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

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The *umuDC* operon of *Escherichia coli*, a member of the SOS regulon, is required for SOS mutagenesis. Following the posttranslational 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 finding 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.

The *umuDC* operon in *Escherichia coli* was originally identified in a genetic screen for mutations that abolished mutagenesis resulting from exposure to UV 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 nonmutability of a Δ*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*$^+$ 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 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 *Eco*K, requires the *umuDC* gene products (22). Finally, the UmuD′C complex functions as an 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

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TABLE 1. Strains and plasmids used

Strain or plasmid	Relevant genotype or description	Reference or source
Strains		
GW1000	*recA441 sulA11 sfiC2*	1
RW82	Δ(*umuDC*)*595*::*cat*	50
JL2301	*lexA300*(Def)::*spc*	21
GW6752	Δ(*recA-srlR*)*306*::Tn*10*	34
GW6900	*recA430 srl*::Tn*10*	34
LMG194	Δ*ara-714 leu*::Tn*10*	20
GW2771	GW1000, *recA*$^+$	J. Kreuger
GW8018	GW1000, *lexA300*(Def)::*spc*	GW1000 × P1(JL2301)
GW8023	GW2771, Δ(*umuDC*)*595*::*cat*	GW2771 × P1(RW82)
GW8024	GW8023, *lexA300*(Def)::*spc*	GW8023 × P1(JL2301)
GW8025	GW8018, Δ(*umuDC*)*595*::*cat*	GW8018 × P1(RW82)
GW8026	GW8018, Δ(*recA-srlR*)*306*::Tn*10*	GW8018 × P1(GW6752)
GW8027	GW8018, *recA430 srl*::Tn*10*	GW8018 × P1(GW6900)
GW8028	GW8023, Δ*ara-714 leu*::Tn*10*	GW8023 × P1(LMG194)
GW8029	GW8025, Δ*ara-714 leu*::Tn*10*	GW8025 × P1(LMG194)
Plasmids		
pBR322		New England Biolabs
pBR322/kan	Km^r, Tc^s	This work
pSE115	*umuDC*; pSC101	15
pSE117	*umuDC*; pBR322	15
pGW3751	*umuD′C*; pBR322	13
pLM206	*umuD*; pBR322	31
pGW2101	*umuDC*; pBR322 Δ*rop*	34
pGW2101/kan	Km^r	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_{BAD} expression vector	20
pTO2	P_{BAD}-*umuDC*	This work
pTO3	pTO2 derivative, Km^r, Ap^s	This work
pZAQ	*ftsQAZ*; pBR322	48

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 described 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 Δ*umuDC* transductants to screen for strains lacking UmuD and on *recA430*, *srl*::Tn*10* (Tc^r) transductants in a *lexA*(Def) background at 42°C to screen for strains that are deficient in coproteolysis of UmuD. Δ(*recA-srlR*) 306::Tn*10* (Tc^r) transductants were screened for UV sensitivity, and *lexA300* (Def)::*spc* (Sp^r) transductants were screened for UmuDC-mediated cold sensitivity. Δ*ara-714 leu*::Tn*10* (Tc^r) 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 Laboratories (Waukegan, Ill.). Ampicillin, tetracycline, kanamycin, spectinomycin, chloramphenicol, 4′,6-diamidino-2-phenylindole (DAPI), and poly-L-lysine were purchased from Sigma Chemical Co. (St. Louis, Mo.). The GeneAmp PCR kit was purchased from Perkin-Elmer Cetus (Norwalk, Conn.). All restriction endonucleases and DNA ligase were purchased from New England Biolabs (Beverly, Mass.). The Western Lights kit for chemiluminescent immunoblot analyses was purchased from Tropix (Bedford, Mass.). 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; and chloramphenicol, 30 μg/ml.

Plasmid constructions. pBR322/kan contains a Km^r gene and a deletion of the Tc^r gene. The Km^r cassette from mini-Tn*5* Km (9) was isolated as a 2.0-kb *Hin*dIII fragment and introduced by ligation into the *Hin*dIII 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-Tn*5* 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 PCR using the following primers: UmuD5′ (5′-CGAATTCATATGTTGTT TATCAAGCC-3′) and Umu3′ (5′-GGAATTCTTATTTGACCCTCAGTAAA TC-3′) (initiation and termination codons are underlined). PCRs were performed with the GeneAmp kit (Perkin-Elmer Cetus). 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, Ohio) and found to be identical to the reported sequence of the *umuDC* operon. The Km^r cassette from mini-Tn*5* 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 previously (15) except that the UV-irradiated cells were plated on selective medium containing 0.2% arabinose.

Quantitative transformation assays. Preparation of competent cells by using $CaCl_2$ and the procedure for transformation of *E. coli* are described by Sambrook et al. (40). In the quantitative transformation assay, 0.3 μg of purified plasmid DNA was added to 100 μl of competent cells. After transformation, the cells were incubated in LB media at 42°C in an air shaker for 2 h without antibiotic selection. Serial 10-fold dilutions of each transformation were made, 10-μl aliquots were spotted on duplicate plates and incubated at 30 or 42°C, and the CFU per milliliter at each temperature was determined. In some cases, the cells from 1 ml of the transformation mix were concentrated, plated, and incubated at 30°C. The ratio of CFU per milliliter at 30°C to that at 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 h 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 of LB (1:125 dilution) containing the appropriate antibiotics in a 500-ml flask and incubated with shaking (~215 rpm) at 42°C for 1 h, at which time the culture was split in half, transferred to 250-ml flasks, and

TABLE 2. Transformation efficiency of *umuDC*-expressing plasmids in *lexA*$^+$ and *lexA*(Def) strains[a]

Strain	Plasmid	Transformants/ml		
		30°C	42°C	30°C/42°C
GW2771 *lexA*$^+$ *recA*$^+$	pBR322/kan	4.3×10^5	3.5×10^5	1.2
	umuDC; pSC101	3.3×10^4	2.6×10^4	1.3
	umuDC; pBR322	6.5×10^4	5.4×10^4	1.2
	umuD′C; pBR322	7.8×10^4	6.2×10^4	1.3
	umuDC; pBR322 Δ*rop*, Km^r	9.5×10^4	9.3×10^4	1.0
	umuD; pBR322	1.6×10^5	1.2×10^5	1.3
GW8024 *lexA*(Def) *recA*$^+$	pBR322/kan	2.4×10^5	2.5×10^5	0.95
	umuDC; pSC101	2.2×10^3	3.4×10^4	0.066
	umuDC; pBR322	3	1.3×10^5	0.000024
	umuD′C; pBR322	4.7×10^2	4.0×10^4	0.012
	umuD; pBR322	1.0×10^5	1.1×10^5	0.94
GW8018 *lexA*(Def) *recA441*	pBR322/kan	1.7×10^6	1.8×10^6	0.96
	umuDC; pSC101	1.0×10^4	3.4×10^4	0.29
	umuDC; pBR322	15	3.4×10^5	0.000044
	umuD′C; pBR322	1.4×10^4	9.2×10^4	0.16
	umuDC; pBR322 Δ*rop*, Km^r	3	770	0.0039
	umuD; pBR322	3.6×10^5	3.8×10^5	0.94
GW8026 *lexA*(Def) Δ*recA*	pBR322/kan	1.6×10^5	3.0×10^5	0.53
	umuDC; pSC101	8.4×10^2	1.6×10^4	0.053
	umuDC; pBR322	0	7.2×10^4	<0.000014
	umuD′C; pBR322	4	3.4×10^4	0.00012
	umuD; pBR322	6.0×10^4	1.1×10^5	0.55
GW8027 *lexA*(Def) *recA430*	pBR322/kan	6.9×10^4	6.9×10^4	1.0
	umuDC; pSC101	7.7×10^2	1.3×10^4	0.061
	umuDC; pBR322	0	3.6×10^4	<0.000028
	umuD′C; pBR322	2	1.4×10^4	0.00014
	umuD; pBR322	6.1×10^4	6.5×10^4	0.93

[a] The data 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 values shown.

incubated with shaking at 42 or 30°C. At various times, samples were removed from the cultures to measure the optical density at 600 nm (OD_{600}), to determine CFU per milliliter 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 sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) loading buffer (40) to a final concentration of 0.005 OD_{600} unit/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_{600} unit) was subjected to SDS-PAGE as described by Sambrook et al. (40). The proteins were transferred from the gel to a polyvinylidene difluoride membrane, with the subsequent antibody reactions and chemiluminescence detection performed as described in the Western Lights manual (Tropix). The anti-UmuD/D′ antibody used in these experiments has been previously described (4).

Microscopic analyses. Sample preparation for microscopy and staining with 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_{600} unit/ml, and 10 μl of these cell suspensions was examined. Samples were examined and photographed with an Axioscope (Carl Zeiss, Inc., Thornwood, N.Y.) 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 milliliter obtained at 30°C to transformants per milliliter obtained 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 and 42°C of cultures growing exponentially at 42°C is used as a measure of cold sensitivity. The results obtained from these two assays were essentially identical. The data in Table 2 show 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) Δ*recA* 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 milliliter at 30°C to transformants per milliliter 42°C is slightly less than 1 in a *lexA*(Def) Δ*recA* 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 Δ*recA* derivatives than it is in a *recA441* derivative (Table 2). The RecA430 protein is deficient in coprotease activity while remaining proficient in recombination (16),

TABLE 3. Transformation efficiencies of noncleavable *umuD* alleles in *lexA*$^+$ and *lexA*(Def) strains[a]

Strain	Plasmid	Transformants/ml		
		30°C	42°C	30°C/42°C
GW2771	pBR322	3.6×10^4	3.9×10^4	0.92
	pGW2101 (*umuDC*)	4.6×10^4	3.5×10^4	1.3
lexA$^+$	pGW2111 (*umuD*(GK25)*C*)	2.0×10^5	1.6×10^5	1.2
recA$^+$	pGW2112 (*umuD*(SA60)*C*)	1.4×10^5	1.1×10^5	1.3
	pGW2115 (*umuD*(KA97)*C*)	3.0×10^5	2.4×10^5	1.2
GW8018	pBR322	1.4×10^5	1.3×10^5	1.1
	pGW2101 (*umuDC*)	9	9.3×10^2	0.0097
lexA(Def)	pGW2111 [*umuD*(GK25)*C*]	44	3.0×10^5	0.00015
recA441	pGW2112 [*umuD*(SA60)*C*]	5	9.0×10^2	0.0056
	pGW2115 [*umuD*(KA97)*C*]	7	3.4×10^4	0.00020

[a] The data 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 values shown.

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*$^+$ 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. 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*$^+$, 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 wild-type *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- 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 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 monitored at 30 and 42°C. The data in Fig. 1 demonstrate that pSE117 (encoding *umuDC*)

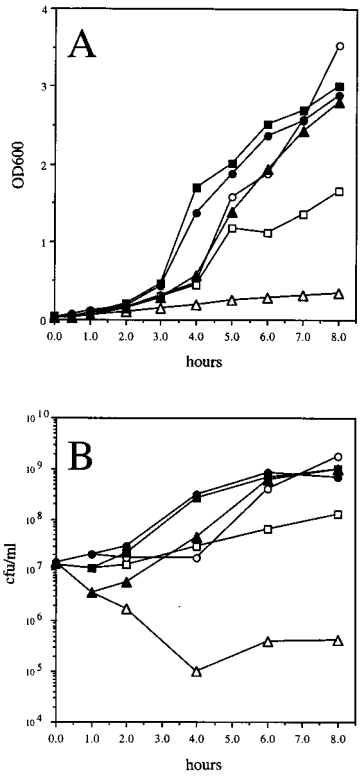

FIG. 1. Inhibition of growth at 30°C mediated by *umuDC* is more severe than that mediated by *umuD′C*. After inoculation, cultures were grown at 42°C for 1 h and then divided in two, with half shifted to 30°C and half remaining at 42°C. Samples were removed at the indicated times for analyses by OD, CFU per milliliter, and immunoblotting (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) Δ*umuDC*] carrying pBR322 (○, ●), pGW3751 (*umuD′C*; pBR322) (□, ■), and pSE117 (*umuDC*; pBR322) (△, ▲) was used. (A) OD_{600} measured as a function of time; (B) CFU per milliliter measured as a function of time.

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′ (see Fig. 3E and D, 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 P_{umuDC} 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*::Tn*5* strain in SOS mutagenesis (data not shown). However, the level of UmuC expressed from this construct, as measured by immunoblotting, 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 (Δ*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 (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 per cell (pBR322) to ~30 copies per cell (*umuDC*; pBR322 origin) (Table 2). The increase in cold sensitivity compared with the vector pBR322 is between 3- and 15-fold for the pSC101-derived plasmid (~5 copies of *umuDC*) and is at least 40,000-fold for pSE117, the pBR322-derived plasmid (~30 copies of *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 Δ*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

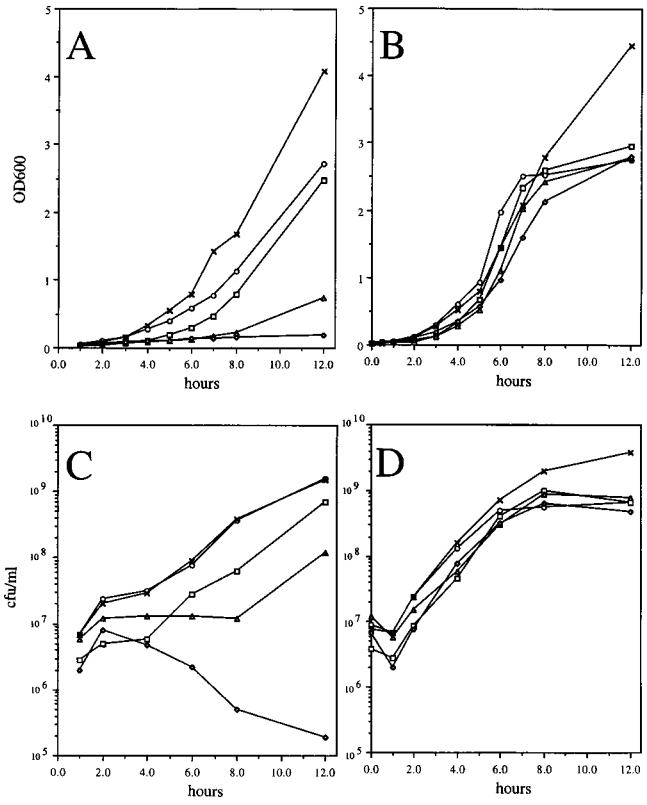

FIG. 2. Correlation of *umuDC* dosage with cold sensitivity for growth. After inoculation, cultures were grown at 42°C for 1 h and then divided in two, with half shifted to 30°C and half remaining at 42°C. Samples were removed at the indicated times for analyses by OD, CFU per milliliter, immunoblotting (see Fig. 3), and microscopy (see Fig. 4 and 5). All strains were *lexA*(Def) and Δ*umuDC*/pBR322 (○), *umuD*$^+$ *C*$^+$/pBR322 (□), Δ*umuDC*/pSE115 (*umuDC*; pSC101) (△), Δ*umuDC*/pSE117 (*umuDC*; pBR322) (◇), and Δ*umuDC*/pLM206 (*umuD*; pBR322) (×). (A) OD_{600} at 30°C measured as a function of time; (B) OD_{600} at 42°C measured as a function of time; (C) CFU per milliliter at 30°C measured as a function of time; (D) CFU per milliliter at 42°C measured as a function of time.

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_{600} and CFU per milliliter, is observed as the copy number of *umuDC* is increased from 0 to ~30 copies per cell (Fig. 2A and C). *lexA*(Def) strains containing pSE117 (~30 copies per 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 D). *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 by immunoblotting using affinity-purified antibodies against UmuD and UmuD′ (Fig. 3A to D). The immunoblot analyses demonstrate that increasing the copy number of *umuDC* from 0 to ~30 copies leads to an increase in the levels of UmuD and UmuD′ in cells growing at 42 and 30°C (Fig. 3A to D). Similar results are obtained in immunoblot analyses using antibodies specific

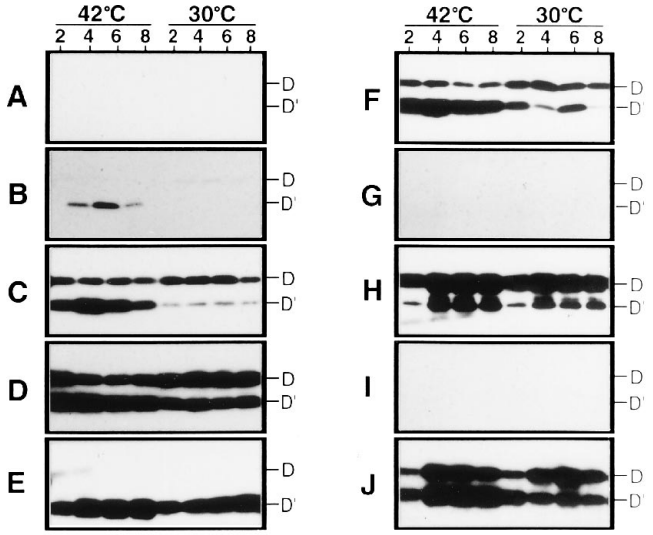

FIG. 3. Immunoblot analyses of UmuD and UmuD′ protein levels. Samples from the growth curves shown in Fig. 1, 2, and 7 were removed at the indicated time, equalized for OD, and analyzed by immunoblotting using antibodies to UmuD and UmuD′. (A) GW8025 [*lexA*(Def) *recA441* Δ*umuDC*]/pBR322; (B) GW8018 [*lexA*(Def) *recA441 umuD*$^+$*C*$^+$]/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*$^+$ *recA*$^+$ Δ*umuDC* Δ*ara*)/pTO3 (P_{BAD}-*umuDC*) in the absence of arabinose; (H) GW8028/pTO3 in the presence of 0.2% arabinose; (I) GW8029 [*lexA*(Def) Δ*umuDC* Δ*ara*]/pTO3 in the absence of arabinose; (J) GW8029/pTO3 in the presence of 0.2% arabinose.

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 morphologies of these strains by using a microscope equipped with Nomarski optics. Fluorescence 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 Materials and Methods, all of the cell samples were equalized to approximately 1 OD_{600} unit/ml before they were prepared for microscopic examination. The majority of cells of a *lexA*(Def) strain containing the Δ*umuDC595* deletion appear as short rods at 30°C, approximately three to four times the length of the cells from the same strain grown at 42°C (Fig. 4E). Fluorescence microscopy revealed that these cells each contain three to four 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 to C). These filaments appear to be nonseptated 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 an *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 between 15 and 45 cell lengths (Fig. 4G 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) from filaments at 42°C (Fig. 4H). This finding 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 that are almost completely devoid of DAPI staining material or contain a single, small mass of DAPI-staining material are evident. 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). Plasmid pZAQ (48), which causes a sevenfold 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 (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, we performed growth curve analyses 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 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*$^+$ Δ*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

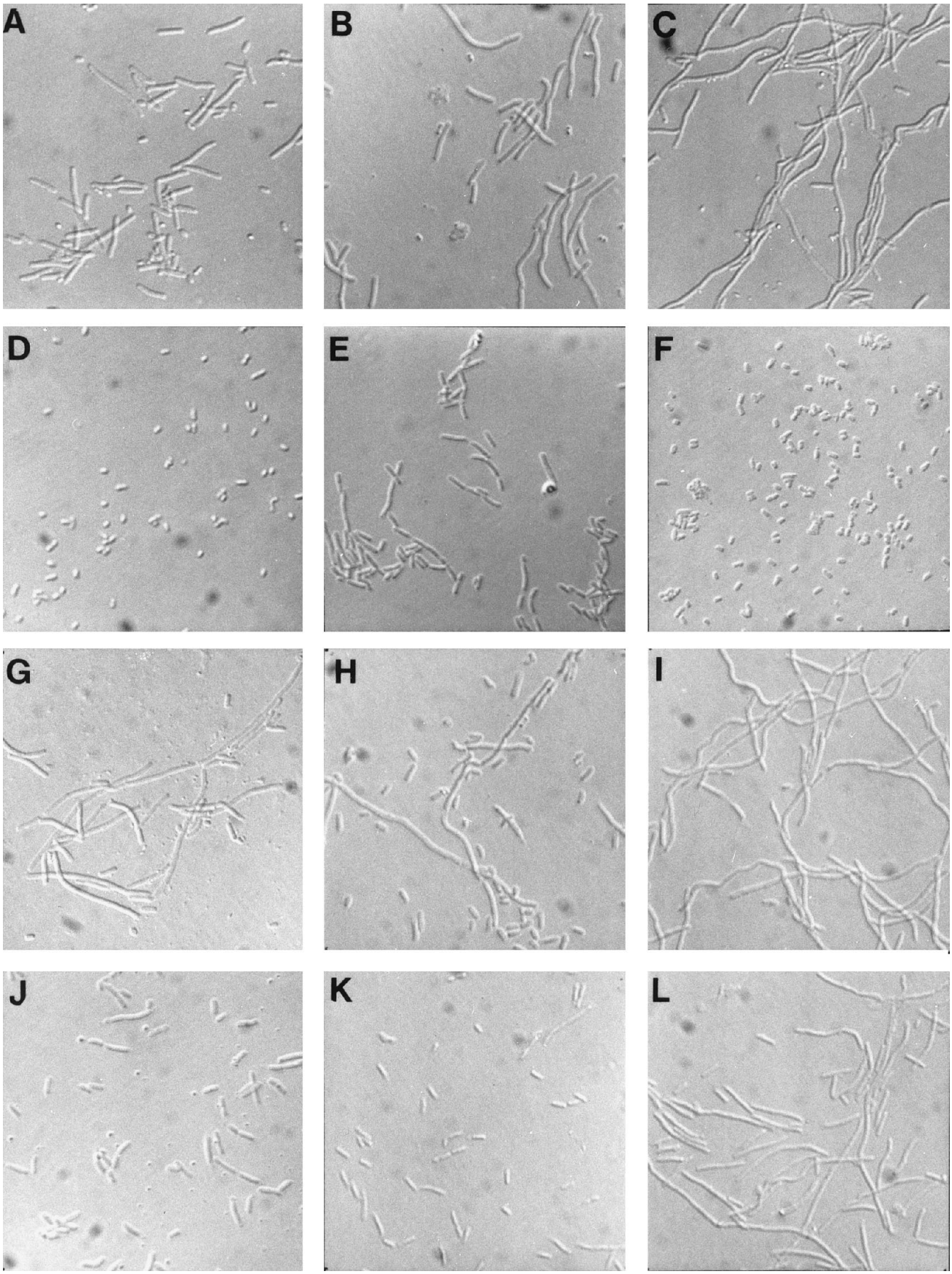
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FIG. 4. Cellular morphologies associated with UmuDC-mediated cold sensitivity. Samples were removed during the growth curve analyses shown in Fig. 2 and 7 at 6 h unless otherwise indicated, equalized for OD, and fixed on microscope slides. Microscopic analyses were done on a Zeiss Axioscope with Nomarski optics. (A) GW8018 [*lexA*(Def) *umuD*$^+$*C*$^+$]/pBR322, 30°C, 4 h; (B) GW8018/pBR322, 30°C, 6 h; (C) GW8018/pBR322, 30°C, 8 h; (D) GW8018/pBR322, 42°C; (E) GW8025 [*lexA*(Def) Δ*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*$^+$ Δ*umuDC* Δ*ara*)/pTO3 [P_{BAD}-*umuDC*) in the presence of 0.2% arabinose, 30°C; (K) GW8029 [*lexA*(Def) Δ*umuDC* Δ*ara*]/pTO3 in the absence of arabinose, 30°C; (L) GW8029/pTO3 in the presence of arabinose, 30°C.

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 Δ*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*$^+$ *recA*$^+$ Δ*umuDC*) and GW8029 [*lexA*(Def) *recA441* Δ*umuDC*] and analyzed growth of these strains at 42 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. 7A and C). Little or no effect on growth is evident at 30°C in the absence of arabinose (Fig. 7A and C) or at 42°C in the presence or absence of arabinose (Fig. 7B and D). Interestingly, the *lexA*$^+$ *recA*$^+$ strain carrying pTO3 incubated at 30°C after growth in the presence of arabinose exhibits an increase in OD_{600} without a concomitant increase in CFU per milliliter. This finding suggests that these cells continue to divide at 30°C but that the progeny cells are not viable. The possibility that the increase in OD 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 by immunoblot analyses. The results are shown in Fig. 3. UmuD is not detectable in cells grown in the absence of arabinose (Fig. 3G and I), but high levels are detected in the presence of 0.2% arabinose at 42 and 30°C (Fig. 3H 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*$^+$ *recA*$^+$ Δ*umuDC*) and GW8029 [*lexA*(Def) *recA441* Δ*umuDC*] containing pTO3 (P_{BAD}-*umuDC*) incubated at 30 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 three to four cell lengths long (Fig. 4J), despite the high levels of UmuD and UmuC in these cells (Fig. 3H). However, the majority of GW8029/pTO3 cells grown in the presence of arabinose at 30°C form filaments of approximately 10 to 30 cell lengths (Fig. 4L). In contrast, GW8029/pTO3 cells incubated in the absence of arabinose at 30°C form short rods of two to three 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 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. Given 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

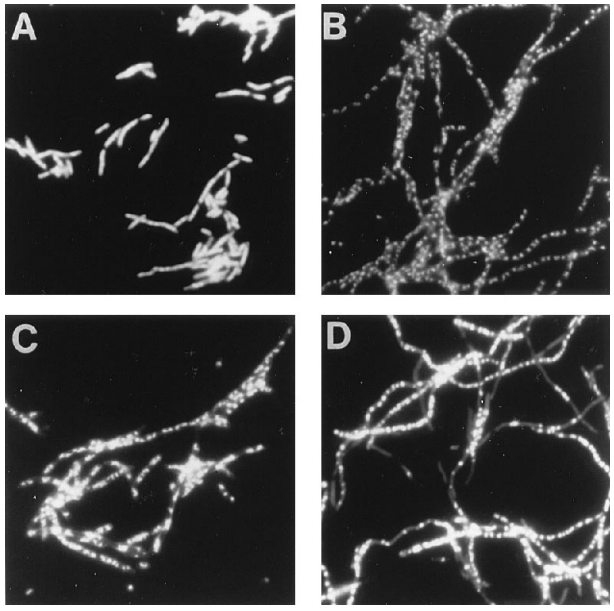

FIG. 5. Nucleoid morphologies associated with UmuDC-mediated cold sensitivity. Samples were removed during the growth curve analyses shown in Fig. 2 and 7 at 6 h unless otherwise indicated, equalized for OD, and fixed on microscope slides. Following the addition of DAPI, fluorescence microscopic analyses were done on a Zeiss Axioscope. (A) GW8025 [*lexA*(Def) Δ*umuDC*]/pBR322, 30°C; (B) GW8018 [*lexA*(Def) *umuD*$^+$*C*$^+$]/pBR322, 30°C, 8 h; (C) GW8025/pSE115 (*umuDC*; pSC101), 30°C; (D) GW8025/pSE117 (*umuDC*; pBR322), 30°C.

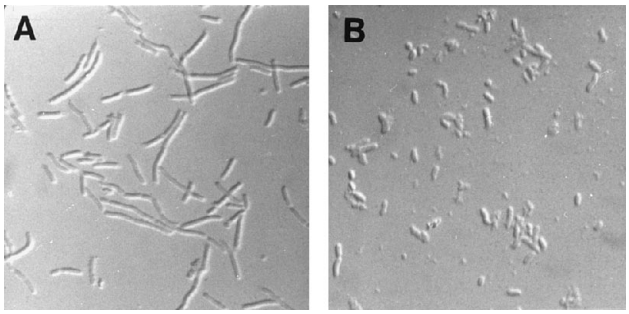

FIG. 6. Overexpression of the *ftsQAZ* operon suppresses UmuDC-mediated filamentation. Samples were removed from cultures of GW8025 [*lexA*(Def) Δ*umuDC*] carrying pSE115 (*umuDC*; pSC101) or pSE115 and pZAQ (*ftsQAZ*; pBR322) after 3.5 h of growth, equalized for OD, and prepared for microscopic analyses 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.

recA allele and that the intact form of UmuD appears to be most active in producing this phenomenon. Δ*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- to 4,000-fold less active in conferring cold sensitivity than plasmids encoding UmuD and UmuC strongly suggest that 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 Δ*recA*, *recA*$^+$, and *recA430* mutations compared with 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 Δ*recA*, *recA*$^+$, and *recA430* strains compared with *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 finding 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 is 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, such as *sfiC* (8) or *dinY* (36), have been shown to be regulated by RecA but not repressed by LexA. 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, plays 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

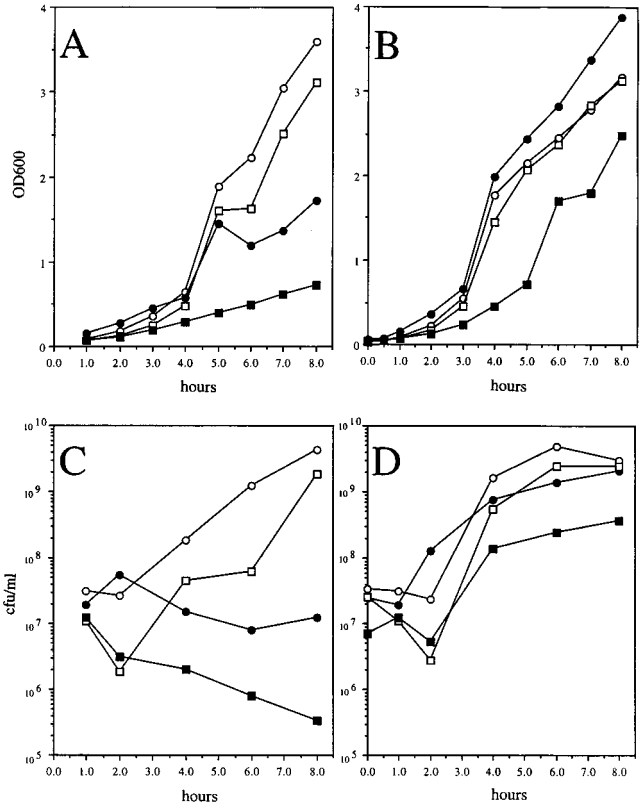

FIG. 7. Constitutive expression of *umuDC* confers cold sensitivity in the absence of any other SOS-regulated proteins. After inoculation, cultures were grown at 42°C for 1 h and divided in half, with half shifted to 30°C and half remaining at 42°C. Samples were removed at the indicated times for analyses by OD, CFU per milliliter, immunoblotting (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 Δ*umuDC* Δ*ara* and *lexA*$^+$/pTO3 [P_{BAD}-*umuDC*] (○, ●), and *lexA*(Def)/pTO3 (□, ■). (A) OD_{600} at 30°C measured as a function of time; (B) OD_{600} at 42°C measured as a function of time; (C) CFU per milliliter at 30°C measured as a function of time; (D) CFU per milliliter at 42°C measured as a function of time.

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 than at 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 finding 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 and GroES both in vivo and in vitro (13) and with DnaK, DnaJ, and GrpE in vitro (37). It is possible that these proteins are 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 and GroES 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 because 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 rifampin (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 rifampin sensitive) suggest that the filamentation that 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 inference 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 result 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 finding 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*$^+$) 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 observation leads us to suggest that UmuD and UmuC may function in cell cycle regulation; more specifically, 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 *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.

On the basis of the lack of an obvious evolutionary selection for proteins that promote mutagenesis and 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 support 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 unknown; however, one interesting possibility is that the elevated levels of UmuD and UmuC that lead to cold sensitivity for growth 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.

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