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Cold Sensitivity Induced by Overproduction of UmuDC in *Escherichia coli*

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The UmuD and UmuC proteins of *Escherichia coli* are essential for mutagenesis by UV and most chemicals. Their mode of action is presently unknown. Strains which lack the LexA repressor [*lexA*(Def)] and contain a pBR322-derived plasmid carrying the *umuDC* operon overexpress UmuD and UmuC and become cold sensitive (growth at 42°C but not at 30°C). Deletion mapping showed that the *umuDC* locus on the plasmid is responsible for conferring cold sensitivity. The conditional lethality appeared due to a rapid and reversible inhibition of DNA synthesis at the nonpermissive temperature. Cold sensitivity was enhanced by the increase of NaCl in the medium to 1% and eliminated by 4% ethanol in the medium. Cold sensitivity was partially suppressed by the *lon-100* mutation and completely suppressed by the *htpR165* mutation.

Mutagenesis by a variety of agents including UV light is not a passive event in *Escherichia coli*. The process requires induction of the SOS regulatory network and, in particular, is dependent on expression of the *umuDC* locus which is regulated by the *lexA* gene product (24). The *umuDC* locus has been cloned and its polypeptide products have been identified in maxicells (4, 21). The role that these proteins play in mutagenesis is unknown. Though models for this process involving modification of DNA polymerase III holoenzyme have been proposed (20), there has been no direct evidence that the UmuD and UmuC proteins interact with the cellular DNA replication machinery.

A plasmid analog of *umuDC*, the *mucAB* locus on plasmid pKM101, has also been cloned and characterized (19). The DNA sequence of *muc* has diverged from *umu* but both are similarly organized as operons consisting of a gene coding for a 16-kilodalton protein followed by a gene coding for a 45-kilodalton protein. Though the intact *muc* locus complements defects in the *umu* locus, the 16-kilodalton protein of *muc* does not complement a defect in the 16-kilodalton protein of *umu* and vice versa. That is, each gene product requires its cognate partner for activity (K. L. Perry, S. J. Elledge, B. Mitchell, L. Marsh, and G. C. Walker, submitted for publication). This finding indicates that the 45- and 16-kilodalton proteins interact. For the purposes of this paper we will refer to the UmuD and UmuC proteins as though they do act together. However, it is possible that the gene products have activities independent of one another.

All of the mutagenesis caused by agents such as UV and a small fraction of the repair of the DNA lesions caused by these agents is dependent on a functional *umuDC* operon (10, 23, 24). In the past this has been the only phenotype associated with the locus. We report here that overproduction of *umu* gene products induces an inability to grow at 30°C (cold sensitivity) as the result of a conditional inhibition of DNA synthesis. This phenotype provides a new tool for the study of the physiology and genetics of UmuD and UmuC.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains used in this study are listed in Table 1. Transductions using P1 *vir* and other standard genetic techniques were performed essentially as described by Miller (17). The *lon-100* and *htpR165* alleles were transduced by selecting for the kanamycin resistance of a linked Tn*5* and subsequently screening for, respectively, mucoid or temperature-sensitive colonies. Plasmid transformations were carried out by using a modification of the $CaCl_2$ method of Mandel and Higa (14). Transformed cells were allowed to express antibiotic resistance in LB broth for 1 h before they were plated on selective media. Cells were maintained at the same temperature during the expression and selection steps of the procedure. When possible, *lexA*(Def) strains were transformed at 43.5°C.

Growth and survival. For growth rate determinations cells were incubated in flasks in a shaking water bath. Samples were withdrawn at various times and the turbidity at 600 nm was determined. The temperature dependence of cell viability was measured by diluting in saline a culture grown at 43.5°C, plating the diluted cells, and incubating the plates at various temperatures.

Since it was not apparent that a *lexA*(Def) *htpR* strain containing pSE117 would be viable at any temperature, it was not possible to construct the strain under known permissive conditions and then test its ability to grow under other conditions. Therefore, a quantitative transformation procedure described in Results was used to determine viability of this strain.

Plasmid manipulations and constructions. Plasmid DNA was prepared as previously described (4). DNA used for plasmid constructions was purified by either a cesium chloride gradient containing ethidium bromide or electroelution from an agarose gel into a trough (15).

Restriction enzymes, T4 DNA ligase, and S1 nuclease were used under conditions suggested by the supplier or by Maniatis et al. (15).

Plasmid pSE117 (S. J. Elledge, Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, 1983) consists of a *Kpn*I-*Eco*RI fragment from pSE115 (4), which contains the entire *umuDC* operon and a kanamycin resistance determinant, cloned into a *Kpn*I-*Eco*RI vector fragment of pL10, a pBR322-derived high-copy-number plasmid. A restriction map of pSE117 is shown in Fig. 1. Deletion plasmids pLM201, pLM202, pLM203, pLM204, pLM205, and pLM206 were constructed by digesting pSE117 DNA with a restriction enzyme with sites in the region of interest, religating the

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TABLE 1. *E. coli* K-12 strains

Strain	Relevant genotype	Source or reference
KM1190	*lexA51*	T. Roberts (5)
MM294A	*lexA*$^+$	4
GW2730	*lexA71*::Tn*5*	J. H. Krueger; P1 *vir* GW2707 × GW1101 (1, 12)
GW5229	*lon-100 tsx*::Tn*10*	L. B. Casson
GW5230	*lexA51 lon-100*	P1 *vir* GW5229 × KM1190
GW5265	*lexA71*::Tn*5 htpR165*	J. H. Krueger and K.-H. Paek; P1 *vir* GW4701 × GW2730 (13)
GW2733	*lexA71*::Tn*5 recA56*	P1 *vir* AB1157 *srl*::Tn*10 recA56* × GW2730

DNA with T4 DNA ligase, transforming *E. coli*, and screening for strains carrying the desired deletion (Fig. 1). Deletion plasmid pLM207 was constructed by the following procedure: DNA of pSE117 was digested to completion with *Eco*RI endonuclease and then partially digested with *Hpa*I endonuclease and treated with S1 nuclease to generate blunt ends. The desired 8.0-kilobase *Hpa*I-*Eco*RI fragment was purified by agarose gel electrophoresis and recircularized by ligating with T4 DNA ligase. The structure of all plasmids was confirmed by restriction enzyme digestion followed by agarose gel electrophoresis.

Incorporation of labeled precursors into macromolecules. Log-phase cultures growing at 42°C were incubated with either 20 μCi of [^{35}S]methionine per ml and 10 μg of unlabeled carrier methionine per ml or 20 μCi of [^{3}H]thymidine per ml with 10 μg of unlabeled carrier thymidine per ml. Cultures were incubated for 60 min at 42°C and then shifted to 30°C, and the incubation was continued. Samples were removed at various times and added to 1 ml of cold 10% trichloroacetic acid to precipitate macromolecules.

Trichloroacetic acid-precipitated samples were collected on Whatman GF/C glass filters which were then washed three times with 0.1 N HCl and once with ethanol and dried. Radioactivity was determined by scintillation counting.

Pulse-labeling and autoradiography of newly synthesized proteins. Cultures growing at 43.5°C were shifted to 30°C for 5 min and then [^{35}S]methionine (60 μCi/ml) was added. The cells were allowed to incorporate radiolabeled methionine for 3 min, rapidly chilled on ice, and then harvested by centrifugation in a microcentrifuge. The pellets were prepared for, and subjected to, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and autoradiography as previously described (4). Maxicell-labeled proteins (4, 21) of GW2733(pSE117) were run on the same gels to provide markers for the positions of genuine UmuD and UmuC proteins. Autoradiograms were scanned with an Ortec model 4310 densitometer.

Media and reagents. LB medium has been previously described (17). It is a rich medium containing 0.5% NaCl and was used in all experiments except where otherwise stated. Kanamycin sulfate was purchased from Calbiochem-Behring Corp. and was used at 25 μg/ml. Ampicillin (sodium salt) was purchased from Sigma Chemical Co. and was used at 100 μg/ml. [^{3}H]thymidine (72 Ci/mmol) was purchased from ICN Biomedicals, Inc. [^{35}S]methionine (>600 mCi/mmol) was purchased from Amersham Corp.

RESULTS

UmuDC-induced cold-sensitivity. *E. coli* strains which are *lexA*(Def) (formerly *spr*) lack functional LexA protein and thus express the *umuD* and *umuC* genes constitutively at fully induced levels (1, 12, 21). These *lexA*(Def) strains are viable at temperatures including the range of 30 to 43.5°C. All of the *lexA*(Def) strains used in this study contain the *sulA11* mutation which suppresses SOS-induced filamentation (9). Plasmid pSE117 contains the *umuDC* genes including putative promotor and "SOS box" (LexA repressor binding site) (Perry et al., submitted for publication) subcloned onto a pBR322-derived multicopy vector. In an attempt to overproduce UmuD and UmuC, we tried to transform *lexA*(Def) strains with pSE117. However, most *lexA*(Def) strains could not be transformed by pSE117 if the selection for transformants was carried out at the usual temperature of 37°C (S. J. Elledge and G. C. Walker, unpublished data). Thus the plasmid either encodes a function that is lethal in a *lexA*(Def) strain or the plasmid cannot be maintained in such a strain. However, surprisingly, all

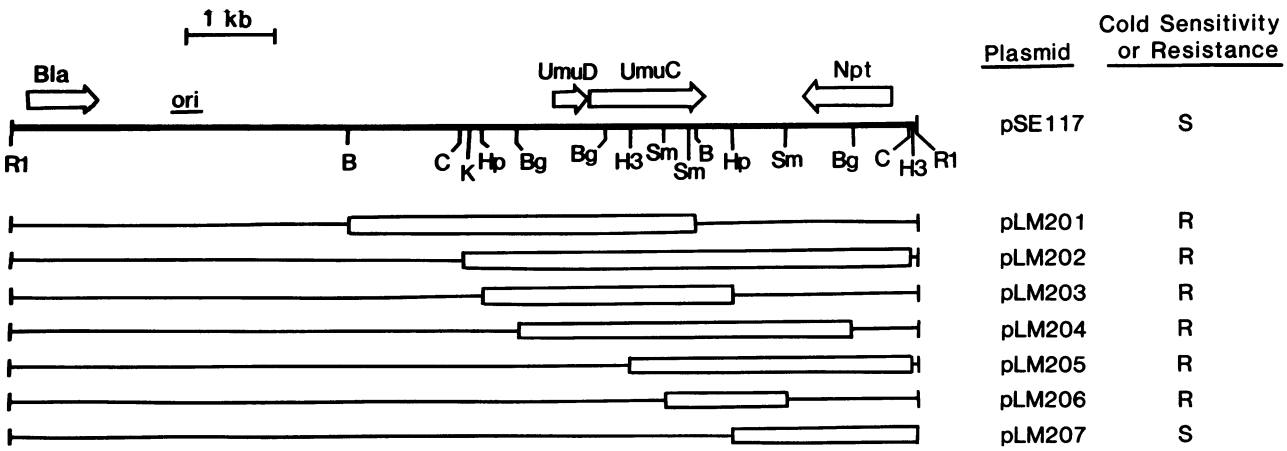

FIG. 1. Linear representation of pSE117 and deletion derivatives. Deleted regions are represented by open boxed regions. Abbreviations for restriction sites are as follows: *Eco*RI, RI; *Bam*HI, B; *Cla*I, C; *Kpn*I, K; *Hpa*I, Hp; *Bgl*II, Bg; *Hin*dIII, H3; *Sma*I, Sm. Also shown are the origin of replication, *ori*; neomycin phosphotransferase gene (kanamycin resistance), Npt; and β-lactamase gene (ampicillin resistance), Bla. Deletions have the following endpoints: pLM201, *Bam*HI-*Bam*HI; pLM202, *Cla*I-*Cla*I; pLM203, *Hpa*I-*Hpa*I; pLM204, *Bgl*II-*Bgl*II; pLM205, *Hin*dIII-*Hin*dIII; pLM206, *Sma*I-*Sma*I; pLM207, *Hpa*I-*Eco*RI. The ability of each plasmid to confer cold sensitivity on KM1190 was determined by constructing the plasmid-bearing KM1190 strains at 43.5°C. In each case cold-sensitive strains were <0.1% viable at 30°C, whereas cold-resistant strains were >50% viable at 30°C. kb, Kilobase.

lexA(Def) strains which we tested could be transformed by pSE117 at 43.5°C. The resulting pSE117-bearing *lexA*(Def) strains were cold sensitive (Table 2). Plasmid pSE117 does not confer cold sensitivity on *lexA*$^+$ strains.

To confirm that the *umuDC* region of pSE117 was responsible for conferring cold sensitivity on *lexA*(Def) strains, we constructed a series of deletions of the plasmid in vitro (Fig. 1). Derivatives of the *lexA*(Def) strain KM1190 bearing plasmids with deletions removing either the entire *umuDC* region or only part of *umuC* were not cold sensitive. From these results one can conclude that at least *umuC* is required for cold sensitivity. It is possible that both *umuC* and *umuD* are necessary. We will tentatively refer to pSE117-caused cold sensitivity as "UmuDC dependent."

Since we routinely use antibiotics to select against plasmid-free segregants of plasmid-containing strains, an apparent cold sensitivity could be caused by an inability to express the pSE117-encoded drug resistance genes at low temperatures. To test this possibility, cultures were plated at 30 and 43.5°C in the absence of antibiotics. The plating efficiency of KM1190(pSE117) at 30°C was only 0.8% even in the absence of selection for the plasmid. Therefore, cold sensitivity does not merely reflect a failure to express the antibiotic resistance determinants of pSE117.

Cold-sensitive strains which cannot tolerate brief exposure to nonpermissive temperatures (e.g., room temperature) are potentially difficult to manipulate. Fortunately, we found that the plating efficiency of KM1190(pSE117) did not decrease during a 2-h preincubation at 30°C before return to 43.5°C. A 6-h preincubation at 30°C lowered viability somewhat (data not shown). All dilutions and plating procedures for this work were carried out rapidly at room temperature.

The frequency of survival of KM1190(pSE117) at 30°C was reproducible within an experiment, but varied somewhat from experiment to experiment. One source of variability may be slight fluctuations in the concentration of the components of the medium. Table 3 shows that doubling the NaCl concentration of the medium lowered viability of KM1190(pSE117) at 30°C threefold.

Cold-resistant mutants arose at a high frequency (10^{-4} to 10^{-3} per cell) when KM1190(pSE117) cells were plated at 30°C, presumably in part because cell growth was not blocked completely. New colonies continued to arise for several days on such plates. For this study the number of colonies was scored between 20 and 24 h of incubation. Colonies which appeared at 30°C were mutants and not simply the result of physiological adaptation since, after subculture at 43.5°C, the suppressed strains retained their cold resistance phenotype (data not shown).

TABLE 2. Relative plating efficiency of strains at 30 versus 43.5°C

Strain	Relevant characterisitics	CFU at 30°C/CFU at 43.5°C[a]
KM1190	*lexA*(Def)	0.9
KM1190(pSE117)	*lexA*(Def), *umuDC* plasmid	1.4×10^{-4}
KM1190(pLM203)	*lexA*(Def), *umuDC* deleted from plasmid	1.0
GW2730	*lexA*(Def)	0.9
GW2730(pSE117)	*lexA*(Def), *umuDC* plasmid	2.4×10^{-3}
GW5230(pSE117)	*lexA*(Def) *lon-100*, *umuDC* plasmid	0.15
MM294A(pSE117)	*lexA*$^+$, *umuDC* plasmid	0.8

[a] CFU on LB plates (with ampicillin and kanamycin for plasmid-containing strains).

TABLE 3. Effect of medium supplements on cold sensitivity

Strain	Supplement	CFU/ml	
		30°C	43.5°C
KM1190	None	5×10^8	7×10^8
	0.5% NaCl[a]	8×10^8	7×10^8
KM1190(pSE117)	None	7×10^5	10×10^8
	0.5% NaCl[a]	2×10^5	8×10^8
KM1190	None	3×10^8	3×10^8
	4% Ethanol	7×10^7	ND[b]
KM1190(pSE117)	None	2×10^4	1×10^8
	4% Ethanol	9×10^7	ND

[a] LB medium contains 0.5% NaCl. The additional salt brings the final concentration to 1.0% NaCl.
[b] ND, No data. Ethanol at 4% is toxic to *E. coli* at 43.5°C.

Growth of KM1190(pSE117) at 30°C. Incubation at 30°C affected the growth of KM1190(pSE117) (Fig. 2). At 43°C the growth of the two strains was nearly identical. After a shift to 30°C the turbidity of the KM1190(pSE117) culture continued to rise for several hours, but slowly. The KM1190(pSE117) and KM1190 cultures were examined microscopically. After the shift to 30°C KM1190(pSE117) cells filamented but KM1190 cells did not. Filament length was roughly proportional to the period of incubation at 30°C.

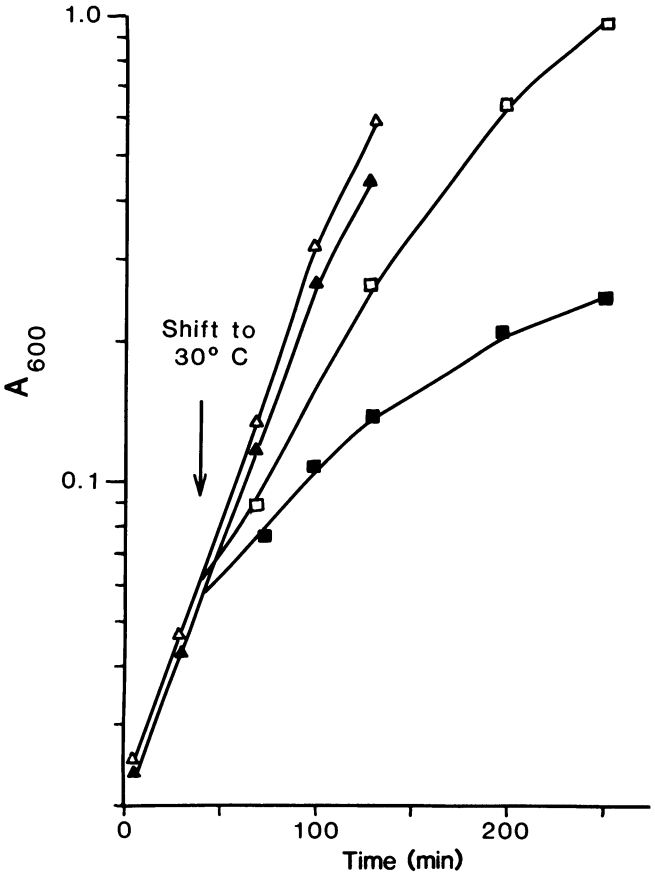

FIG. 2. Growth of KM1190 and KM1190(pSE117) at 43 and 30°C. Log-phase cultures growing at 43°C were shifted to 30°C or allowed to continue growing at 43°C. Symbols: △, KM1190, 43°C; □, KM1190, 30°C; ▲, KM1190(pSE117), 43°C; ■, KM1190(pSE117), 30°C. A_{600}, Absorbance at 600 nm.

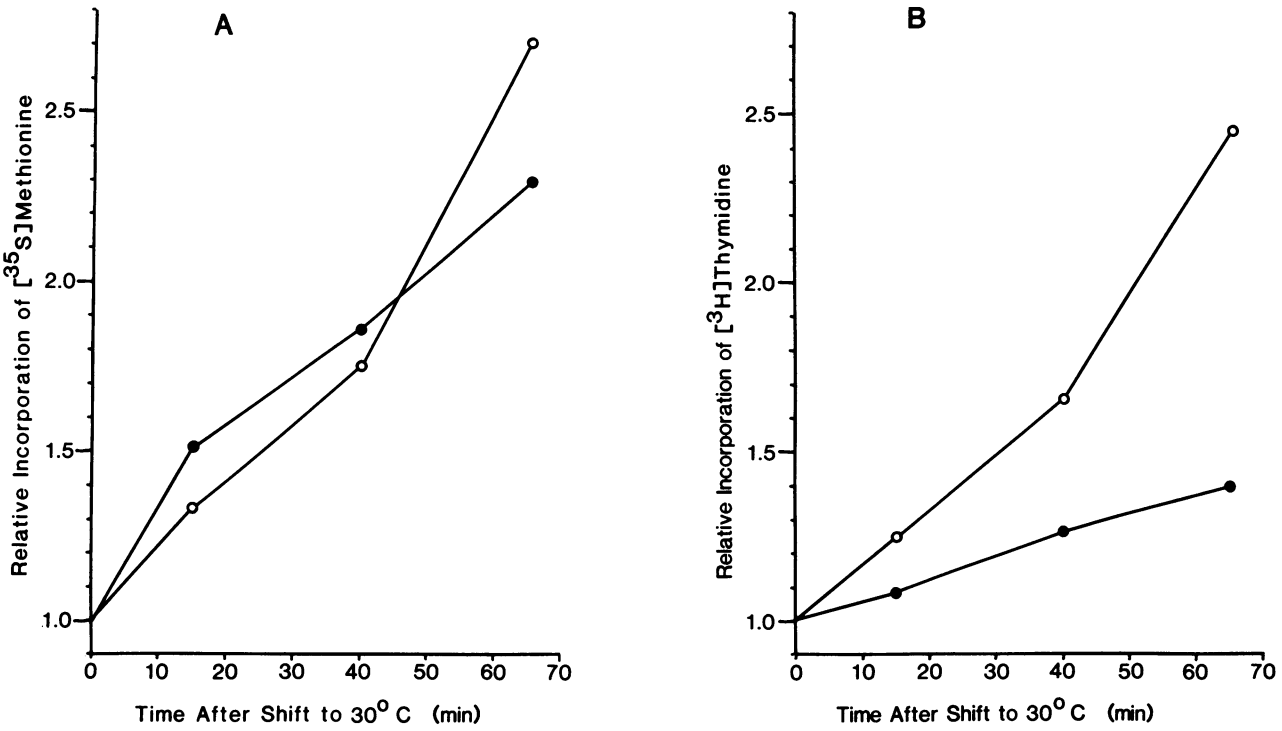

FIG. 3. Incorporation of [^{35}S]methionine and [^{3}H]thymidine into KM1190 and KM1190(pSE117) at 30°C. Labeling was begun at 43°C. Cultures were shifted to 30°C at time zero. (A) Incorporation of [^{35}S]methionine; (B) incorporation of [^{3}H]thymidine. Symbols: ○, KM1190 (wild type); ●, KM1190(pSE117) (cold sensitive).

We also compared the growth of a *lexA*$^{+}$ strain to that of a *lexA*(Def) strain. Neither strain carried the cloned *umuDC* genes but both contained a wild-type *umuDC* locus on the chromosome. The strains grew at similar rates at 42°C, but at 30°C the doubling time of the *lexA*(Def) strain was 80% longer than that of the *lexA*$^{+}$ strain. We do not know whether this temperature effect is due to the expression of UmuDC or of some other LexA-controlled protein(s).

DNA and protein syntheses at 30°C. KM1190 and KM1190(pSE117) were incubated with radioactively labeled precursors of DNA or protein (Fig. 3). At 30°C incorporation of [^{3}H]thymidine was depressed in the KM1190(pSE117) strain relative to KM1190, whereas incorporation of [^{35}S]methionine was unaffected. Incorporation of [^{3}H]uridine was also not reduced at 30°C (data not shown). This suggests that cold sensitivity results from a temperature-dependent block in the pathway of DNA synthesis.

DNA synthesis inhibition and recovery. After transfer of a KM1190(pSE117) culture to 30°C, no lag period was discernible before the rate of [^{3}H]thymidine incorporation fell. To more accurately determine how rapidly DNA synthesis responded to a temperature shift, we pulse-labeled aliquots of a culture for 5 min with [^{3}H]thymidine at various times while cells were subjected to a permissive/nonpermissive/permissive temperature regimen (Fig. 4). The test strain was KM1190(pSE117) and the control strain was KM1190 (pLM203); pLM203 is a derivative of pSE117 from which the *umuD* and *umuC* genes have been deleted (Fig. 1). Thus we were able to grow both strains in the presence of kanamycin and ampicillin to rule out the possibility that the antibiotics might be affecting DNA synthesis in a temperature-dependent fashion. The rate of DNA synthesis in KM1190(pSE117) cells slowed at 30°C and recovered at 43°C (Fig. 4). Both the inhibition and the recovery of DNA synthesis were very rapid. Note that the growth rate of KM1190 at 30°C was about half of its rate at 43°C (Fig. 2). As expected, at 30°C the rate of DNA synthesis in the control strain dropped to about 50% of its rate at 43°C.

Stability of *umuD* and *umuC* gene products. Preliminary pulse-chase studies of maxicell-labeled UmuDC proteins indicated that they were unstable (data not shown). This in turn suggested that they might not accumulate even if they were being overproduced in pSE117-containing *lexA*(Def) strains. The promoter of the *umuDC* operon is relatively strong when fully induced as judged by the expression of a *umuC'-lacZ*$^{+}$ operon fusion located on the chromosome (1). However, the *umuD* and *umuC* gene products are not visible upon Coomassie blue staining of whole-cell proteins of KM1190(pSE117) displayed on an SDS-polyacrylamide gel (data not shown). To find out if the UmuD and UmuC polypeptides were overproduced in KM1190(pSE117) at 30°C, cells were shifted from 43.5 to 30°C, pulse-labeled for 3 min with [^{35}S]methionine, chilled, and immediately prepared for loading on an SDS-polyacrylamide gel (Fig. 5). Proteins that migrate to the positions expected for UmuD and UmuC are labeled more heavily during this brief pulse in KM1190(pSE117) than in the control strain, KM1190(pLM203). This suggests that UmuD and UmuC are indeed overproduced from plasmid pSE117 in a *lexA*(Def) genetic background at 30°C even though they do not accumulate.

The expression of UmuD and UmuC did not appear to be temperature dependent. Similar overexpression of UmuD and UmuC polypeptides occurred during brief labeling periods at 37 and 42°C (data not shown). To further examine the effect of temperature on expression of the *umuDC* operon, we introduced a *lacZ* fusion to the *umu* operon on pSE143 into KM1190(pSE117). pSE143 is a high-copy-number

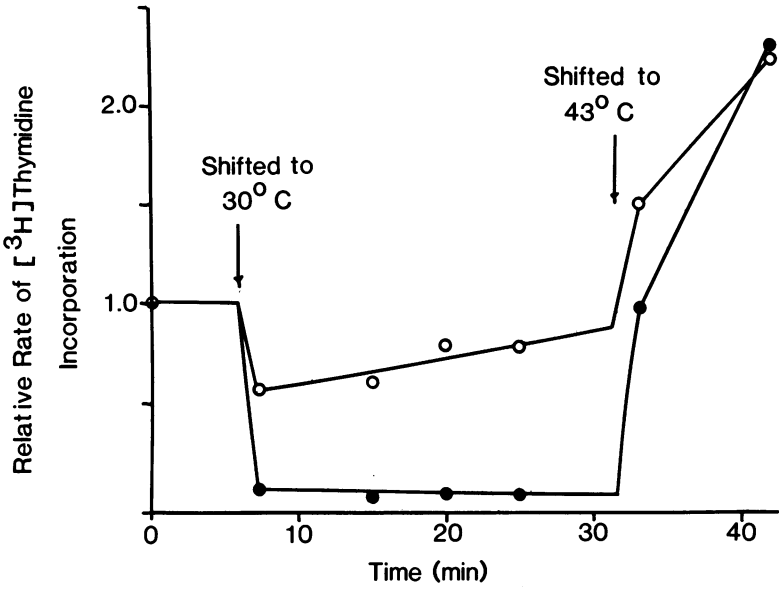

FIG. 4. Inhibition and recovery of DNA synthesis. Log-phase cultures growing at 43°C were shifted first from 43 to 30°C and then from 30 to 43°C. Points represent incorporation during a 5-min labeling period begun at the indicated time. Arrows show the time of temperature shifts. The experiment was begun at 43°C. Symbols: ○, KM1190(pLM203) (control, *umuDC*⁻ plasmid); ●, KM1190(pSE117) (*umuDC*⁺ plasmid).

plasmid compatible with pSE117 and carrying a protein fusion of β-galactosidase to *umuC'* and a wild-type copy of *umuD* and upstream sequences (4). The resulting strain remained cold sensitive and did not accumulate β-galactosidase activity after a temperature shift from 43.5 to 30°C (data not shown). Therefore, increased transcription or translation of the *umu* operon at low temperatures seems unlikely.

Suppression of cold sensitivity. The effects of ethanol on cells in some ways mimic an increase in temperature. Therefore, we tested the effect of 4% ethanol on survival of KM1190(pSE117) at 30°C. This concentration of ethanol induces a heat shock response in *E. coli* at 30°C equivalent to growth at 42°C (F. C. Neidhardt, R. A. VanBogelen, and V. Vaughn, submitted for publication). Remarkably, 4% ethanol also completely suppressed the cold sensitivity of KM1190(pSE117) (Table 3). In addition, it was possible to construct pSE117-containing derivatives of the *lexA*(Def) strain KM1190 by transforming with pSE117 and selecting for the plasmid at 30°C on medium containing the appropriate antibiotics and 4% ethanol.

Mutations suppressing the cold sensitivity of each of the *lexA*(Def)(pSE117) strains arose spontaneously at a high frequency. Many of these suppressed strains exhibited a mucoid phenotype at 30°C but not at 43.5°C. Since some *lon* mutations are known to behave in this fashion (16), the ability of a known *lon* mutation to suppress the cold sensitivity of KM1190(pSE117) was tested. The *lon-100* mutation was introduced into strain KM1190 by P1 transduction and the resulting strain was transformed with plasmid pSE117 DNA. GW5232, the *lon-100* derivative of KM1190(pSE117), was only slightly cold sensitive (Table 2). Thus *lon-100* largely suppresses *umuDC*-induced cold sensitivity.

E. coli cells that carry *htpR* mutations are temperature sensitive and defective in the induction of the heat shock response (18). Cells which carry the *htpR165* mutation are also deficient in Lon or a Lon-like protease activity (2, 6). Therefore, it was of interest to determine whether the *htpR165* mutation would suppress pSE117-mediated cold sensitivity (via its Lon⁻ character) or enhance it (via its defect in inducing heat shock proteins). Because *htpR* cells are temperature sensitive, an *htpR*(pSE117) strain could not be constructed at 43.5°C. Therefore, we examined the ability of an *htpR lexA*(Def) strain to be transformed by pSE117 at 30°C. Parallel transformations of the *lexA*(Def) strain GW2730 and its *htpR165* derivative, GW2765, were carried out with measured amounts of pBR322 DNA (as a control for transformability) and pSE117 DNA. Table 4 shows that the *htpR165* strain but not the *htpR*⁺ strain could be efficiently transformed by pSE117 at 30°C. We wished to confirm that the *htpR165* allele in GW2765 was responsible for suppressing cold sensitivity in GW2765(pSE117). Colonies of GW2765(pSE117) were streaked heavily on LB plates and incubated at 43.5°C. Spontaneous temperature-resistant revertants were restreaked and incubated at 30°C to test for

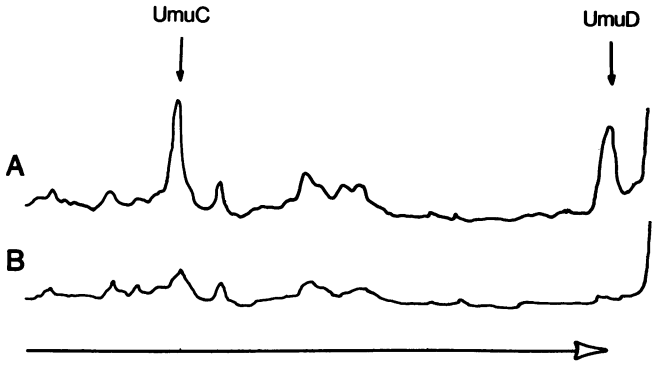

FIG. 5. Densitometric tracings of autoradiogram of an SDS gel analysis of ^{35}S-labeled whole-cell extracts. Cell cultures were pulse-labeled for 3 min at 30°C. (A) Extract from KM1190(pSE117); *umuDC*⁺ plasmid. (B) Extract from KM1190(pLM203); *umuDC* deleted from plasmid. Maxicell-labeled proteins provided migration standards for UmuD and UmuC.

TABLE 4. Viability of *htpR*$^+$ and *htpR165 lexA*(Def) pSE117 transformants[a]

Recipient strain	Marker	Incubation temp (°C)	No. of transformants with pSE117/no. of transformants with pBR322
GW2730	*htpR*$^+$	43.5	1.5
GW2730	*htpR*$^+$	30	$<5 \times 10^{-4}$
GW2765	*htpR165*	30	2:0

[a] Strains were transformed with 0.2 μg of plasmid DNA.

new cold sensitivity. The majority (13 of 14) of the temperature-resistant colonies had simultaneously acquired cold sensitivity. Thus, it appears that *htpR* can suppress *umuDC*-induced cold sensitivity.

DISCUSSION

Introduction of a multicopy plasmid carrying the *umuDC* genes into *E. coli* strains which are *lexA*(Def) resulted in overproduction of the *umuDC* gene products and cold sensitivity. This cold sensitivity may be due to an increase in the stability of the *umuDC* gene products at 30°C, a temperature-dependent change in protein activity, or some more indirect effect. Our experiments suggest that the cold sensitivity is unlikely to be due to an increase in the expression of the *umuDC* operon at 30°C.

Cell death in *lexA*(Def)(pSE117) strains shifted to 30°C appeared to be due to a reduction in the rate of DNA synthesis which in turn led to filamentation and cell lysis. The *lexA*(Def) strains used in these studies are *sulA* and therefore lack a component of the SOS-inducible filamentation system of wild-type *E. coli* (9). Though we are not certain whether KM1190 carries other mutations affecting SOS-dependent filamentation (3), we know that KM1190 and our other stable *lexA*(Def) strains do not significantly filament at any temperature under normal growth conditions. The filamentation observed in the KM1190(pSE117) strain at 30°C may be a response similar to the filamentation of *lexA*(Ind$^-$) *dna*(Ts) strains shifted to their nonpermissive temperature (3) and may be mediated by the process responsible for linking cell septation to the completion of chromosomal DNA synthesis during normal replication.

The *umuD* and *umuC* gene products are required in *E. coli* for mutagenesis by many agents including UV radiation. However, the role that they play and the mechanism of the entire process is not understood at the biochemical level. That DNA synthesis in intact cells decreased rapidly when a *lexA*(Def)(pSE117) strain was shifted to 30°C and recovered rapidly at 43°C may indicate that a *umuDC* gene product(s) reversibly interacts with some protein component(s) of the replication machinery. Alternatively, the UmuD and UmuC polypeptides may interfere with replication by sequestering a DNA substrate. Whatever the actual mechanism of inhibition proves to be, the phenotype of a *lexA*(Def)(pSE117) strain is the same as a "quick stop" *dna*(Cs) mutant.

UmuD and UmuC appear to be very well expressed from pSE117 in a *lexA*(Def) background as judged by incorporation of labeled methionine during a brief labeling pulse. However, neither accumulates enough to be visible on an SDS-polyacrylamide gel after Coomassie blue staining. From this evidence and also from pulse-chase studies in maxicells (unpublished data), both gene products appear to be unstable. This supports the physiological studies of the mutagenic process in *E. coli* by Witkin (25), who suggested that some inducible factor required for mutagenesis decayed rapidly after SOS induction.

The *sulA* gene product, which is responsible for the inhibition of cell division observed after induction of the SOS response, has also been shown to be very unstable (22). The *umuDC* gene products are thus a second example of SOS-induced proteins controlled at the level of both transcription and degradation. This rapid turnover of gene products may allow a temporally limited response to DNA damage which includes a repertoire of activities not compatible with normal cell division. Though we have no evidence that constitutive expression of UmuDC from the *E. coli* chromosome is deleterious to the cell, it may slow replication at low temperatures.

The cold sensitivity of KM1190(pSE117) was suppressed by 4% ethanol. Ethanol exerts diverse influences on the cell, including the induction of the heat shock response. It is not immediately clear how ethanol suppresses the cold sensitivity of KM1190(pSE117). The enhancing effect of NaCl in the medium is intriguing in light of the finding that NaCl has a generally stabilizing effect on temperature-sensitive proteins (11). Perhaps one or both of the Umu proteins are similarly stabilized by NaCl. Alternatively, the most frequent classes of cold-resistant revertants may be NaCl sensitive.

Two conditions that induce the heat shock response, high temperature and the presence of ethanol, both suppressed UmuDC-dependent lethality. However, a simple induction of the heat shock response is apparently not involved in this suppression since introduction of the *htpR165* mutation which renders cells unable to induce heat shock proteins not only did not prevent suppression but instead allowed the cell to grow at 30°C. Still, we cannot rule out more complicated models which would involve the heat shock response.

We had originally expected that cold-resistant mutants of *lexA*(Def)(pSE117) might arise in which some cellular protease activity was increased. Therefore, it was somewhat surprising to find that *lon* and *htpR* mutations could suppress the cold sensitivity of *lexA*(Def)(pSE117) strains; these mutations decrease the rate of proteolysis of some substrates (3, 6, 8). However, the relationship between various protease activities in *E. coli* is complex, and it is possible that the UmuD or UmuC protein or both are less stable in *lon* and *htpR* strains. The cII protein of phage lambda, for example, is degraded more rapidly in a *lon*$^-$ than in a *lon*$^+$ background (7). However, the half-lives of UmuD and UmuC polypeptides were identical in maxicells derived from GW2733 (pSE117) or from one representative mucoid, cold-resistant revertant, presumably a *lon* mutant (data not shown). It is possible that the *lon* protease is responsible for degrading *umuD* or *umuC* polypeptides to a toxic fragment which is stable only at low temperature. Another possibility is that *lon* is responsible for degrading some protein with which *umuDC* gene products interact. Since this cold sensitivity was seen only when *umuDC* gene products are overproduced, it may be that *umuDC* gene products are titrating out some essential factor which itself accumulates in *lon* cells.

We are currently using the cold-sensitive phenotype to probe the *umuDC* locus and the genes whose products interact with the UmuD and UmuC proteins to understand how the activities required for cold sensitivity relate to the activities required for mutagenesis. In particular, we would like to know whether UmuD as well as UmuC is required for cold sensitivity. Preliminary experiments with a plasmid carrying *umuC* alone under the *lac* promotor suggest that, by itself, UmuC is not capable of inducing cold sensitivity (Elledge and Walker, unpublished data). Our experiments

also indicate that some mutants of pSE117 previously isolated by us as having an altered mutagenesis phenotype do not induce cold sensitivity.

The mechanism by which UmuD and UmuC contribute to mutagenesis and repair is still unknown. That these gene products, in excess, can conditionally block normal DNA replication supports models in which the UmuD or UmuC products or both interact with replication proteins. We are currently attempting to duplicate this inhibition of DNA synthesis in an in vitro replication system.

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