit growth and thereby increase survival of stationary phase cultures exposed to UV irradiation by counteracting a Fis-mediated activity.

We also examined whether the counteraction of a Fis-dependent activity is involved in the UV resistance conferred by the *umuD<sup>+</sup>C<sup>+</sup>* gene products in exponentially growing cultures (Fig. 5B). Similar to the result in stationary phase cultures, the inactivation of *fis* suppresses the UV sensitivity of  $\Delta$ *umuDC* cultures in exponential growth (Fig. 5B). This suggests that the same mechanism of inhibiting growth by antagonizing a Fis-dependent activity may be central to *umuD<sup>+</sup>C<sup>+</sup>*-mediated UV resistance in exponentially growing cells as well.

## Discussion

The inhibition of DNA synthesis and growth by treatment with a DNA damaging agent appears to be a ubiquitous response (14). In eukaryotes, it has been clearly demonstrated that this is a regulated response that increases survival by arresting the cell cycle and allowing DNA repair to occur (10, 35, 46). This ensures that DNA replication and chromosome segregation are completed with high fidelity (10). The observation that UV survival is increased by artificially inhibiting DNA replication in *E. coli* (7, 8) suggests that conceptually similar but as yet unidentified mechanisms may play an important role in DNA damage tolerance in prokaryotes. The data presented in this paper suggest that the *umuD<sup>+</sup>C<sup>+</sup>* gene products participate in such a regulatory mechanism that inhibits growth temporarily and thereby increases survival in cells that have accumulated DNA damage while in stationary phase and are subsequently given the opportunity to resume growth.

The natural environments of prokaryotic cells are often characterized by limited amounts of nutrients, in which the opportunity to replicate exponentially, due to an increase in the available nutrients, is experienced only occasionally (17). Thus, the majority of prokaryotic cells in nature exist in a quiescent state that is not unlike stationary phase of cultures grown under laboratory conditions. Stationary phase cells, although more resistant to DNA damaging agents such as hydrogen peroxide or alkylating agents than exponentially growing cells (17), nevertheless might accumulate DNA lesions while quiescent due to exposure to endogenous or exogenous DNA damaging agents. This is consistent with the finding that the SOS response, and presumably the *umuD<sup>+</sup>C<sup>+</sup>* operon, is induced in a cAMP-dependent manner in a fraction of the cells in a stationary phase culture that has not been exposed to exogenous DNA damaging treatments (45). The accumulation of DNA damage in stationary phase cells may pose a significant problem when the bacteria experience a nutrient upshift and attempt to rapidly divide, unless there is a mechanism to inhibit growth until DNA repair has been completed.

The proposed activity of the *umuD<sup>+</sup>C<sup>+</sup>* gene products, in inhibiting the transition to exponential growth of stationary phase cells exposed to a DNA damaging treatment,

conceptually parallels the DNA damage checkpoint at the G1 to S transition, which is controlled by the *RAD9* gene product in *S. cerevisiae* (27, 41). The *recA*<sup>+</sup> gene product acts as the sensor of DNA damage in *E. coli*, analogous to *RAD9* or *POL2* in *S. cerevisiae* (27), that transduces the signal in this case by mediating the cleavage of LexA which results in the induction of the SOS regulon, including the *umuD*<sup>+</sup>*C*<sup>+</sup> operon (14). The *umuD*<sup>+</sup>*C*<sup>+</sup> gene products are functionally similar to the as yet unidentified effectors in *S. cerevisiae* that inhibit the initiation of DNA synthesis after DNA damaging treatments and are regulated by the signal transduction pathway activated by *RAD9* (10). Both prokaryotic and eukaryotic mechanisms delay growth and DNA replication and increase cellular survival, presumably by allowing DNA repair to occur (14) prior to the transition to exponential growth in *E. coli* and S phase in *S. cerevisiae*, respectively. Stationary phase in *E. coli* may more closely resemble G0 than G1 in eukaryotic cells. It is tempting to speculate that the *umuC*-like genes recently identified in eukaryotes (20, 21) may play a role in delaying entry into the normal mitotic cell cycle in response to DNA damage.

Our results suggest that the mechanism by which the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products increase UV resistance in stationary phase cells involves the antagonism of a Fis-dependent activity, which results in the inhibition of growth (Fig. 5). The Fis protein, the levels of which increase dramatically within the first two cell divisions after nutrient upshift (1), has been implicated in the regulation of the transition from stationary phase to exponential growth (34). In another conceptual parallel to the eukaryotic cell cycle, the pattern of expression and activities of the Fis protein resemble those of a eukaryotic-like cyclin that promotes a growth phase transition in *E. coli*. Fis, a small DNA-binding protein, plays many roles in the regulation of cell growth (13). For example, Fis is involved in DNA replication (12, 16), the growth-phase specific regulation of the expression of approximately 40 genes (15, 52), and the regulation of the synthesis of components of the translational machinery in response to growth rate (28, 37). Counteraction of one or more Fis-dependent functions such as these by the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products would lead to the inhibition of growth.

Mutations that inactivate *fis* cause an increase in the duration of the lag phase and a reduction in growth rate following nutrient upshift (34). This parallels the physiological consequence of constitutive expression of *umuD<sup>+</sup>C<sup>+</sup>* from a multicopy plasmid, which inhibits the transition to exponential growth at 30°C resulting in cold sensitivity (Fig. 1). The fact that the inactivation of *fis* exacerbates *umuD<sup>+</sup>C<sup>+</sup>*-mediated cold sensitivity (Table 3) suggests that the inhibition of the transition from stationary phase to exponential growth is mediated through Fis. In addition, Fis levels have been shown to decrease markedly during prolonged starvation (34). Thus, the time-dependent decrease of Fis during prolonged incubation in stationary phase could account, at least in part, for the increase in *umuD<sup>+</sup>C<sup>+</sup>*-dependent growth inhibition at 30°C observed when stationary phase cultures incubated for prolonged periods experience a nutrient upshift.

The rapid, growth-phase specific inhibition of DNA synthesis conferred by high levels of the *umuD<sup>+</sup>C<sup>+</sup>* gene products at 30°C suggests that the *umuD<sup>+</sup>C<sup>+</sup>*-dependent inhibition of growth at 30°C is a consequence of the inhibition of DNA synthesis in lag phase cells. Although the mechanism of the *umuD<sup>+</sup>C<sup>+</sup>*-dependent inhibition of DNA synthesis is unclear, the rapid kinetics of inhibition suggests that the *umuD<sup>+</sup>C<sup>+</sup>* gene products inhibit the activity of DNA polymerase III during the elongation step of DNA replication (24). This suggests that the *umuD<sup>+</sup>C<sup>+</sup>* gene products do not inhibit the activity of Fis that may be required for the initiation of DNA replication (12), although at present this cannot be entirely discounted since the exact role(s) of Fis in DNA replication has not been clearly elucidated. Interestingly, exponentially growing cells are resistant to the *umuD<sup>+</sup>C<sup>+</sup>*-mediated rapid inhibition of DNA synthesis at 30°C. This suggests that DNA polymerase III holoenzyme in exponentially growing cells is different than in lag phase cells.

The physiological relevance of the activity of the *umuD<sup>+</sup>C<sup>+</sup>* gene products that leads to an inhibition of DNA synthesis is not clear, since this effect has been observed only when the *umuD<sup>+</sup>C<sup>+</sup>* gene products are expressed at abnormally high levels. However, previous experimental analyses of SOS mutagenesis suggest that UmuD' and UmuC interact with DNA

polymerase III during translesion synthesis (14, 36). The combination of the results presented in this paper and previous data (33) are consistent with the hypothesis that intact UmuD and UmuC analogously interact with DNA polymerase III resulting in the inhibition of DNA synthesis and the inhibition of growth.

We have shown that physiologically relevant levels of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products regulate the kinetics of growth and survival of stationary phase cultures exposed to UV irradiation. The *umuD*<sup>+</sup>*C*<sup>+</sup> gene products expressed from the single chromosomal copy of the operon and regulated in an SOS-dependent manner increase survival of UV irradiated stationary phase cells (Fig. 5A) which is correlated with a significant increase in the length of the lag phase upon nutrient upshift (Fig. 4). The increase in the length of the lag phase appears to be due to the antagonism of a Fis-dependent function. This is supported by the observation that inactivation of *fis* suppresses the UV sensitivity of  $\Delta$ *umuDC* mutants in both stationary phase (Fig. 5A) and exponential growth (Fig. 5B). These results are consistent with the model that the UV resistance conferred by the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products in *E. coli* is predominantly due to the temporary inhibition of growth via the counteraction of a Fis-dependent activity, which may also be associated with an inhibition of DNA synthesis. Thus, a mechanism conceptually similar to the checkpoint in eukaryotes that blocks cell cycle progression after DNA damage (10) appears to increase cellular survival in *E. coli* exposed to DNA damaging agents.

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**Table 1.** List of strains and plasmids.

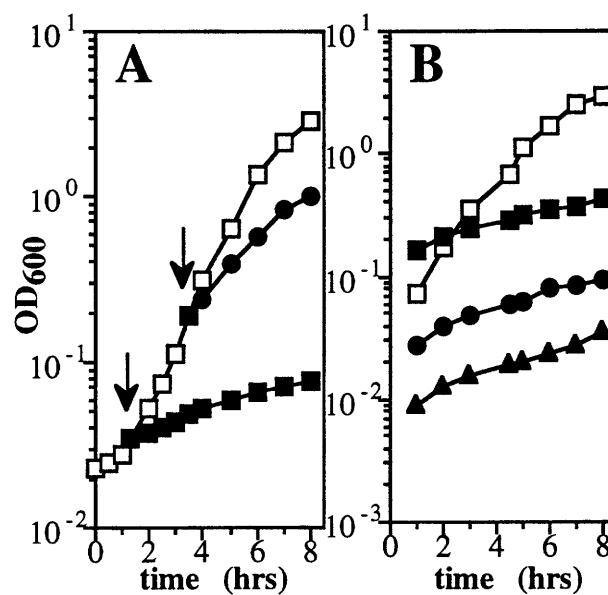
Strain	Relevant Genotype	Source/Reference
GW2771	<i>lexA</i> <sup>+</sup> , <i>recA</i> <sup>+</sup> , <i>umuD</i> <sup>+</sup> <i>C</i> <sup>+</sup>	(33)
GW8018	<i>lexA</i> (Def):: <i>spc</i> , <i>recA441</i> , <i>sulA11</i> , <i>sfiC2</i>	(33)
GW8023	GW2771, $\Delta$ ( <i>umuDC</i> )595:: <i>cat</i>	(33)
GW8025	GW8018, $\Delta$ ( <i>umuDC</i> )595:: <i>cat</i>	(33)
ZK1000	<i>rpoS</i> :: <i>kan</i>	(3)
GC2880	$\Delta$ <i>cya</i> , <i>ilv</i> ::Tn5	(45)
RJ1802	<i>fis</i> ::767, <i>kan</i> <sup>r</sup>	(1)
RJ4013	<i>frg-733</i> ::Tn <i>phoA</i> '-4	(52)
RJ4015	<i>proP-104</i> ::Tn <i>phoA</i> '-4	(51)
GW8030	GW8018, <i>rpoS</i> :: <i>kan</i>	GW8018 x P1(ZK1000)
GW8033	GW8018, $\Delta$ <i>cya</i> , <i>ilv</i> ::Tn5	GW8018 x P1(GC2880)
GW8034	GW8018, <i>fis</i> ::767, <i>kan</i> <sup>r</sup>	GW8018 x P1(RJ1802)
GW8035	GW8018, <i>frg-733</i> ::Tn <i>phoA</i> '-4	GW8018 x P1(RJ4013)
GW8036	GW8018, <i>proP-104</i> ::Tn <i>phoA</i> '-4	GW8018 x P1(RJ4015)
GW8037	GW2771, <i>fis</i> ::767, <i>kan</i> <sup>r</sup>	GW2771 x P1(RJ1802)
GW8038	GW8023, <i>fis</i> ::767, <i>kan</i> <sup>r</sup>	GW8023 x P1(RJ1802)
Plasmid	Comments	Source/Reference
pBR322/ <i>kan</i>	<i>Km</i> <sup>r</sup> , <i>Tc</i> <sup>s</sup>	(33)
pSE115	<i>umuD</i> <sup>+</sup> <i>C</i> <sup>+</sup> ; pSC101	(11)
pSE117	<i>umuD</i> <sup>+</sup> <i>C</i> <sup>+</sup> ; pBR322	(11)
pRJ4000	<i>P</i> <sub>lac</sub> - <i>fis</i> , <i>Ap</i> <sup>r</sup> , <i>ColE1</i>	(52)
pGW3751	<i>umuD</i> <sup>+</sup> <i>C</i> <sup>+</sup> ; pBR322	(6)
pTO4	<i>umuD</i> (SA60) <i>C</i> <sup>+</sup> ; pBR322	This work

**Table 2.** Effect of *rpoS* on the transformation efficiency of *umuD<sup>+</sup>C<sup>+</sup>*-expressing plasmids in *lexA(Def)* strains.

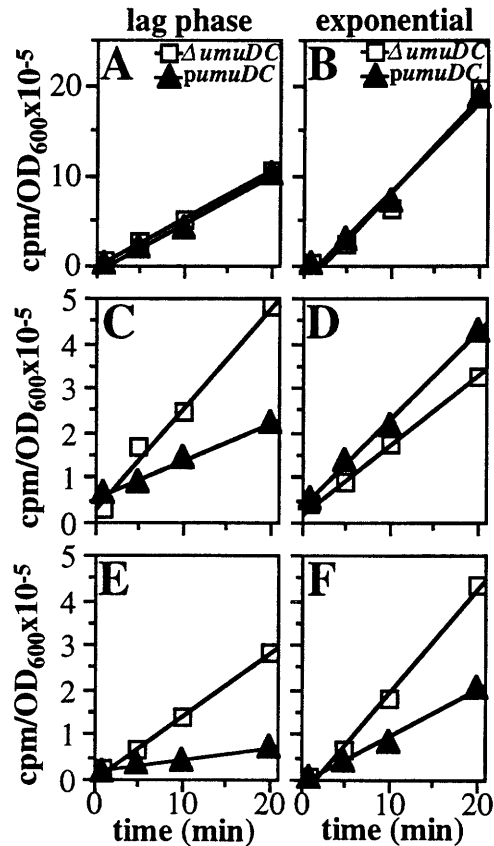
strain	plasmid	transformants per ml 30°C/42°C
GW8018[ <i>lexA(Def) rpoS<sup>+</sup></i> ]	pBR322	1.04 ± 0.01
	<i>umuD<sup>+</sup>C<sup>+</sup></i> ; pBR322	0.029 ± 0.001
GW8030[ <i>lexA(Def) rpoS</i> ]	pBR322	1.05 ± 0.04
	<i>umuD<sup>+</sup>C<sup>+</sup></i> ; pBR322	0.025 ± 0.005

**Table 3.** Effect of *fis* on the transformation efficiency of *umuD*<sup>+</sup>*C*<sup>+</sup>-expressing plasmids in *lexA*(Def) strains.

strain	plasmid	transformants per ml 30°C/42°C
GW8018[ <i>lexA</i> (Def) <i>fis</i> <sup>+</sup> ]	pBR322	1.04 ± 0.01
	<i>umuD</i> <sup>+</sup> <i>C</i> <sup>+</sup> ; pBR322	0.029 ± 0.001
GW8034[ <i>lexA</i> (Def) <i>fis</i> ]	pBR322	0.98 ± 0.02
	<i>umuD</i> <sup>+</sup> <i>C</i> <sup>+</sup> ; pBR322	0.000069 ± 0.000005

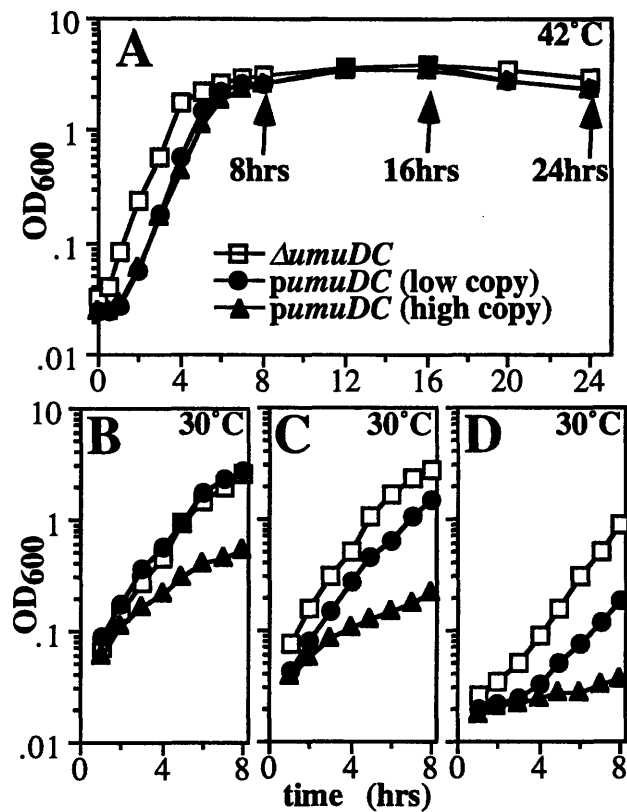


**Fig. 1.** *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent inhibition of growth at 30°C is growth-phase specific. (A) A stationary phase culture of a *lexA*(Def) strain carrying a multicopy *umuD*<sup>+</sup>*C*<sup>+</sup> plasmid [GW8025 (pSE117)] was diluted 1:125 into fresh LB media and growth at 42°C was monitored by OD<sub>600</sub> (□). A portion of this culture was shifted to 30°C (time of shift indicated by arrows) during lag phase (■) and exponential growth (●) and subsequent growth was monitored by OD<sub>600</sub>. (B) Stationary phase cultures of [GW8025 (pBR322kan)] and [GW8025 (pSE117)] were diluted into fresh LB media, grown at 42°C for 1 hr, and shifted to 30°C. Growth at 30°C was monitored by OD<sub>600</sub>. □, GW8025(pBR322kan) 1:125 dilution; ■, GW8025(pSE117) 1:20 dilution; ●, GW8025(pSE117) 1:125 dilution; ▲, GW8025(pSE117) 1:400 dilution.

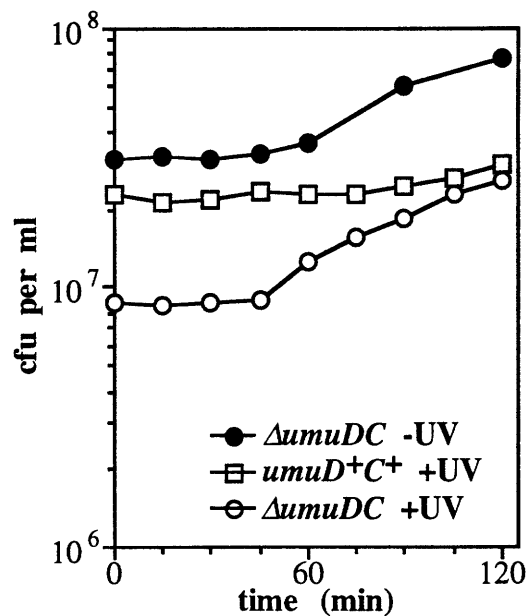


**Fig. 2.** *umuD*<sup>+</sup>*C*<sup>+</sup>-mediated inhibition of DNA synthesis at 30°C. Stationary phase cultures of GW8025(pBR322kan) (□) and GW8025(pSE117) (▲) were diluted 1:125 in fresh LB media and grown at 42°C. A portion of these cultures were shifted to 30°C during lag phase and exponential growth. At various times subsequently, the rate of DNA synthesis at 42°C and 30°C was determined. Incorporation of [<sup>3</sup>H-methyl]thymidine was normalized at each time point to the OD<sub>600</sub> of the culture. The ratio of the rates of DNA synthesis of the  $\Delta umuDC$  to *umuD*<sup>+</sup>*C*<sup>+</sup> cultures are indicated in brackets. (A) 42°C, lag phase [1.0]; (B) 42°C, exponential growth [1.0]; (C) 30°C for 10 mins after shift in lag phase [2.7]; (D) 30°C for 10 mins after shift in exponential growth [0.8]; (E) 30°C for 2 hrs after shift in lag phase [5.0]; (F) 30°C for 2 hrs after shift in exponential growth [2.0].

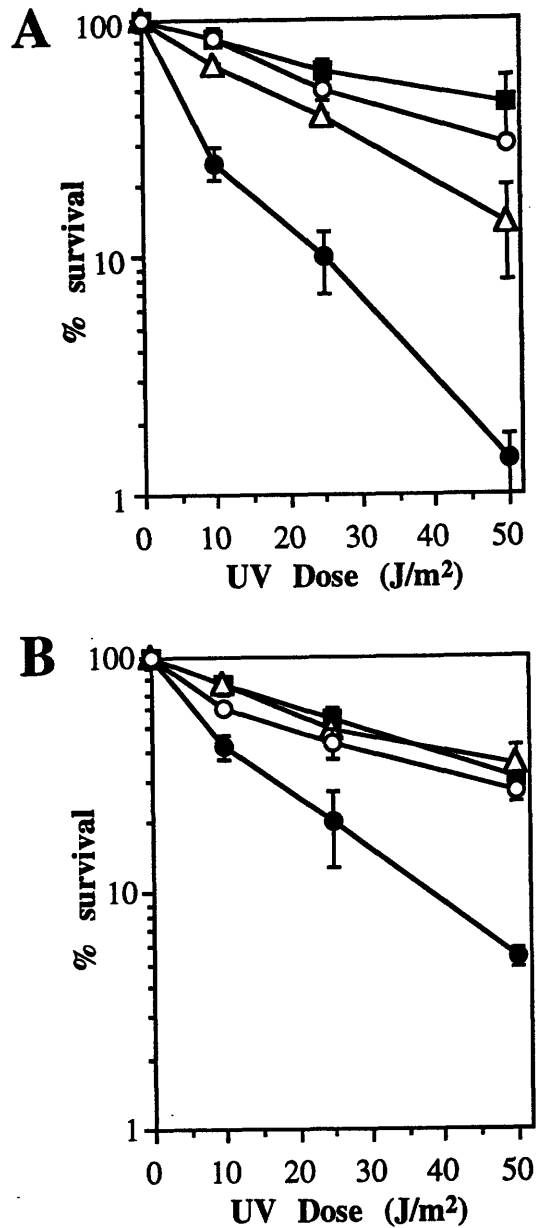




**Fig. 3.** Effect of prolonged starvation on *umuD*<sup>+</sup>*C*<sup>+</sup>-mediated growth inhibition at 30°C. (A) The following strains were grown in LB media at 42°C: GW8025(pBR322kan) (□), GW8025(pSE115) (●) and GW8025(pSE117) (▲). At various times during growth (as indicated by arrows), a portion of each culture was diluted 1:125 in fresh LB media, grown at 42°C for 1 hr, and shifted to 30°C. Subsequent growth at 30°C was monitored by OD<sub>600</sub>. (B) Cultures 8 hrs old at time of nutrient upshift; (C) Cultures 16 hrs old at time of nutrient upshift; (D) Cultures 24 hrs old at time of nutrient upshift.



**Fig. 4.** Effect of the  $umuD^+C^+$  gene products on survival and growth of UV-irradiated stationary phase cultures after nutrient upshift. Cultures were grown at 37°C. GW2771 ( $umuD^+C^+$ ) and GW8023 ( $\Delta umuDC$ ) were grown in M9 media to stationary phase. 1 ml of each culture was exposed to a UV dose of 50J/m<sup>2</sup>. UV-irradiated and unirradiated cultures were diluted 1:100 in fresh M9 media, and grown in the dark. At various times during growth, serial dilutions were plated to quantify CFU per ml. □, GW2771 ( $umuD^+C^+$ ) +UV; ●, GW8023 ( $\Delta umuDC$ ) -UV; ○, GW8023 ( $\Delta umuDC$ ) +UV.



**Fig. 5.** Effect of *fis* ::767 on survival after UV irradiation of stationary phase and exponentially growing cultures. Cultures were grown at 37°C. ■, GW2771 (*fis*<sup>+</sup>, *umuD*<sup>+</sup>*C*<sup>+</sup>); ●, GW8023 (*fis*<sup>+</sup>,  $\Delta$ *umuDC*); Δ, GW8037 (*fis*::767, *umuD*<sup>+</sup>*C*<sup>+</sup>); ○, GW8038 (*fis*::767,  $\Delta$ *umuDC*). (A) Survival of cells UV irradiated after 11 hours in stationary phase. (B) Survival of cells UV irradiated in exponential growth.

## **Chapter 4**

### **A Second Role for the *umuD*<sup>+</sup>*C*<sup>+</sup> Gene Products of *E. coli* in DNA Damage Tolerance: The Regulation of DNA Synthesis after UV Irradiation**

**This work was done in collaboration with Tim Opperman.**

## Abstract

Recent analyses suggest that the *umuDC* gene products in *Escherichia coli* have a novel role in DNA damage tolerance which is distinct from their well-characterized role in SOS mutagenesis. In this paper, we present direct evidence that an activity of intact UmuD, the form inactive in SOS mutagenesis, and UmuC increases UV resistance in exponentially growing cells. Furthermore, this activity appears to increase DNA damage tolerance by regulating the inhibition and subsequent recovery of DNA synthesis after UV irradiation. The time course of induction of UmuD and UmuD' suggests that intact UmuD and UmuC could function to inhibit growth and DNA synthesis immediately after UV irradiation. Subsequently, the RecA\*-mediated cleavage of UmuD to UmuD', the 12 kDa carboxy terminal fragment of UmuD, would switch the prevalent activity of the *umuDC* gene products from the inhibition of DNA synthesis and growth to SOS mutagenesis or other activities of UmuD' and UmuC. The *umuC125* mutation, which specifically interferes with the ability of UmuC to inhibit growth at 30°C without affecting its capacity to participate in SOS mutagenesis, also interferes with the UmuDC-mediated inhibition of DNA synthesis after UV irradiation. This finding indicates that the exaggeration of this previously uncharacterized activity of UmuD and UmuC caused by overexpression results in UmuDC-dependent inhibition of growth at 30°C.

## Introduction

The inhibition of DNA replication in response to DNA damage and the regulation of its subsequent recovery have been shown to play an important role in damage tolerance in eukaryotes (10, 16). For example, the products of the *RAD9* and *POL2* genes in *Saccharomyces cerevisiae* have been shown to constitute cell cycle checkpoints that sense DNA damage and block progression of the cell cycle (26, 36, 38), thereby allowing DNA repair processes to be completed prior to attempts at replication (16). Currently it is unclear whether similar mechanisms exist in prokaryotic organisms to increase survival after DNA damage by temporarily inhibiting DNA replication. In *Escherichia coli*, Doudney, *et al.* (7, 8) have demonstrated that resistance to UV irradiation is increased if DNA replication is artificially blocked prior to exposure to UV light. Thus, it seems feasible that a regulated mechanism to inhibit DNA replication in response to DNA damage would increase DNA damage tolerance in *E. coli*. Our recent analyses suggest that the *umuDC* gene products in *E. coli* may have a previously uncharacterized role in regulating DNA synthesis in response to DNA damaging treatments, thereby increasing resistance.

The *umuDC* operon was identified in two independent genetic screens for mutations that abolish UV and 4-nitroquinoline-1-oxide mutagenesis in *E. coli* (18, 37). The *umuDC* operon is a member of the SOS regulon whose expression is regulated by the *recA* and *lexA* gene products (16). Following a DNA damaging treatment, the attempt by the cell to replicate damaged templates leads to the creation of single-stranded DNA gaps which, in the presence of nucleotide triphosphate, serve to activate RecA to RecA\* (33). RecA\* mediates the autodigestion of the LexA repressor (22) which leads to the induction of the genes and operons that make up the SOS regulon (16). RecA\* also mediates the autodigestion of UmuD to UmuD', the 12kDa carboxyl fragment of UmuD which is the active species in SOS mutagenesis (2, 27, 35). The activity of the *umuDC* gene products that has been most extensively characterized is their participation in the process of SOS mutagenesis, which appears to result from the introduction of errors during

the process of translesion synthesis performed by DNA polymerase III modified by UmuC, UmuD' and RecA (16, 32).

On the basis of our characterization of the physiological consequences of constitutively expressing the *umuDC* operon from a multicopy plasmid, we have proposed a novel activity for the *umuDC* gene products that is distinct from their role in SOS mutagenesis (23, 24, 29) (Murli *et al.*, manuscript in preparation). We have previously shown that higher levels of the *umuDC* gene products than are encountered physiologically inhibit DNA synthesis and growth at 30°C in *E. coli* (24, 29). This *umuDC*-mediated inhibition of growth at 30°C is specifically due to an inhibition of the ability of cells to make the transition from stationary phase to exponential growth (Murli *et al.*, manuscript in preparation). The *umuDC* gene products appear to inhibit this growth-phase transition by counteracting an activity of the *fis* gene product (Murli *et al.*, manuscript in preparation). The *fis* gene product has been shown to play an important role in mediating the transition from stationary phase to exponential growth (30) and in several other cellular processes, including DNA replication (14). At present, it is unclear whether the *fis* gene product is involved in the inhibition of DNA synthesis at 30°C associated with high levels of the *umuDC* gene products. Our earlier work has demonstrated that UmuC and intact UmuD, the form inactive in SOS mutagenesis, are the *umuDC* gene products responsible for growth inhibition at 30°C (29).

Several lines of evidence suggest that the growth inhibition at 30°C associated with abnormally high levels of intact UmuD and UmuC reflects a novel, physiologically relevant activity of the *umuDC* gene products in DNA damage tolerance in *E. coli*. First, the increased sensitivity to UV irradiation exhibited by  $\Delta umuDC$  mutants in stationary phase is suppressed by a mutation in *fis* (Murli *et al.*, manuscript in preparation), suggesting that the *umuDC* gene products increase DNA damage tolerance by counteracting an activity of Fis. Furthermore, physiologically relevant levels of the *umuDC* gene products inhibit the transition to exponential growth of stationary phase cells exposed to UV light (Murli, *et al.*, manuscript in preparation). This activity of the *umuDC* gene products is correlated with increased UV resistance and may

involve the inhibition of a Fis-dependent activity. Taken together, these observations have led us to propose that the *umuDC* gene products regulate DNA synthesis and growth in bacteria exposed to DNA damaging agents, thereby increasing survival (Murli *et al.*, manuscript in preparation). In this paper, we present direct evidence that an activity of intact UmuD and UmuC increases UV resistance in exponentially growing cells. Furthermore, this activity appears to increase DNA damage tolerance by modulating the inhibition and subsequent recovery of DNA synthesis after UV irradiation.



## Materials and Methods

**Strains and plasmids.** The strains and plasmids used in these experiments and their relevant features are listed in Table 1. Genetic markers were transferred between strains using P1 transduction (25). pTO4 was constructed by replacing the 1 kb Bgl II fragment of pSE117 with the corresponding restriction fragment from pGW2112 (27) containing the *umuD*(SA60) mutation. The presence of the *umuD*(SA60) mutation in pTO4 was verified by sequence analysis.

**Reagents.** Bacterial strains were grown in LB or M9 supplemented with 0.4% Casamino acids (25). Ampicillin, chloramphenicol, tetracycline, and kanamycin were purchased from Sigma Chemical Corp. (St. Louis, MO) and were used at the following concentrations: ampicillin (150 µg/ml), chloramphenicol (30 µg/ml), tetracycline (12.5 µg/ml), and kanamycin(25 µg/ml). <sup>3</sup>H-methyl thymidine (83 Ci/mmol) was purchased from Amersham (Arlington Heights, Ill.). The anti UmuD/D' antibodies used in these experiments were previously described (1). The Western Lights kit for chemiluminescence development of immunoblots was purchased from Tropix (Bedford, MA).

**Procedures.** UV survival curves were performed as previously described (5). Cultures growing exponentially in M9 supplemented with 0.4% casamino acids were irradiated in 1 ml aliquots of growth media in a 60 mm petri dish. The UV irradiation source was a GE 15W germicidal lamp. Stationary phase cultures were diluted 1:10 in 0.85% saline solution prior to UV irradiation. Measurement of induced replisome reactivation was performed essentially as described by Khidir, *et al.* (19). Briefly, overnight cultures grown in M9 media supplemented with 0.4% casamino acids were diluted 1:200 in fresh, prewarmed media and incubated at 37°C until the culture was growing exponentially (~ 0.1 OD<sub>600</sub>). The OD<sub>600</sub> and rate of DNA synthesis was measured at the indicated times before and after UV irradiation as described by Khidir, *et al.* (19). A 25 ml aliquot of each exponentially growing culture in growth media was irradiated with

UV ( $10 \text{ J/m}^2$ ) in a 150 mm Petri dish to minimize the interruption to exponential growth. Similarly, to measure steady-state induction kinetics of UmuD and UmuD' a culture growing exponentially in M9 (0.4% casamino acids) was UV irradiated ( $20 \text{ J/m}^2$ ) and samples were taken at various times after irradiation and prepared for immunoblot analyses as previously described (29).

## Results

**Intact UmuD and UmuC increase UV resistance.** Our previous results demonstrated that the *umuD<sup>+</sup>C<sup>+</sup>* gene products increase DNA damage tolerance by counteracting an activity of the *fis* gene product (Murli, *et al.*, manuscript in preparation). However, we could not distinguish whether this is due to the activity of UmuD' and UmuC in SOS mutagenesis or a novel activity of intact UmuD and UmuC, as suggested by the parallels to *umuD<sup>+</sup>C<sup>+</sup>*-mediated growth inhibition at 30°C (29). To address this question, we compared the UV sensitivity of a strain that expresses intact UmuD and UmuC in the absence of UmuD' (GW8027) to an isogenic strain lacking *umuDC* (GW8040). Both of these strains carry the *recA430* allele, which is proficient in recombination but deficient in the coprotease activity that is required for the post-translational processing of UmuD to yield UmuD' (12). Since the coprotease activity of RecA\* is also required for the cleavage of LexA and the induction of the SOS response, these strains carry a *lexA(Def)* allele, resulting in constitutive expression of the SOS regulon. In addition, we compared the UV sensitivity of a *lexA(Def) recA<sup>+</sup>* strain (in which UmuD cleavage occurs normally) that expresses the *umuD<sup>+</sup>C<sup>+</sup>* operon (GW8039) to one that is lacking *umuDC* (GW8024). Strikingly, we observed that the  $\Delta$ *umuDC* mutation increases UV sensitivity in the *recA430* as well as in the *recA<sup>+</sup>* background, suggesting that intact UmuD and UmuC confer UV resistance (Fig. 1).

The overall increase in UV sensitivity of *recA430* strains compared to *recA<sup>+</sup>* strains (Fig. 1) may be due to an inability to induce one or more of the DNA-damage-inducible genes that are not directly repressed by LexA but require activated RecA (RecA\*) for expression (21). At least one gene regulated in this manner, the *hga* gene, has been shown to play an important role in UV survival (3). The gene product of *hga* (2-keto-4-hydroxyglutarate aldolase) is required for resumption of cellular respiration after UV irradiation (3).

In a parallel experiment, we examined whether intact UmuD and UmuC increase UV resistance in *recA<sup>+</sup>* strains to establish whether the UV resistance conferred by intact UmuD and UmuC is unique to *recA430* backgrounds. We analyzed UV sensitivity in *lexA<sup>+</sup> recA<sup>+</sup>*

*ΔumuDC* strains that differ only in the genotype of the *umuDC* operon carried on a plasmid (Fig. 2). As has previously been reported, we observed an increased UV resistance in the strain that carries *umuD<sup>+</sup>C<sup>+</sup>* on a plasmid [GW8023/pSE117] relative to a strain that carries the plasmid vector alone [GW8023/pBR322kan]. A more modest increase in UV resistance is conferred by *umuD'C<sup>+</sup>* on a plasmid [GW8023/pGW3751]. Similarly, the strain carrying *umuD(SA60)C<sup>+</sup>* on a plasmid [GW8023/pTO4] exhibits an increased resistance to UV irradiation relative to the control strain carrying the vector alone, but this increased UV resistance is significantly lower than that conferred by *umuD<sup>+</sup>C<sup>+</sup>*. The UmuD(SA60) protein is refractive to RecA\*-mediated coproteolysis and is therefore inactive in translesion synthesis (27, 32), but is proficient in the UmuDC-dependent inhibition of growth (29). Previously, the resistance to UV irradiation conferred by *umuD<sup>+</sup>C<sup>+</sup>* has been attributed to the SOS mutagenesis activity of the *umuD<sup>+</sup>C<sup>+</sup>* gene products (9, 39). However, the observation that wildtype *umuD<sup>+</sup>C<sup>+</sup>* confers a greater degree of UV resistance than either *umuD'C<sup>+</sup>* or *umuD(SA60)C<sup>+</sup>* suggests that both the activity of UmuD' and UmuC in SOS mutagenesis and a novel activity of intact UmuD and UmuC are responsible for conferring UV resistance.

**Intact UmuD and UmuC modulate the kinetics of recovery of DNA replication following UV irradiation.** The UV survival curves demonstrate that intact UmuD and UmuC function to increase survival after UV irradiation (Figs. 1 and 2). We have previously shown that the growth inhibition at 30°C conferred by high levels of the *umuDC* gene products is associated with an inhibition of DNA synthesis (24) (Murli, *et al.*, manuscript in preparation). We therefore examined the possibility that the resistance to UV irradiation conferred by intact UmuD and UmuC was due to a previously uncharacterized activity of these proteins in the regulation of the kinetics of the recovery of DNA synthesis. Immediately following UV irradiation of an exponentially growing *E. coli* culture, there is a rapid decrease in the rate of DNA synthesis (19). This is followed by a recovery period after which the rate of DNA synthesis returns to the initial rate of increase (19). The inhibition of DNA synthesis occurs in *recA* mutant backgrounds, but

recovery requires *recA*<sup>+</sup> and the induction of the SOS response (19). The data suggest that the process of the recovery of DNA synthesis after a DNA damaging treatment, known as induced replisome reactivation (IRR), occurs at existing replication forks rather than through new rounds of initiation (19).

Our model predicts that intact UmuD and UmuC regulate the kinetics of the recovery of DNA synthesis and thereby increase UV resistance. To test this model, we compared the rates of DNA synthesis before and after UV irradiation of strain GW8027 [*lexA*(Def) *recA430*] to that of the isogenic strain lacking *umuDC* [GW8040, *lexA*(Def) *recA430*  $\Delta$ *umuDC*]. Any differences observable between these two strains can be attributed to the activity of intact UmuD and UmuC, as discussed earlier. We found a significant, reproducible increase in the magnitude of the inhibition of DNA synthesis in the presence of intact UmuD and UmuC (Fig. 3). Since GW8027, which expresses intact UmuD and UmuC, is also more UV resistant than an isogenic  $\Delta$ *umuDC* strain (Fig. 1), the data is consistent with a model in which intact UmuD and UmuC increase survival after UV irradiation by modulating the kinetics of the reactivation of DNA synthesis.

To directly compare the activities of UmuD and UmuC to UmuD' and UmuC in the regulation of DNA synthesis after UV irradiation, we examined the rates of DNA synthesis before and after UV irradiation in a set of derivatives of strain GW8023 [*lexA*<sup>+</sup> *recA*<sup>+</sup>  $\Delta$ *umuDC*] which differed in the genotype of *umuDC* expressed from a pBR322-derived plasmid (Fig. 4). In a strain carrying pSE117 (*umuD*<sup>+</sup>*C*<sup>+</sup>), the rate of DNA synthesis recovers approximately 40 minutes after UV irradiation (Fig. 4A). The time of recovery is significantly reduced in the strain carrying pGW3751 (*umuD'* *umuC*<sup>+</sup>) (Fig. 4B). The recovery of DNA synthesis in the presence of UmuD' and UmuC to the levels observed prior to UV irradiation occurs within approximately 20 minutes. Conversely, the presence of noncleavable intact UmuD(SA60) and UmuC increases the delay in the recovery of DNA synthesis after UV irradiation (Fig. 4C). In the presence of plasmid pTO4, which encodes the noncleavable derivative of UmuD *umuD*(SA60) and *umuC*<sup>+</sup>, complete recovery of DNA synthesis is not observed in the first 60 minutes following UV

irradiation. The presence of pTO4 protects the strain GW8023 from the lethal effects of UV irradiation relative to the vector alone, but this UV protection is significantly less than that conferred by *umuD*<sup>+</sup>*C*<sup>+</sup> (Fig. 2). The inability of RecA\*-mediated proteolysis to alleviate the inhibitory effects on DNA replication and growth of the noncleavable UmuD(SA60) and UmuC may partially account for the decreased resistance conferred by *umuD*(SA60)*C*<sup>+</sup> relative to that conferred by wildtype *umuD*<sup>+</sup>*C*<sup>+</sup>. Thus, the induced replisome reactivation (IRR) data and the UV survival curves support the hypothesis that the presence of intact UmuD and UmuC increases survival after UV irradiation by delaying the recovery of DNA synthesis.

**UV irradiation induces the accumulation of significant levels of UmuD prior to the accumulation of UmuD'.** If significant levels of UmuD are induced prior to the accumulation of UmuD', then UmuD could function with UmuC to regulate the recovery of DNA synthesis after UV irradiation in the absence of the capacity to undergo SOS mutagenesis. The accumulation of UmuD prior to UmuD' has been previously suggested by the reduced ability of UmuD, relative to LexA, to undergo RecA\*-mediated proteolysis *in vivo* and *in vitro* (2, 35). We examined the steady-state induction kinetics of UmuD and UmuD' by a UV dose of 20 J/m<sup>2</sup> in the wildtype strain GW2771 [*recA*<sup>+</sup> *lexA*<sup>+</sup> *umuD*<sup>+</sup>*C*<sup>+</sup>] by immunoblot analysis (Fig. 5). Significant levels of UmuD are detectable within the first ten minutes after UV induction while the accumulation of UmuD' is only observed twenty minutes after UV irradiation. This is consistent with the observation by Defais, *et al.* (4) that maximal SOS mutagenic activation is observed thirty minutes after UV irradiation. A higher UV dose of 50 J/m<sup>2</sup> does not change the temporal pattern of UmuD/D' induction although the steady state levels of UmuD/D' are higher (data not shown). The accumulation of significant levels of UmuD within the first ten minutes after UV irradiation is consistent with the comparison of the IRR in the presence and absence of intact UmuD and UmuC in Fig. 3 which suggests that the activity of intact UmuD and UmuC in regulating the recovery of DNA replication occurs rapidly after UV induction. The temporal pattern of UmuD/D' induction by UV irradiation suggests that there is a window immediately after UV

irradiation in which intact UmuD could act in concert with UmuC to regulate DNA synthesis in the absence of significant levels of UmuD'.

**Regulation of DNA synthesis after UV irradiation contributes to UV resistance.** The *umuC125* mutation does not cause UmuDC-dependent growth inhibition at 30°C, but is proficient in SOS mutagenesis, suggesting that these are genetically separable functions of the UmuC protein (23). Interestingly, the presence of *umuD<sup>+</sup>umuC125* on a plasmid confers an increased UV sensitivity in *lexA<sup>+</sup>* strains relative to the vector alone (23). We have previously suggested that UmuDC-dependent growth inhibition at 30°C is due to a novel and physiologically relevant activity of the *umuD<sup>+</sup>C<sup>+</sup>* gene products that is exaggerated by their constitutive expression (29). The accumulated evidence suggests that this novel activity involves the regulation of DNA synthesis after UV irradiation. Since the *umuC125* mutation interferes with the ability of UmuC to confer growth inhibition at 30°C (23), we examined whether the UmuC125 protein, in combination with UmuD, is also defective in the regulation of DNA synthesis after UV irradiation (Fig. 4D). Surprisingly, we found that the *umuC125* mutation not only abolishes the regulation of the kinetics of the recovery of DNA synthesis observed in the presence of wildtype *umuD<sup>+</sup>C<sup>+</sup>*, but also abolishes the immediate inhibition of DNA synthesis after UV irradiation. Since the pattern of IRR in the presence of *umuD<sup>+</sup>* and *umuC125* is significantly different than that observed in the absence of the *umuDC* gene products (see Fig. 3), the UmuC125 protein appears to have a novel interaction with the DNA replication apparatus which prevents the inhibition of DNA synthesis characteristically seen after UV irradiation. Unregulated DNA replication of damaged DNA could result in the increased UV sensitivity observed in the presence of the *umuC125* mutation (23). These data are consistent with the hypotheses that intact UmuD and UmuC regulate DNA synthesis after UV exposure and that this activity is important for UV resistance. Moreover, this activity is correlated with the activity of the *umuD<sup>+</sup>C<sup>+</sup>* gene products in UmuDC-dependent inhibition of growth at 30°C.

## Discussion

Our previous studies had led us to propose that intact UmuD and UmuC regulate cell division and growth in cells exposed to DNA damaging agents in order to allow DNA repair to take place before the resumption of growth (29) (Murli, *et al.*, manuscript in preparation). The results reported in this paper support and extend this hypothesis by demonstrating that intact UmuD and UmuC function to modulate the kinetics of the recovery of DNA synthesis after UV irradiation and thus increase survival. This is consistent with previously reported results that demonstrated that the artificial inhibition of DNA replication by treatment with chloramphenicol increases cell survival after UV irradiation (8). These results had led Doudney, *et al.* (7) to speculate that a factor induced in an SOS-dependent manner inhibits replication upon UV irradiation and allows the repair of DNA damage, thereby increasing survival. Our data suggest that intact UmuD and UmuC are SOS-controlled factors that function in this manner to confer UV resistance. In addition, our results suggest that RecA\*-mediated proteolytic cleavage of UmuD to yield UmuD' reverses the inhibition of DNA synthesis by this complex, thereby switching the activity of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products to SOS mutagenesis mediated by UmuD' and UmuC.

Several lines of evidence suggest parallels between our earlier characterization of the growth inhibition at 30°C caused by overexpression of the *umuD*<sup>+</sup>*C*<sup>+</sup> operon (29) (Murli *et al.*, manuscript in preparation) and our current analyses of UV survival and the kinetics of recovery of DNA synthesis after UV irradiation. First, intact UmuD rather than UmuD' appears to be the active species, in combination with UmuC, in conferring both growth inhibition at 30°C (29) and in inhibiting DNA replication after UV irradiation (Fig. 4). Secondly, the *umuC125* mutation, which has previously been shown to abolish growth inhibition at 30°C without interfering with SOS mutagenesis (23), blocks the ability of UmuC to delay the resumption of DNA replication after UV irradiation (Fig. 4D). In fact, there is no inhibition of DNA synthesis in the presence of *umuC125* after UV irradiation, which is interesting to consider in light of the increased UV sensitivity conferred by this mutation (23). The data are consistent with the hypothesis that



unregulated DNA synthesis after UV irradiation due to the absence of this novel activity of UmuC and UmuD leads to UV sensitivity in the presence of the *umuC125* mutation.

The phenotype of the *umuC125* mutation raises interesting questions about the mechanism of UmuDC-dependent inhibition of DNA synthesis after UV irradiation. One possibility is that intact UmuD and UmuC inhibit ongoing replication by DNA polymerase III after UV irradiation through a direct mechanism that involves an interaction between intact UmuD, UmuC and DNA polymerase III. This is supported by the results from our analyses of the *umuD<sup>+</sup>C<sup>+</sup>*-dependent growth inhibition at 30°C caused by overexpression of the *umuD<sup>+</sup>C<sup>+</sup>* operon, which is associated with a rapid, reversible inhibition of DNA synthesis at the restrictive temperature (24) (Murli *et al.*, manuscript in preparation). These data are consistent with the inhibition being at the level of elongation rather than initiation (24), suggesting that there is a direct interaction between UmuD, UmuC and DNA polymerase III holoenzyme at the replication fork. Previous results have suggested that the inhibition and subsequent recovery of DNA synthesis after UV irradiation is likely to occur at existing replication forks rather than through new rounds of initiation (19). After UV irradiation, intact UmuD and UmuC could inhibit DNA replication by decreasing the rate of nucleotide addition by an elongating DNA polymerase III holoenzyme. Alternatively, UmuD and UmuC may inhibit the dissociation or the reinitiation downstream of a DNA polymerase stalled at the site of a DNA lesion, resulting in an apparent inhibition of DNA synthesis. The resumption of DNA synthesis would require the alleviation of the inhibitory activity of UmuD and UmuC by the conversion of UmuD to UmuD', or by the repair of the DNA lesions. A second possibility is that UmuD and UmuC act to inhibit DNA synthesis through an indirect mechanism, e.g. by acting in a signal transduction pathway, although the accumulated evidence suggests a more direct mechanism (24). Finally, the inhibition of an activity of *fis<sup>+</sup>* has been shown to be central to the UV resistance conferred by the *umuD<sup>+</sup>C<sup>+</sup>* gene products in both stationary phase and exponential growth (Murli, *et al.*, manuscript in preparation). The role of *fis<sup>+</sup>* in *umuD<sup>+</sup>C<sup>+</sup>*-mediated inhibition of DNA synthesis after UV irradiation is unclear, although the involvement of Fis in DNA replication (13) suggests

a possible mechanistic connection. Further experiments will be required to determine the exact mechanism by which UmuD and UmuC inhibit DNA synthesis after UV irradiation.

The *umuC125* mutation renders the intact UmuD - UmuC complex inactive in the regulation of DNA synthesis after UV irradiation. Since the inhibition of DNA synthesis by UV irradiation is observed even in  $\Delta umuDC$  backgrounds (see Fig. 3), the absence of an inhibition of DNA synthesis caused by the *umuC125* mutation suggests the possibility that a novel interaction occurs between the UmuD and UmuC125 proteins and the replication apparatus. This interaction could make the DNA polymerase III holoenzyme insensitive to the inhibition of replication by DNA lesions or modify the polymerase such that it increases the likelihood of reinitiation downstream of a lesion. Alternatively, the absence of an inhibition of DNA synthesis after DNA damage in the presence of the *umuC125* mutation raises the possibility that the inhibition of DNA synthesis observed in *recA* mutants (19) is functionally different than that observed in *recA*<sup>+</sup> cells. It is possible that the absence of the *recA* gene product results in a passive inhibition of DNA synthesis when DNA polymerase III holoenzyme encounters DNA lesions that would normally be repaired in a *recA*<sup>+</sup>-dependent manner. The inhibition of DNA synthesis in *recA*<sup>+</sup> cells may be due to an active mechanism that requires the activity of the *recA*<sup>+</sup> gene product that is counteracted by the *umuC125* mutation.

The data presented here support the model that the modulation of the inhibition and recovery of DNA synthesis by intact UmuD and UmuC plays a physiologically significant role in increasing survival after UV irradiation. We propose that this novel activity of intact UmuD and UmuC and the activity of UmuD' and UmuC in SOS mutagenesis are temporally regulated by the activity of the RecA\* coprotease. The induction of the *umuD*<sup>+</sup>*C*<sup>+</sup> operon requires the presence of activated RecA\*, a sensor that indicates the presence of DNA damage in the cell (16). The inherent inefficiency of RecA\*-mediated UmuD cleavage relative to LexA cleavage (2, 35) results in a time window after the SOS regulon is induced by UV irradiation in which intact UmuD could accumulate in the cell along with UmuC, in the absence of significant levels of UmuD'. The time course of UmuD and UmuD' induction after UV irradiation shows that intact

UmuD is the dominant species detectable for the first twenty minutes (Fig. 5), during which time it could function with UmuC to regulate DNA synthesis (Fig. 6). These data correlate well with the induced replisome reactivation data which show that the inhibition of DNA synthesis by intact UmuD and UmuC occurs rapidly after UV irradiation and persists for approximately twenty minutes (Fig. 3). The RecA\*-mediated conversion of UmuD to UmuD' could function as a molecular switch that leads to a change from the activity of intact UmuD and UmuC in the inhibition of DNA synthesis after UV irradiation to translesion synthesis or other activities of UmuD' and UmuC (Fig. 6). Maximal UmuD' levels are detected thirty minutes after UV irradiation (Fig. 5) which is consistent with the finding that maximal SOS mutagenic activity is observed thirty minutes after UV irradiation (4). Finally, the steady state levels of these complexes could be regulated by additional mechanisms that include the proteolysis of UmuD and UmuC by the Lon protease and of UmuD/D' heterodimers by the ClpXP protease (15).

The importance of RecA\*-mediated temporal regulation of the activity of UmuD and UmuC is underscored by the finding that the presence of non-cleavable UmuD(SA60) and UmuC confers decreased UV resistance at higher UV doses relative to wildtype *umuD*<sup>+</sup>*C*<sup>+</sup>. In the presence of *umuD*(SA60)*C*<sup>+</sup>, the inability to cleave UmuD(SA60) to UmuD' (27) prevents the alleviation of DNA synthesis inhibition within the sixty minutes after UV irradiation (Fig. 4C). At lower doses of UV, lower levels of UmuD(SA60) and UmuC are induced which are able to confer a comparable degree of UV resistance to that conferred by *umuD*<sup>+</sup>*C*<sup>+</sup> or *umuD'**C*<sup>+</sup> (Fig. 2). At a high UV dose, the presence of larger amounts of UmuD(SA60) and UmuC lead to an increased UV sensitivity relative to *umuD*<sup>+</sup>*C*<sup>+</sup> or *umuD'**C*<sup>+</sup> (Fig. 2), possibly due to the inability of RecA\*-mediated proteolysis to alleviate the inhibition of DNA synthesis and growth.

The control of DNA replication and the progression of the cell cycle after DNA damage has been shown to play an important role in survival after DNA damaging treatments in eukaryotes (10, 16). Our data suggest that intact UmuD and UmuC play a role in the regulation of the inhibition and recovery of DNA synthesis after UV irradiation and thereby increase survival in *E. coli*. This activity of UmuD and UmuC seems to serve as a functional equivalent

of the eukaryotic S phase DNA damage checkpoint that inhibits DNA synthesis and cell cycle progression in response to DNA damage (26, 31). Such a role of intact UmuD and UmuC in DNA damage tolerance could partially account for the ubiquitousness of *umuDC*-like genes in prokaryotes (28, 34), some of which are non-mutable by UV irradiation (34). Finally, it is possible that this previously uncharacterized role of UmuD and UmuC in the control of the cell cycle in response to DNA damage could account for the presence of *umuC* homologs in the genomes of eukaryotes and archeae (20).

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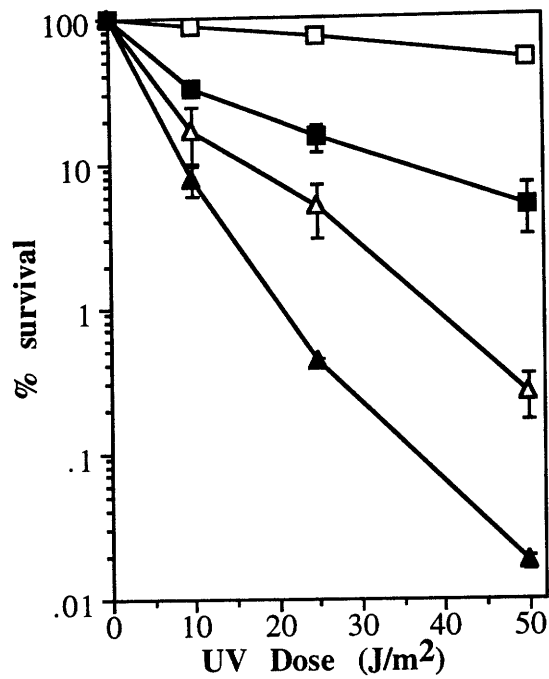
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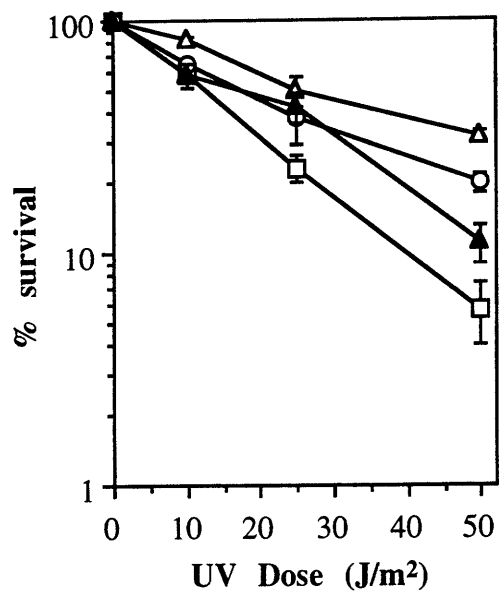
**Table 1. Strains and Plasmids.**

Strain	Relevant Genotype	Source/Reference
JL2301	<i>lexA300(Def)::spc</i>	(17)
GW6900	<i>recA430, srl::Tn10</i>	(27)
GW2771	<i>lexA<sup>+</sup>, recA<sup>+</sup>, umuD<sup>+</sup>C<sup>+</sup></i>	(29)
GW8023	<i>lexA<sup>+</sup>, recA<sup>+</sup>, Δ(umuDC)595::cat</i>	(29)
GW8025	<i>lexA(Def), recA441, Δ(umuDC)595::cat</i>	(29)
GW8039	<i>lexA(Def), recA<sup>+</sup>, umuD<sup>+</sup>C<sup>+</sup></i>	GW2771 x P1(JL2301)
GW8024	<i>lexA(Def), recA<sup>+</sup>, Δ(umuDC)595::cat</i>	(29)
GW8027	<i>lexA(Def), recA430, umuD<sup>+</sup>C<sup>+</sup></i>	(29)
GW8040	<i>lexA(Def), recA430, Δ(umuDC)595::cat</i>	GW8025 x P1(GW6900)
Plasmid	Comments	Source/Reference
pBR322kan	Km <sup>r</sup> , Tc <sup>r</sup>	(29)
pSE117	<i>umuD<sup>+</sup>C<sup>+</sup></i> ; pBR322	(11)
pGW3751	<i>umuD<sup>+</sup>C<sup>+</sup></i> ; pBR322	(6)
pLM109	<i>umuD<sup>+</sup>C125</i> ; pBR322	(23)
pTO4	<i>umuD(SA60)C<sup>+</sup></i> ; pBR322	This work

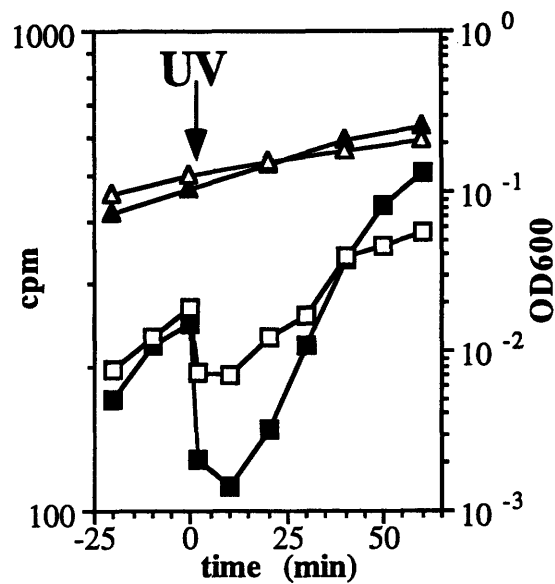




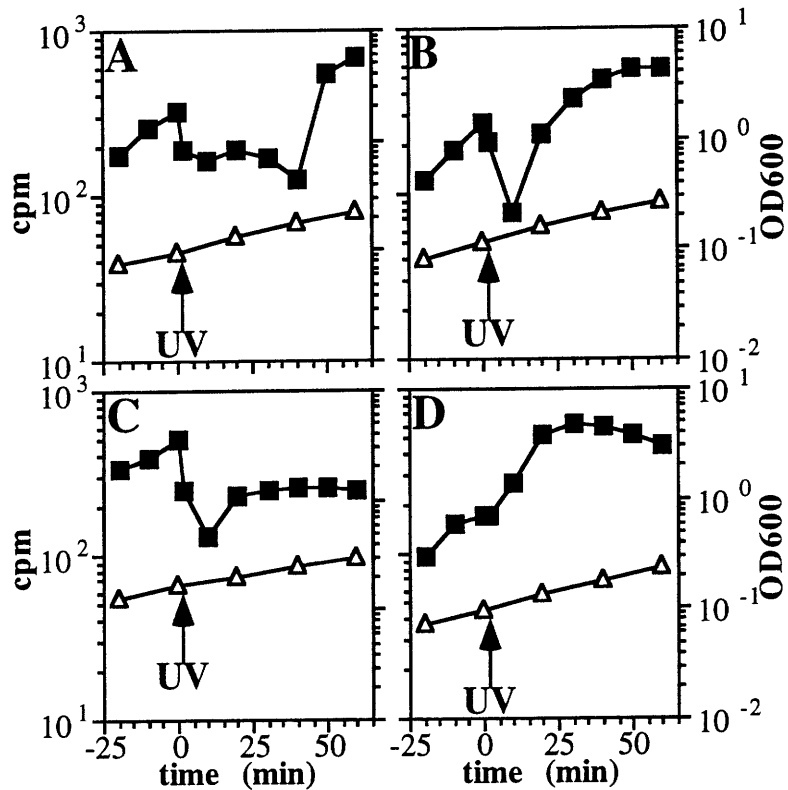
**Figure 1.** Effect of intact UmuD and UmuC on UV survival. (□) GW8039 [*lexA*(Def) *recA*<sup>+</sup> *umuD*<sup>+</sup>*C*<sup>+</sup>]; (■) GW8024 [*lexA*(Def) *recA*<sup>+</sup>  $\Delta$ (*umuDC*)595::*cat*]; (Δ) GW8027 [*lexA*(Def) *recA*430 *umuD*<sup>+</sup>*C*<sup>+</sup>]; (▲) GW8040 [*lexA*(Def) *recA*430  $\Delta$ (*umuDC*)595::*cat*].



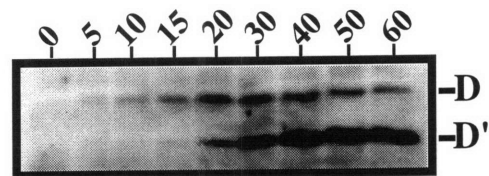
**Figure 2.** UV survival in isogenic strains of GW8023 [*lexA*<sup>+</sup> *recA*<sup>+</sup>  $\Delta$ (*umuDC*)595::*cat*] that differ in the genotype of *umuDC* carried on a plasmid. ( $\Delta$ ) pSE117 [*umuD*<sup>+</sup>C<sup>+</sup>]; (○) pGW3751 [*umuD*'C<sup>+</sup>]; ( $\blacktriangle$ ) pTO4 [*umuD*(SA60)C<sup>+</sup>]; (□) pBR322kan.



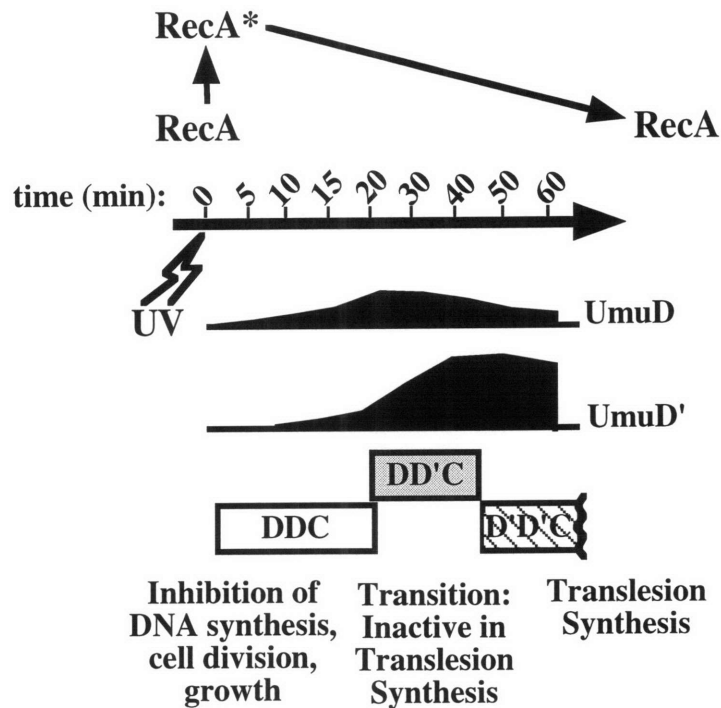
**Figure 3.** Intact UmuD and UmuC modulate the inhibition and recovery of DNA synthesis after UV irradiation. The rate of DNA synthesis (squares) and OD<sub>600</sub> (triangles) were monitored at various times before and after UV irradiation (dose=10 J/m<sup>2</sup>). The time of UV irradiation is indicated by the arrow. (■, ▲) GW8027 [*lexA*(Def) *recA430 umuD*<sup>+</sup>*C*<sup>+</sup>]; (□, △) GW8040 [*lexA*(Def) *recA430*  $\Delta$ (*umuDC*)595::*cat*].



**Figure 4.** The inhibition and recovery of DNA synthesis after UV irradiation in isogenic strains of GW8023 [*lexA*<sup>+</sup> *recA*<sup>+</sup>  $\Delta$ (*umuDC*)595::*cat*] that differ in the genotype of *umuDC* carried on a plasmid. The rate of DNA synthesis (squares) and OD<sub>600</sub> (triangles) were monitored at various times before and after UV irradiation (dose=10 J/m<sup>2</sup>). The time of UV irradiation is indicated by the arrow. A) pSE117 [*umuD*<sup>+</sup>*C*<sup>+</sup>]; B) pGW3751 [*umuD'**C*<sup>+</sup>]; C) pTO4 [*umuD*(SA60)*C*<sup>+</sup>]; D) pLM109 [*umuD*<sup>+</sup>*C*125].



**Figure 5.** Time course of UV induction of UmuD/D'. Steady state levels of UmuD/D' at various times after UV irradiation (indicated above figure) were detected on immunoblots using anti-UmuD/D' antibodies. The positions of UmuD and UmuD' are indicated.



**Figure 6.** Model for the temporal regulation of the different activities of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products by RecA\*. The activation of RecA to RecA\* by treatment with a DNA damaging agent leads to the cleavage of LexA and the induction of the SOS regulon, including the *umuD*<sup>+</sup>*C*<sup>+</sup> operon. The relative steady state levels of UmuD and UmuD' after UV induction (see Fig. 5), represented below the time line, suggest that the repression of DNA synthesis and growth mediated by the UmuDC complex is the predominant activity immediately after SOS induction. At later times, the activity of the UmuD'C complex in SOS mutagenesis is predicted to predominate. The slow kinetics of RecA\*-mediated cleavage of UmuD to UmuD' relative to LexA (2, 35) allows intact UmuD to accumulate and function as an inhibitor of cell growth and division in combination with UmuC immediately after UV irradiation. The cleavage of UmuD to UmuD' alleviates the inhibition of growth by UmuD and UmuC and switches the activity of these proteins to SOS mutagenesis. The decay of activated RecA\* to RecA as the DNA damage is repaired leads to the returned repression of the SOS regulon (16). In addition, since RecA\* is required for a third role in SOS mutagenesis (16), the decay of RecA\* to RecA would also lead directly to the inhibition of SOS mutagenesis.

## **Chapter 5**

### **Conclusions**

The data presented in this thesis support the hypothesis that intact UmuD and UmuC play a novel role in the regulation of DNA synthesis and growth in response to DNA damage in *E. coli*. Regulated mechanisms that repress DNA replication and cell cycle progression in response to DNA damage have been the subject of detailed analyses in eukaryotes (3, 7, 12, 14). These mechanisms, which allow time for DNA repair to take place before the resumption of growth, have been shown to be central to DNA damage tolerance in these organisms. Much less is known about analogous processes in prokaryotes. The regulation of the *sulA* and *sfhC* genes by *recA*, the sensor of DNA damage in *E. coli*, is one of the few well characterized links between DNA damage and the bacterial cell cycle (2, 8). The *sulA* and *sfhC* gene products inhibit cell division upon treatment with a DNA damaging agent. The evidence presented in this thesis suggests that UmuD and UmuC participate in a previously uncharacterized mechanism to inhibit DNA replication and growth after DNA damage in *E. coli*, thereby increasing survival (Fig. 1).

This model grew out of the further characterization of the phenotype of *umuDC*-mediated cold sensitivity that is manifested in cells that overexpress the *umuDC* gene products (9, 10). These analyses revealed that UmuD and UmuC inhibit specific aspects of cell growth, in addition to interacting with another LexA-regulated gene product to inhibit cell division (Fig. 1). These results suggested that the growth inhibition at 30°C is due to a novel activity of intact UmuD and UmuC that is exaggerated by the overexpression of these proteins. The importance of this activity was indicated by the finding that physiologically relevant levels of the *umuDC* gene products, i.e. expressed from the single chromosomal copy of the operon and regulated in a normal SOS-dependent manner, inhibit the transition to exponential growth of UV irradiated stationary phase cells, which appears to be the result of counteracting a Fis-dependent activity. In addition, UmuD and UmuC inhibit DNA synthesis after treatment with a DNA damaging agent. These results are consistent with the model that UmuD and UmuC play a novel role in DNA damage tolerance in *E. coli* via the inhibition of growth and DNA synthesis (Fig. 1).

The *umuDC* operon of *E. coli*, which is regulated as a member of the SOS regulon, has been shown to be required for the mutagenesis resulting from treatment with DNA damaging



agents such as UV light, termed SOS mutagenesis (7). Constitutive expression of the *umuDC* operon from a multicopy plasmid was shown to confer growth inhibition at 30°C which was associated with a rapid inhibition of DNA synthesis at the restrictive temperature (10). The isolation of a mutation in *umuC*, *umuC125*, which inhibited the ability of UmuC to confer cold sensitivity without interfering with its ability to participate in SOS mutagenesis suggested that these were separable activities of the UmuC protein (9). Further analyses supported the hypothesis that the activity of the *umuDC* gene products that conferred cold sensitivity is distinct than that required for SOS mutagenesis (see Chapter 2). These experiments demonstrate that intact UmuD, the form inactive in SOS mutagenesis, is the active species, in combination with UmuC, in conferring growth inhibition at 30°C when overexpressed. Furthermore, the finding that physiologically relevant levels of the *umuDC* gene products (expressed constitutively from the chromosome) are capable of inhibiting septation at 30°C suggested that *umuDC*-mediated cold sensitivity is the result of an exaggeration of a previously uncharacterized activity of UmuD and UmuC.

Further characterization of the growth inhibition at 30°C conferred by constitutive expression of *umuDC* from a multicopy plasmid led to the discovery of a physiologically relevant inhibition by the *umuDC* gene products of the transition from stationary phase to exponential growth (see Chapter 3). This finding is supported by the observation that the rapid inhibition of DNA synthesis previously associated with *umuDC*-mediated cold sensitivity (10) is only observed in cells shifted to the restrictive temperature during lag phase. The accumulated results suggest that *umuDC*-mediated growth inhibition at 30°C may be a reflection of an activity of the *umuDC* gene products that increases survival by temporarily inhibiting growth upon SOS induction by DNA damage. This hypothesis was confirmed by the observation that the presence of a properly regulated, chromosomal copy of the *umuDC* operon is sufficient to significantly lengthen the lag phase upon nutrient upshift of UV irradiated stationary phase cells. The observation that the *umuDC* gene products inhibit the transition to exponential growth led to the identification of the participation of the Fis protein in the resistance to UV irradiation conferred

by the *umuDC* operon. The Fis protein, which has many activities that are involved in cell growth (5), has been implicated in the regulation of the transition from stationary phase to exponential growth (11). The inactivation of *fis* essentially completely suppresses the UV sensitivity associated with the deletion of the *umuDC* operon without restoring the ability to undergo SOS mutagenesis, demonstrating that the antagonism of a Fis-dependent activity, which presumably leads to the inhibition of growth, is central to the UV resistance conferred by the *umuDC* gene products.

The analysis of the inhibition and resumption of DNA synthesis after UV irradiation revealed that physiologically relevant levels of intact UmuD and UmuC inhibit DNA synthesis in exponentially growing cells that have experienced DNA damage (see Chapter 4). This activity is correlated with a previously unrecognized UV resistance conferred by intact UmuD and UmuC. Thus, the UV protection associated with the presence of the *umuDC* operon is not solely the result of the ability of UmuD' and UmuC to facilitate translesion synthesis by DNA polymerase III. Both the importance of this activity of intact UmuD and UmuC and the hypothesis that *umuDC*-mediated growth inhibition at 30°C is an exaggeration of this activity are supported by the analysis of the *umuC125* mutation. No inhibition of DNA synthesis is observed after UV irradiation in the presence of the *umuC125* mutation. In addition, the *umuC125* mutation confers UV sensitivity in a *lexA*<sup>+</sup> background (9). These results are consistent with the hypothesis that the prevention of unregulated DNA replication of damaged DNA by UmuD and UmuC results in increased UV resistance.

### **Model for the regulation of the activities of the *umuDC* gene products**

The activation of RecA to RecA\* upon sensing DNA damage leads to the cleavage of the transcriptional repressor LexA and the induction of the SOS regulon, including the *umuDC* operon. Since the RecA\*-mediated cleavage of UmuD to UmuD' is significantly less efficient than that of LexA (1, 13), UmuD accumulates prior to UmuD'. Therefore, UmuD and UmuC function to repress DNA replication and growth immediately after SOS induction. The cleavage

of UmuD to UmuD' mediated by RecA\* alleviates this inhibition of growth and switches the activity of these proteins to the SOS mutagenic activity of UmuD' and UmuC. Thus, at later times after SOS induction, the SOS mutagenesis activity predominates. The decay of RecA\* to RecA as DNA damage is repaired inhibits these activities by two mechanisms. First, LexA levels rise to reestablish repression of the SOS regulon. Second, SOS mutagenesis is inhibited directly since RecA\* is required for a third, direct role in SOS mutagenesis in addition to LexA and UmuD cleavage (7). In addition, proteolysis of UmuD and UmuC by the Lon protease and UmuD'/D heterodimers by the ClpXP protease (6) would reduce the levels of these activities in the cell.

### **Future directions**

One of the immediate questions raised by my work is whether the activities after UV irradiation of the *umuDC* gene products in counteracting a Fis-dependent activity and inhibiting DNA synthesis are mechanistically related, especially in light of the involvement of Fis in DNA replication (4). This question could be addressed by some straightforward experiments. For example, the role of the *fis* gene product in inhibition and recovery of DNA synthesis after UV irradiation in exponentially growing cells and in stationary phase cells after nutrient upshift could be examined using the experimental techniques described in chapter 4. It will be interesting to see whether the inhibition of DNA synthesis and the counteraction of a Fis-dependent activity by the *umuDC* gene products are separate pathways or a single pathway leading to increased DNA damage tolerance in *E. coli*. In addition, the parallels between *umuDC*-mediated inhibition of growth at 30°C and the activity of the *umuDC* gene products in the inhibition of DNA synthesis and growth after DNA damage, suggest that further characterization of *umuDC*-mediated growth inhibition could provide useful insights into the mechanistic basis of this novel activity of UmuD and UmuC. Thus, a screen for both multicopy and loss of function suppressors of *umuDC*-mediated growth inhibition at 30°C may identify other gene products that interact with UmuD and UmuC to regulate growth after DNA damage. A preliminary screen has identified *dnaQ*,

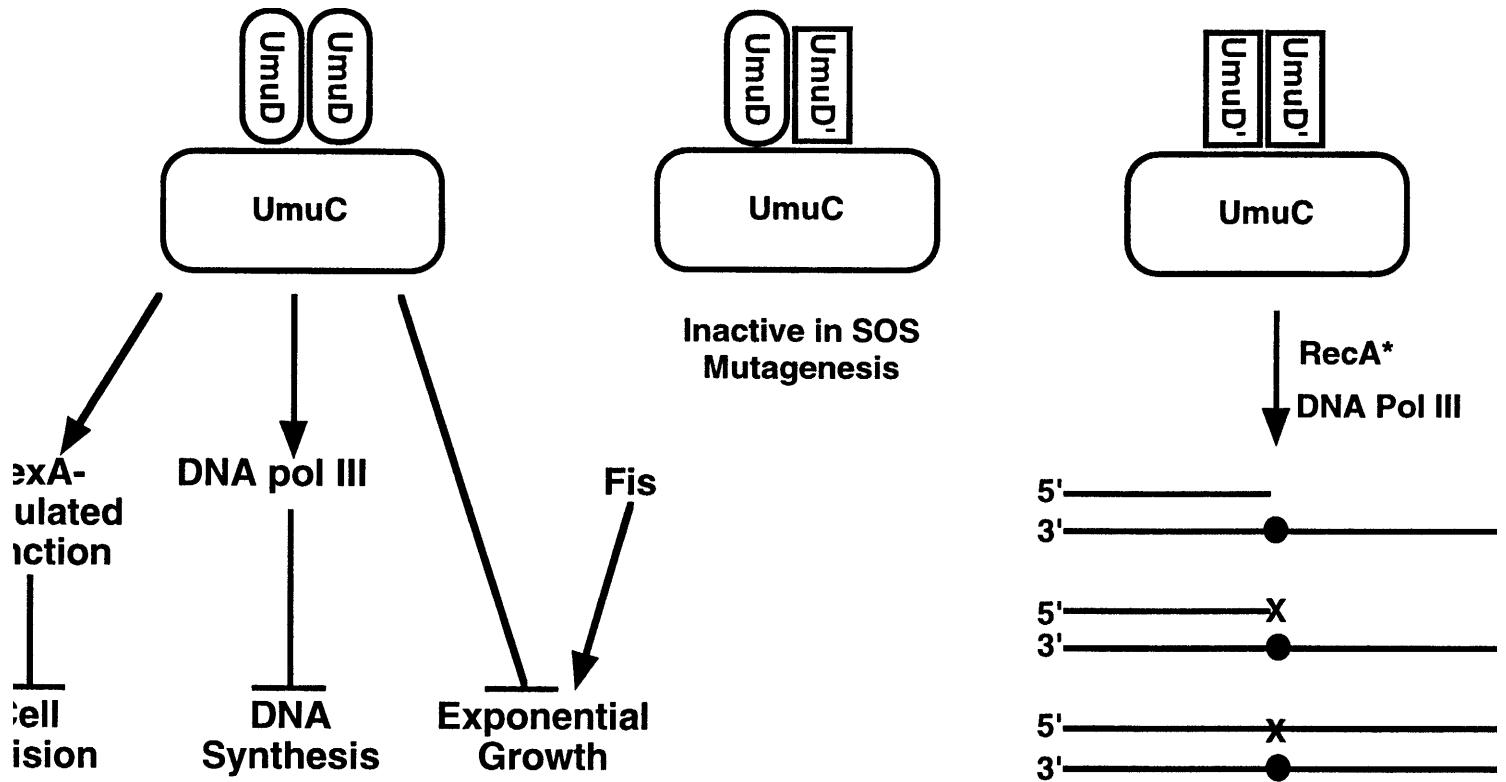
which encodes the  $\epsilon$  or proofreading subunit of DNA polymerase III, as a multicopy suppressor of *umuDC*-mediated growth inhibition at 30°C. Although more detailed analyses are required, this result suggests that intact UmuD and UmuC interact with DNA polymerase III directly to inhibit DNA synthesis. It will also be interesting to examine other SOS-regulated gene products, the functions of some of which are unknown, to determine whether any are involved in regulating DNA synthesis and growth after DNA damage through either a *umuDC*-dependent or independent mechanism.

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**Fig. 1** Model of the activities of the *umuDC* gene products of *E. coli*. Intact UmuD and UmuC increase survival after DNA damage by inhibiting DNA replication and growth. Upon RecA\*-mediated cleavage of UmuD to UmuD', UmuD' and UmuC modify DNA polymerase III in the presence of RecA\* to facilitate translesion synthesis which is associated with the introduction of errors (SOS mutagenesis).

## UmuDC-mediated cold sensitivity reflects a novel activity for UmuD and UmuC



UmuD and UmuC inhibit DNA synthesis and growth after DNA damage

Translesion Synthesis/  
SOS Mutagenesis

## **Appendix A**

The *groE* Gene Products of *Escherichia coli* Are Dispensable for  
*mucA+B*-dependent UV Mutagenesis

Published in *Mutation Research*, **309**: 225-233. This work was done in collaboration with  
Caroline Donnelly.



## Abstract

UV mutagenesis in *Escherichia coli* requires the *groES<sup>+</sup>EL<sup>+</sup>* chaperonins as well as the *umuD<sup>+</sup>C<sup>+</sup>* SOS-regulated genes. GroES and GroEL appear to be required to stabilize UmuC. The *mucA<sup>+</sup>B<sup>+</sup>* genes, which are encoded on a broad host range plasmid, are functionally analogous and structurally similar to the *umuD<sup>+</sup>C<sup>+</sup>* genes of *E. coli*. While these gene pairs are quite similar, differences have been reported in the functioning of these gene products. We tested whether *mucA<sup>+</sup>B<sup>+</sup>* function requires the *groE<sup>+</sup>* gene products as well. We show that *mucA<sup>+</sup>B<sup>+</sup>*-induced UV mutagenesis, UV resistance, phage reactivation and cold sensitivity do not require the *groE<sup>+</sup>* heat shock genes. These findings suggest that the requirement of UmuC for *groES<sup>+</sup>EL<sup>+</sup>* function is not shared by its analog, MucB.

## Introduction

The GroEL and GroES heat shock proteins of *Escherichia coli*, known as chaperonins, are involved in the proper folding and, perhaps, assembly of subunit proteins (2, 8, 9, 26, 30). Like other heat shock genes, the *groEL* gene is highly conserved in eukaryotic as well as prokaryotic cells (20). The eukaryotic homolog of GroEL, hsp60, is thought to mediate proper folding of chloroplast and mitochondrial proteins by either promoting protein folding or by blocking folding pathways which lead to aggregate formation (10, 18, 23).

Our work has focused on the molecular role(s) of GroEL and GroES in a DNA repair process in *E. coli*. We have demonstrated that *groEL* and *groES* mutants are defective in UV mutagenesis, which is the cellular processing of UV induced lesions in the DNA that leads to the induction of mutations in the genome (3, 33). Liu and coworkers have shown that *groE* mutants are also defective in Weigle reactivation of UV damaged bacteriophage (15). Both UV mutagenesis and Weigle reactivation require the SOS-regulated *umuD*<sup>+</sup>*C*<sup>+</sup> genes, as well as the SOS regulatory pathway which is dependent on the *recA*<sup>+</sup> and *lexA*<sup>+</sup> gene products in *E. coli* (6, 13, 24).

We have studied, both genetically and biochemically, the basis of the functional requirement for *groES* and *groEL* in UV mutagenesis (3). The defect in *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent mutability of these strains appears to be at the level of expression of UmuC since increased expression of the *umuC* gene will suppress the nonmutability of *groEL* and *groES* strains. We have shown that the half-life of UmuC is shortened in a *groEL* mutant strain (3). Overexpression of the *umuC* gene most likely suppresses the nonmutability of *groE* strains by increasing the steady state levels of UmuC.

More recently, we have obtained evidence which indicates that *groES*<sup>+</sup> and *groEL*<sup>+</sup> play a role in UV mutagenesis at an early step in the pathway and the requirement for *groE*<sup>+</sup> in this process can be virtually eliminated (4). Coexpression of UmuD' (a posttranslational cleavage product of UmuD which is the active form in UV mutagenesis) with UmuC will suppress the

nonmutability of *groES* and *groEL* strains. We have shown that expression of UmuD' (instead of its precursor, UmuD) stabilizes the UmuC protein in the *groE* strains (4).

In order to further investigate the role of GroES and GroEL in UV mutagenesis, we tested whether other proteins which can substitute for *umuD*<sup>+</sup>*C*<sup>+</sup> also require the *groE* gene products. An analog of the *umuD*<sup>+</sup>*C*<sup>+</sup> gene pair, *mucA*<sup>+</sup>*B*<sup>+</sup>, is encoded on a broad host range plasmid pKM101 which is a derivative of the naturally occurring plasmid R46 (19, 33). *E. coli* strains which lack functional *umuD*<sup>+</sup>*C*<sup>+</sup> genes are nonmutable by UV irradiation. However, the presence of the *mucA*<sup>+</sup>*B*<sup>+</sup> genes restores UV mutability to such a strain (34). Like the *umuD*<sup>+</sup>*C*<sup>+</sup> genes, the *mucA*<sup>+</sup>*B*<sup>+</sup> genes are organized in an operon controlled by the SOS regulatory genes *recA*<sup>+</sup> and *lexA*<sup>+</sup> (5, 28, 33).

Analysis of the nucleotide sequence of the *umuD*<sup>+</sup>*C*<sup>+</sup> and *mucA*<sup>+</sup>*B*<sup>+</sup> genes indicates that a similarity of 41% exists at the nucleotide level for the *mucA/umuD* gene pair and a similarity of 55% exists for the *mucB/umuC* gene pair (24). However, despite the fact that the *mucA*<sup>+</sup>*B*<sup>+</sup> operon can suppress the nonmutability of *umuD* or *umuC* mutants, the MucA protein alone cannot substitute for UmuD in a *umuD umuC*<sup>+</sup> host and the MucB protein alone cannot substitute for UmuC in a *umuD<sup>+</sup>umuC* host (24). These results suggest that there is a physical interaction between the two gene products of each operon and that the pairs of proteins have diverged sufficiently so that interactions between MucA and UmuC, and between UmuD and MucB, are excluded.

Functional differences between *mucA*<sup>+</sup>*B*<sup>+</sup> and *umuD*<sup>+</sup>*C*<sup>+</sup> were examined by Blanco and coworkers who have concluded that the *mucA*<sup>+</sup>*B*<sup>+</sup> gene products are more active in mutagenesis than are the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products (1). In fact, of the five cloned *umuDC*-like operons, the *mucA*<sup>+</sup>*B*<sup>+</sup> operon is the most efficient at promoting UV mutagenesis (27). The efficiency of posttranslational processing is much greater for MucA than UmuD (12), possibly contributing to the enhanced ability of *mucA*<sup>+</sup>*B*<sup>+</sup> to promote UV mutagenesis. Other differences between *mucA*<sup>+</sup>*B*<sup>+</sup> and *umuD*<sup>+</sup>*C*<sup>+</sup> have been reported as well (11).

We report here that *mucA<sup>+</sup>B<sup>+</sup>*-dependent UV mutagenesis does not require the *groES<sup>+</sup>EL<sup>+</sup>* genes of *E. coli*. These results are discussed both in the context of the functional role of the molecular chaperones, GroEL and GroES, in the proper folding of proteins involved in UV mutagenesis, and in the context of the dissimilarities between the *umuD<sup>+</sup>C<sup>+</sup>* gene products and the *mucA<sup>+</sup>B<sup>+</sup>* gene products.

## Materials and Methods

### *Bacterial strains and plasmids*

The bacterial strains and plasmids are listed in Table 1. The *groE* mutations were introduced into GW3200 and GW2100 by P1 transduction (21). pKM101 was introduced into various strains by conjugation; other plasmids were introduced by treatment of recipient cells with calcium chloride and transformation (16). Bacterial strains were selected on LB agar plates containing ampicillin (200 µg/ml), tetracycline (10 µg/ml), or kanamycin (40 µg/ml).

### *φXK9 growth*

φXK9 is a host range mutant of φX174 that is capable of growth on *E. coli* K12 strains. To generate a phage stock, AB1157 was grown to a density of  $1-3 \times 10^8$  cells per ml in 20 ml LB broth and 10 mM CaCl<sub>2</sub>. Phage was added at a multiplicity of infection of 0.01 and the culture was incubated with shaking at 37°C for 2 h. The culture lysed, cell debris was collected and the supernatant was discarded. The pellet was resuspended in phage dilution buffer (PDB) (0.1 M NaCl, 0.05 M Na-tetraborate, and 0.002 M EDTA) and incubated at 4°C for 12 h. Most of the phage were released and the debris was removed by centrifugation.

### *UV mutagenesis*

UV mutagenesis was assayed as described previously (3, 6). Cells (5 or 10 ml) were grown to OD<sub>600</sub> = 0.5, pelleted, and resuspended in the same volume of 0.85 % saline. The suspension was irradiated at a UV fluence of 1 J/m<sup>2</sup>/s for various amount of time. Cells were diluted in saline and spread on M9 agar plates containing threonine, histidine, leucine, and proline (100 µg/ml). In addition plates contained either limiting arginine (1 µg/ml) to measure reversion of the *argE3* mutation, or excess arginine (100 µg/ml) to measure survival. Colonies were counted after 48 h at 37°C. The number of Arg<sup>+</sup> revertants was compared to the number of survivors.

### *UV killing*

Cells (5 or 10 ml) were grown in LB broth to OD<sub>600</sub> = 0.5, collected by centrifugation and resuspended in the same volume of 0.85% saline. The suspension was irradiated at a fluence of 1 J/m<sup>2</sup>/s for various amounts of time. The cells were diluted in saline and plated on LB agar plates. Colonies were counted after 20 hours at 37°C.

#### *Weigle reactivation*

Five ml of  $\phi$ XK9 in PDB (10<sup>7</sup> pfu/ml) was irradiated at a fluence of 10J/m<sup>2</sup>/s for 10 s. Cells (5 ml) were grown to a density of 2 X 10<sup>8</sup> cells/ml in LB broth, collected by centrifugation, and resuspended in an equal volume of 10 mM CaCl<sub>2</sub>. The cell suspension was divided and one half was irradiated at a fluence of 1 J/m<sup>2</sup>/s for 25 s. 100  $\mu$ l of irradiated phage and 100  $\mu$ l of either irradiated or nonirradiated bacteria were mixed (m.o.i. = 0.05) and incubated for 20 minutes at room temperature without shaking. This solution was diluted, mixed with the indicator strain, SR58 (31), and plated on LB agar plates which were incubated at 37°C overnight.

#### *Cold sensitivity*

Cells were made competent by the calcium chloride method (16) and transformed with pGW1700 (*mucA*<sup>+</sup>*B*<sup>+</sup>) or pSE117 (*umuD*<sup>+</sup>*C*<sup>+</sup>) DNA. The transformation mixture was allowed to grow at 37°C for 90 minutes in LB broth lacking antibiotics. One half of the transformation mixture was spread on LB agar plates containing the appropriate antibiotics and incubated at 30°C. The other half was spread on similar plates and incubated at 42°C. Growth of colonies was recorded after 18 h at 42°C; growth of colonies at 30°C was recorded after 36 h.

## Results

### *groES* and *groEL* mutants are competent for *mucA<sup>+</sup>B<sup>+</sup>*-dependent UV mutagenesis

The presence of the plasmid pKM101 in wild type *E. coli* cells results in the enhancement of several phenotypes associated with the processing of damaged DNA. These cells exhibit an increased frequency of UV mutagenesis, an increased resistance to killing by UV irradiation, and also an increased capacity to reactivate of UV-irradiated phage (19, 22, 31, 32, 33). These phenotypes associated with the presence of pKM101 all require the function of the *mucA<sup>+</sup>B<sup>+</sup>* genes of pKM101 (25, 28). In *umuD* and *umuC* mutants, UV mutagenesis (25), the increase in resistance to killing by UV irradiation (34), and Weigle reactivation of UV-irradiated single stranded bacteriophage (31, 34) is dependent on the *mucA<sup>+</sup>B<sup>+</sup>* function of pKM101.

We were interested in determining whether these *mucA<sup>+</sup>B<sup>+</sup>*-dependent phenotypes were also affected by mutations at the *groE* locus of *E. coli*. As in previous studies, we monitored the capacity of cells to undergo UV mutagenesis by measuring UV-induced reversion of an *argE3* mutation to Arg<sup>+</sup>. We constructed *groEL100* and *groES30* derivatives of the *groE<sup>+</sup>* strain AB1157 and also of the *groE<sup>+</sup>* strain GW3200 (a *umuD44* derivative of AB1157). We then measured the UV-induced reversion of *argE3* in these strains in the presence and absence of pKM101. As previously reported (34), the presence of pKM101 in a *groE<sup>+</sup>umuD44* background increases the frequency of UV mutagenesis to a level which is significantly higher than than observed in a *groE<sup>+</sup>umuD<sup>+</sup>* strain lacking pKM101 (Fig. 1A). However, in contrast to *umuD<sup>+</sup>C<sup>+</sup>*-dependent UV mutagenesis, which is greatly reduced in *groEL100* and *groES30* strains (3), the *mucA<sup>+</sup>mucB<sup>+</sup>*-dependent mutagenesis seen in pKM101-containing derivatives of GW3200 was not affected by the *groEL100* and *groES30* mutations (Fig. 1B and 1C). The *groE*-independence of *mucA<sup>+</sup>mucB<sup>+</sup>*-dependent mutagenesis does not appear to be due to the presence of *groE*-like functions on the plasmid pKM101 since UV mutagenesis promoted by the recombinant plasmid pGW1700, which only carries the *mucA<sup>+</sup>mucB<sup>+</sup>* genes of pKM101, was similarly unaffected by the *groEL100* and *groES30* mutations (data not shown).

Since the products of the *groEL* and *groES* genes are essential for the viability of *E. coli* (7), the alleles of *groEL* and *groES* thus far isolated have been partial loss of function alleles. We therefore analyzed several other *groE* alleles in order to test the hypothesis that *mucA<sup>+</sup>mucB<sup>+</sup>*-dependent mutagenesis is *groE*-independent. As shown in Fig. 2, *mucA<sup>+</sup>mucB<sup>+</sup>*-dependent UV mutagenesis was essentially unaffected by any of a variety of *groEL* and *groES* alleles. The small differences between the strains are not statistically significant.

#### *pKM101 increases the resistance of groE strains to killing by UV irradiation*

We tested whether the *mucA<sup>+</sup>mucB<sup>+</sup>*-dependent increase in resistance to killing by UV irradiation seen in *umuC* strains (34) is influenced by *groEL100* or *groES30* mutations. As shown in Fig. 3, the survival of *groEL100* and *groES30* derivatives of the *umuD44* strain GW3200 that contained pKM101 were indistinguishable from the survival of the *groE<sup>+</sup>* derivative.

#### *mucA<sup>+</sup>B<sup>+</sup>*-dependent Weigle reactivation of UV-irradiated phage

We also tested whether *mucA<sup>+</sup>B<sup>+</sup>*-dependent reactivation of bacteriophage required *groE<sup>+</sup>* function. We measured reactivation of a UV-irradiated derivative of  $\phi$ X174 ( $\phi$ XK9, which infects *E. coli* K12 cells) in *groE<sup>+</sup>* and *groE* backgrounds, in the presence or absence of pKM101. The reactivation of the phage was measured in an *umuD44* background to eliminate *umuD<sup>+</sup>C<sup>+</sup>*-dependent reactivation. In the absence of pKM101 function, the phage was not reactivated regardless of the *groE* genotype (Table 2). In the presence of *mucA<sup>+</sup>B<sup>+</sup>*, the phage was reactivated irrespective of the *groE* genotype (Table 2). If anything the phage was reactivated slightly more efficiently in the *groE* mutants than in the *groE<sup>+</sup>* strain. Thus, we have found that three phenotypes associated with expression of the *mucA<sup>+</sup>B<sup>+</sup>* genes, i.e. UV mutagenesis, resistance to killing by UV irradiation and Weigle reactivation of UV-irradiated single-stranded bacteriophage, do not require *groE<sup>+</sup>* function.

#### *Cold sensitivity*



Another phenotype which the *umuD<sup>+</sup>C<sup>+</sup>* genes have in common with the *mucA<sup>+</sup>B<sup>+</sup>* genes is a cold sensitivity for growth when these gene products are overexpressed. A multicopy plasmid carrying the *umuD<sup>+</sup>C<sup>+</sup>* genes in a *lexA(Def)* strain results in overexpression of UmuD and UmuC. Such a strain will grow at 42°C but will not grow at 30°C (17). The cold sensitivity appears to be due to a reduction in the rate of DNA synthesis when this strain is shifted to 30°C (17). In fact, it was the observation that mutations in *groEL* and *groES* suppress the cold sensitive lethality associated with *umuD<sup>+</sup>C<sup>+</sup>* overexpression that first alerted us to the possibility that *umuD<sup>+</sup>C<sup>+</sup>*-dependent UV mutagenesis might be *groE*-dependent (3).

The severity of the cold sensitivity due to overexpression of *mucA<sup>+</sup>B<sup>+</sup>* is not as great as that seen when *umuD<sup>+</sup>C<sup>+</sup>* is overexpressed which may reflect inherent differences in the molecular activities of the proteins in the two systems. We tested whether the *mucA<sup>+</sup>B<sup>+</sup>*-dependent cold sensitivity could be suppressed by *groE* mutations in the same fashion as as the *umuD<sup>+</sup>C<sup>+</sup>*-dependent cold sensitivity. Plasmid pGW1700 which encodes *mucA<sup>+</sup>B<sup>+</sup>* is a pBR322 derivative and causes a cold sensitive phenotype if transformed into a *lexA(Def)* host. We transformed pGW1700 into a *lexA71::Tn5* derivative which also carried the *groEL100* mutation. This strain could grow at 42°C but grew very poorly at 30°C. These results were indistinguishable from the growth of the *groE<sup>+</sup>* derivative, suggesting that the *groE* mutation has no effect on the cold sensitivity of the *mucA<sup>+</sup>B<sup>+</sup>* overexpressing strain. These results are summarized in Table 3.

## Discussion

The simplest interpretation of the data presented here is that *groE*<sup>+</sup> function is not required for MucAB-dependent processing of UV damaged DNA. Our previous results showed that similar processing of UV damaged DNA by the *umuD*<sup>+</sup>*C*<sup>+</sup> gene products of *E. coli* does require *groEL* and *groES* function (3). These results are somewhat surprising given the degree of homology between these gene pairs. The dissimilarity in requirement for *groE*<sup>+</sup> function in *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent versus *mucA*<sup>+</sup>*B*<sup>+</sup>-dependent UV mutagenesis may be indicative of a folding or assembly problem for UmuC and/or UmuD which MucA and MucB do not share.

GroEL and GroES are members of a ubiquitous class of molecular chaperone proteins found in all cells (9). Lorimer and his colleagues (10) have shown that GroEL is able, in a Mg-ATP-dependent fashion, to promote the conversion of unfolded or partly folded ribulose 1,6-bisphosphate carboxylase subunits into active enzyme. They conclude that the role of GroEL and GroES is to sequester partially folded, unstable intermediates before these intermediates assemble into an aggregated state which molecular chaperones cannot act upon (10). Martin, *et al.* (18), have gone on to show some of the biochemical events during this process and these results suggest that folding takes place on the surface of the GroEL molecule. The folding polypeptide is stabilized in a conformation characteristic of a "molten globule" and Mg-ATP and GroES then promote the proper folding of the molecule to its final native structure (18).

Our previous studies have helped us to focus on the part of the *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent UV mutagenesis pathway that requires GroEL and GroES. First of all, we have shown that UmuC is most likely the target of *groE*<sup>+</sup> action (3). In addition, we have shown that GroEL and UmuC physically interact *in vivo* (4). It is possible that GroEL and GroES are required to promote the proper folding of UmuC or to stabilize UmuC until interaction with UmuD' is possible. By analogy, we propose that *groE*<sup>+</sup> function is not required for *mucA*<sup>+</sup>*B*<sup>+</sup> -dependent activities because MucB does not require GroEL and GroES for proper folding. It is possible that, because the *mucAB* genes are on a broad host range plasmid which can enter a variety of bacterial strains, there has been selective pressure for the MucB protein to have evolved in such a way that it does

not require a specific interaction with a host molecular chaperone. Furthermore, since MucB and UmuC are highly homologous, we may be able to identify regions of UmuC which confer a *groE*<sup>+</sup>-dependence by exchanging regions of the *umuC*<sup>+</sup> gene with *mucB*<sup>+</sup> sequences.

While the molecular basis for the requirement of molecular chaperones for *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent mutagenesis are quite intriguing, another interesting aspect of these experiments is one that questions the biochemical processes in which the UmuDC proteins and the MucAB proteins are involved. Liu and Tessman have reported that, while the majority of the mutagenesis that accompanies the SOS repair of UV-damaged single-stranded bacteriophage DNA is eliminated when the *groES* or the *groEL* gene is defective, reactivation of the damaged template is not affected substantially by mutations at *groE* (15). These findings suggest that UV mutagenesis and reactivation of single-stranded phage can be uncoupled by *groE* mutations. Taken together with our results which indicate that *groE* mutations appear to have the effect of decreasing the amount of active UmuC protein in the cell, these findings imply that reactivation of UV-damaged templates without concomitant mutagenesis may require less UmuC than does the mutagenic processing of the template. An alternative explanation might be that different UmuDC/MucAB-dependent phenomena are carried out by different multiprotein assemblies. If this were true, then it is possible that the assembly of a particular multiprotein assembly might require GroEL and GroES in the case of UmuDC but not in the case of MucAB, for example to complex with SSB or perhaps DNA pol II for one phenomenon (29) and with DNA pol III for another.

Our results presented here indicate that *groE*<sup>+</sup> function is not required for MucAB-dependent mutagenesis or reactivation of single stranded bacteriophage as it is for UmuDC-dependent mutagenesis. However, these results and those of Liu and Tessman must be qualified since the *groE* genes are essential for *E. coli* and the mutants used in all these studies retain some *groE*<sup>+</sup> function. Thus, *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent repair may require less *groE*<sup>+</sup> function than does *umuD*<sup>+</sup>*C*<sup>+</sup>-dependent mutagenesis, and *mucA*<sup>+</sup>*B*<sup>+</sup>-dependent repair and mutagenesis may also require only the partial *groE*<sup>+</sup> function present in *groE* strains.

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**TABLE 1**

**BACTERIAL STRAINS, PHAGE AND PLASMIDS**

Strains	Relevant Genotype	Reference
<u>Bacteria</u>		
AB1157	<i>argE3 umuD<sup>+</sup>C<sup>+</sup></i>	(6)
GW2100	As AB1157 but <i>umuC122::Tn5</i>	(6)
GW2730	<i>lexA(Def)71::Tn5 sulA11</i>	(14)
GW3200	As AB1157 but <i>umuD44</i>	(6)
GW7503	As AB1157 but <i>groEL100 purA::Tn10</i>	(3)
GW7504	As AB1157 but <i>groES30 purA::Tn10</i>	(3)
GW7511	As GW2730 but <i>groEL100 purA::Tn10</i>	(3)
GW8002	As AB1157 but <i>groEL100 purA::Tn10 umuC122::Tn5</i>	This work
GW8003	As AB1157 but <i>groES30 purA::Tn10 umuC122::Tn5</i>	This work
GW8006	As AB1157 but <i>groEL35 purA::Tn10 umuC122::Tn5</i>	This work
GW8007	As AB1157 but <i>groEL46 purA::Tn10 umuC122::Tn5</i>	This work
GW8009	As AB1157 but <i>groEL619 purA::Tn10 umuC122::Tn5</i>	This work
GW8010	As AB1157 but <i>groES7 purA::Tn10 umuC122::Tn5</i>	This work
GW8011	As AB1157 but <i>groES24 purA::Tn10 umuC122::Tn5</i>	This work
GW8012	As AB1157 but <i>groES36 purA::Tn10 umuC122::Tn5</i>	This work
GW8013	As AB1157 but <i>groES97 purA::Tn10 umuC122::Tn5</i>	This work
GW8020	As GW3200 but <i>groEL100 purA::Tn10</i>	This work
GW8021	As GW3200 but <i>groES30 purA::Tn10</i>	This work
SR58	<i>uvrB5, recA56</i>	A.J. Clark
<u>Phage</u>		
φXK9	host range mutant of φX174	R. Maurer
<u>Plasmids</u>		
pKM101	<i>mucA<sup>+</sup>B<sup>+</sup></i> , R46 derivative	(33)
pGW1700	<i>mucA<sup>+</sup>B<sup>+</sup></i> , pBR322 derivative	(25)
pSE117	<i>umuD<sup>+</sup>C<sup>+</sup></i> , pBR322 derivative	(17)

**TABLE 2**

pKM101-MEDIATED WEIGLE REACTIVATION OF UV-IRRADIATED  $\phi$ XK9 IN *groE*<sup>+</sup> AND *groE* STRAINS

Strain infected by phage	Ratio of phage production from UV-irradiated cells/ phage production from non-irradiated cells <sup>a</sup>	
	No plasmid	pKM101
GW3200	0.75	4.4
GW8020	0.85	6.0
GW8021	1.0	7.2

<sup>a</sup>  $\phi$ XK9 was irradiated at a fluence of 10J/m<sup>2</sup>/s for 10 s. Cell cultures of the appropriate density were divided in half with one half irradiated at a fluence of 1J/m<sup>2</sup>/s for 25 s. The ratio of phage production from UV-irradiated versus non-irradiated cells (the Weigle reactivation factor) was determined as discussed in Materials and Methods.



**TABLE 3**COLD SENSITIVITY DUE TO *mucA*<sup>+</sup>*B*<sup>+</sup> IS NOT SUPPRESSED BY *groE* MUTATIONS<sup>a</sup>

Genotype/plasmid	Growth of transformants at:	
	30°C	42°C
<i>lexA</i> (Def)71::Tn5 / pSE117	.b	+++ <sup>c</sup>
<i>lexA</i> (Def)71::Tn5 <i>groEL100</i> / pSE117	+++	+++
<i>lexA</i> (Def)71::Tn5 / pGW1700	+/- <sup>d</sup>	+++
<i>lexA</i> (Def)71::Tn5 <i>groEL100</i> / pGW1700	+/-	+++

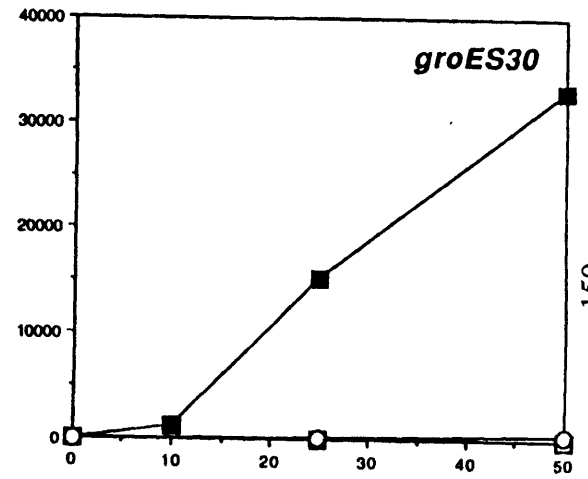
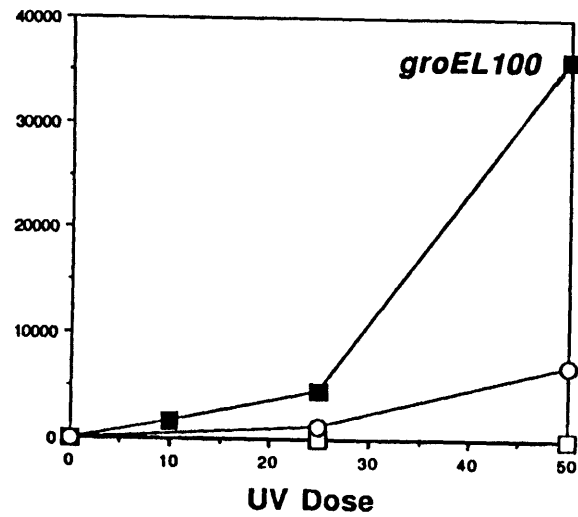
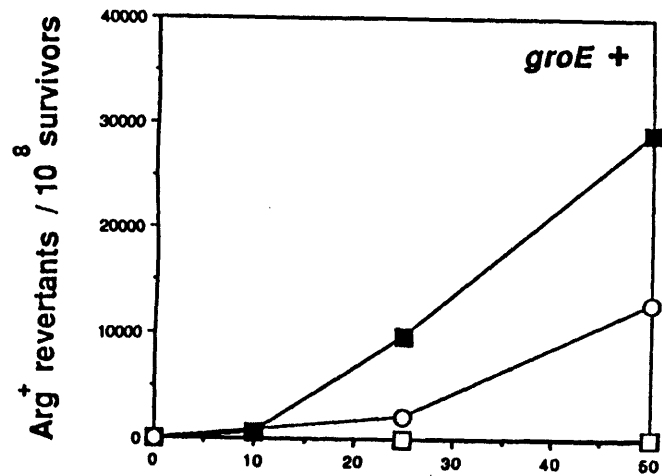
<sup>a</sup> The indicated strains were transformed with either pSE117 or pGW1700 and allowed to grow at 37°C for 90 minutes in LB broth without antibiotics. The transformation mixture was subsequently divided in two and spread on LB agar plates containing the appropriate antibiotics at 30°C and 42°C. The number of transformants was recorded after the indicated times.

<sup>b</sup> "-": Colonies do not form after 36 h.

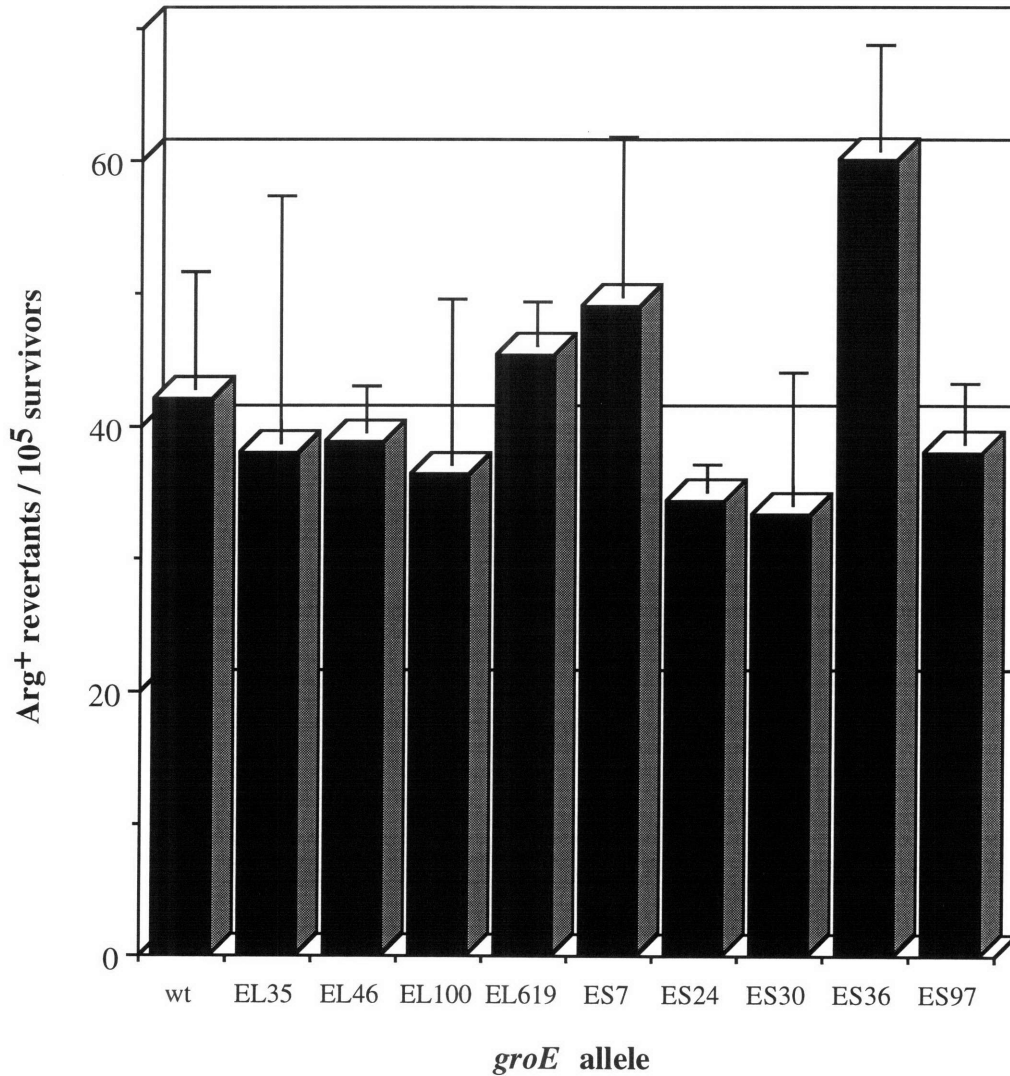
<sup>c</sup> "+++": Colonies grow very well after 18 h.

<sup>d</sup> "+/-": Small colonies form after 36 h.

**FIG. 1.** UV mutagenesis in *groE*<sup>+</sup> and *groE* strains, with and without pKM101. Cultures were grown at 37°C in LB broth to OD<sub>600</sub> = 0.5. Cells were centrifuged and resuspended in 0.85% saline. The cells were then transferred to a glass petri dish, UV irradiated, diluted as appropriate and plated as described in Materials and Methods. A.) O, AB1157; □, GW3200: ■, GW3200(pKM101). B.) O, GW7503; □, GW8020; ■, GW8020(pKM101). C.) O, GW7504; □, GW8021; ■, GW8021(pKM101).



## MucAB-dependent UV mutagenesis in *groE* strains



**FIG. 2.** MucAB-dependent UV mutagenesis in *groE*<sup>+</sup>, *groEL* and *groES* strains. The *groE* mutations were transduced into GW2100 which was nonmutable due to an *umuC122::Tn5* mutation. The cultures were grown at 37°C in LB broth to OD<sub>600</sub> = 0.5, centrifuged and resuspended in 0.85% saline. The cells were then transferred to a petri dish, UV irradiated, diluted as appropriate and plated as described in Materials and Methods. Survival was measured on plates containing limiting arginine (1μg/ml). The reversion frequencies shown are the average of three experiments.

**FIG. 3.** UV survival in *groE*<sup>+</sup> and *groE* strains, with and without pKM101. Cultures were grown and irradiated as in Fig. 1. Irradiated cells were spread on agar plates as described in Materials and Methods. A.) □, GW3200; ■, GW3200(pKM101). B.) □, GW8020; ■, GW8020(pKM101). C.) □, GW8021; ■, GW8021(pKM101).

