Genetic Interactions between the *Escherichia coli umuDC* Gene Products and the β Processivity Clamp of the Replicative DNA Polymerase

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The *Escherichia coli umuDC* gene products encode DNA polymerase V, which participates in both translesion DNA synthesis (TLS) and a DNA damage checkpoint control. These two temporally distinct roles of the *umuDC* gene products are regulated by RecA–single-stranded DNA-facilitated self-cleavage of UmuD (which participates in the checkpoint control) to yield UmuD′ (which enables TLS). In addition, even modest overexpression of the *umuDC* gene products leads to a cold-sensitive growth phenotype, apparently due to the inappropriate expression of the DNA damage checkpoint control activity of UmuD$_2$C. We have previously reported that overexpression of the ε proofreading subunit of DNA polymerase III suppresses *umuDC*-mediated cold sensitivity, suggesting that interaction of ε with UmuD$_2$C is important for the DNA damage checkpoint control function of the *umuDC* gene products. Here, we report that overexpression of the β processivity clamp of the *E. coli* replicative DNA polymerase (encoded by the *dnaN* gene) not only exacerbates the cold sensitivity conferred by elevated levels of the *umuDC* gene products but, in addition, confers a severe cold-sensitive phenotype upon a strain expressing moderately elevated levels of the *umuD′C* gene products. Such a strain is not otherwise normally cold sensitive. To identify mutant β proteins possibly deficient for physical interactions with the *umuDC* gene products, we selected for novel *dnaN* alleles unable to confer a cold-sensitive growth phenotype upon a *umuD′C*-overexpressing strain. In all, we identified 75 *dnaN* alleles, 62 of which either reduced the expression of β or prematurely truncated its synthesis, while the remaining alleles defined eight unique missense mutations of *dnaN*. Each of the *dnaN* missense mutations retained at least a partial ability to function in chromosomal DNA replication in vivo. In addition, these eight *dnaN* alleles were also unable to exacerbate the cold sensitivity conferred by modestly elevated levels of the *umuDC* gene products, suggesting that the interactions between UmuD′ and β are a subset of those between UmuD and β. Taken together, these findings suggest that interaction of β with UmuD$_2$C is important for the DNA damage checkpoint function of the *umuDC* gene products. Four possible models for how interactions of UmuD$_2$C with the ε and the β subunits of DNA polymerase III might help to regulate DNA replication in response to DNA damage are discussed.

The *umuDC* genes encode a DNA polymerase, DNA polymerase V (Pol V), that has a remarkable ability to copy over abasic sites (43, 63), cyclobutane dimers (62), and pyrimidine-pyrimidone [6-4] photoproducts (62), a process referred to as translesion DNA synthesis (TLS). This ability, however, comes at the cost of reduced fidelity. Thus, replication by Pol V is inherently less accurate, leading to the formation of mutations, even when replicating undamaged templates (29, 62). Therefore, to limit the ability of Pol V to introduce mutations into the host genome, expression of the *umuDC* genes is tightly regulated as part of the *Escherichia coli* stress-induced SOS response (12). This highly regulated response helps the cell maintain the integrity of its genome following treatments that lead either directly or indirectly to DNA damage (12).

The *E. coli* SOS response consists of at least 30 unlinked genes (9, 12), collectively referred to as the SOS regulon. Expression of the various SOS-regulated genes is coordinately regulated at the level of their transcription by the LexA and RecA proteins (28). In the absence of DNA damage, LexA acts to repress the expression of the members of the SOS regulon (27). RecA protein, the main bacterial recombinase required for essentially all homologous recombination (reviewed in reference 22), binds to single-stranded DNA (ssDNA) generated by the cell's failed attempts to replicate past lesions in its genome, thus forming RecA-ssDNA nucleoprotein filaments (47). These RecA-ssDNA filaments, in addition to acting in homologous recombination, also act to facilitate the latent capacity of LexA to autodigest (26). Autodigestion of LexA serves to inactivate it as a transcriptional repressor, leading to the concomitant increase in expression of LexA-regulated genes (12).

The UmuD protein similarly undergoes a RecA-ssDNA-facilitated autodigestion that serves to remove its first 24 residues to yield UmuD′ (3, 34, 48). The UmuD′$_2$ homodimer then interacts with UmuC (18, 59, 68), which has an ability to catalyze the formation of phosphodiester bonds, in such a way that the UmuD′$_2$C complex is able to participate in TLS (43). In addition to participating in TLS, UmuC together with the full-length UmuD$_2$ homodimer participates in a DNA damage checkpoint control that acts to regulate DNA replication in response to DNA damage, thereby allowing additional time for nucleotide excision repair to accurately remove lesions in the

DNA prior to continued replication (37). Thus, self-cleavage of UmuD to UmuD′ can be regarded as a molecular switch that acts to temporally regulate these two distinct activities of the UmuD$_2$C and UmuD′$_2$C complexes (37, 57).

In addition to participating in a DNA damage checkpoint control and enabling TLS, overexpression of the *umuDC* gene products confers a cold sensitivity for growth (30, 38, 59). Our recent characterizations of *umuDC*-mediated cold sensitivity indicated that (i) moderately elevated levels of the *umuDC* gene products confer a cold-sensitive growth phenotype, while similarly elevated levels of the *umuD′C* gene products do not (59), and (ii) the catalytic DNA polymerase activity of UmuC is not required for this cold sensitivity (59). These findings, together with others, suggest that the cold sensitivity conferred by elevated levels of the *umuDC* gene products is a manifestation of the inappropriate expression of UmuD$_2$C functions involved in the DNA damage checkpoint control (37, 38, 59). Therefore, in an effort to better characterize the components of the UmuD$_2$C-dependent checkpoint control, we have embarked on an analysis of the genetic requirements of *umuDC*-mediated cold sensitivity.

We have previously suggested that interactions of the *umuDC* gene products with components of the *E. coli* replicative DNA polymerase, DNA polymerase III holoenzyme (Pol III), could serve as a convenient mechanism for regulating the checkpoint and TLS roles of the *umuDC* gene products (37, 57). On the basis of this hypothesis, we reasoned that if interactions involving the *umuDC* gene products and components of Pol III were important for the checkpoint role of UmuD$_2$C, we might then be able to observe an effect on the extent of the cold sensitivity conferred by *umuDC* by the simultaneous overexpression of certain (i.e., relevant) components of Pol III. Using such an approach, we have recently reported that overproduction of the ε proofreading subunit of Pol III or deletion of its structural gene (*dnaQ*) suppresses *umuDC*-mediated cold sensitivity (56). A systematic analysis of the remaining nine Pol III subunits indicated that the homodimeric β processivity clamp was the only other Pol III subunit that when overexpressed affected the extent of *umuDC*-mediated cold sensitivity (56).

In this report, we describe how overexpression of the β processivity clamp (encoded by *dnaN*) strongly exacerbates *umuDC*-mediated cold sensitivity. We have exploited this ability of the β clamp to confer a cold-sensitive growth phenotype upon a *umuD′C*-expressing *E. coli* strain (a strain that is not normally cold sensitive [59]) to identify novel *dnaN* alleles unable to confer this phenotype. Our genetic characterizations of these novel *dnaN* alleles indicate that they are also unable to exacerbate the cold sensitivity conferred by elevated levels of the *umuDC* gene products. Our results described in this report, taken together with others (37, 38, 56, 57, 59), suggest that the UmuD$_2$C-dependent DNA damage checkpoint control is a manifestation of protein-protein interactions involving UmuD$_2$C and the ε and the β subunits of Pol III.

MATERIALS AND METHODS

Bacteriological techniques. *E. coli* strains and plasmid DNAs used in this study are described in Table 1. Bacterial strains were routinely grown in Luria-Bertani (LB) medium as described previously (46), unless otherwise stated. When necessary, LB medium was supplemented with the following antibiotics at the indi-

TABLE 1. *E. coli* strains and plasmid DNAs used in this study

Strain or plasmid	Relevant genotype or characteristics	Source (reference)
E. coli strains[a]		
AB1157	*lexA⁺ recA⁺ dnaN⁺*	Laboratory stock
HC123	*lexA⁺ recA⁺ dnaN59*(Ts)	CGSC[b]
Plasmid DNAs		
pBR322	ColE1 *oriV*; cloning vector	Laboratory stock
pJRC210	pBR322; β overproducer	Charles McHenry
pGB2	pSC101; cloning vector	Roger Woodgate (5)
pSE115	pSC101; *o⁺umuD⁺ umuC⁺*	Laboratory stock (8)
pGY9739	pSC101; *oᶜ₁umuD⁺ umuC⁺*	Suzanne Sommer and Adriana Bailone (52)
pGYDΔC	pSC101; *oᶜ₁umuD⁺ ΔumuC*	Laboratory stock (59)
pGY9738	pSC101; *oᶜ₁umuD′ umuC⁺*	Suzanne Sommer and Adriana Bailone (51)
pGYD′ΔC	pSC101; *oᶜ₁umuD′ ΔumuC*	This work

[a] The complete genotype for AB1157 is *xyl-5 mtl-1 galK2 rpsL31 kdgK51 lacY1 tsx-33 supE44 thi-1 leuB6 hisG4*(Oc) *mgl-51 argE3*(Oc) *rfbD1 proA2 ara-14 thr-1 qsr′ qin-111*. The complete genotype for HC123 is *fhuA22 ompF627 relA1 metB1 dnaN59*(Ts) Hfr Cavalli *PO2A*.

[b] CGSC, *E. coli* Genetic Stock Center.

cated concentrations: ampicillin, 150 μg/ml; spectinomycin, 60 μg/ml; and kanamycin, 80 μg/ml. Because strains expressing elevated levels of the *umuDC* gene products do not grow well at 30°C (30, 38, 59), the strains bearing a *umuDC*-expressing plasmid in this study were routinely grown at 42°C, unless otherwise stated. pGYD′ΔC was constructed by the same method used for construction of pGYDΔC (59). Briefly, pGY9738 was digested with *Mlu*I, followed by end filling of the linear DNA with all four deoxynucleoside triphosphates and the Klenow fragment of DNA Pol I (New England BioLabs) prior to its ligation with T4 DNA ligase (New England BioLabs). Bacterial transformation was by either calcium chloride treatment (46) or electroporation using a GenePulser (Bio-Rad) as per the manufacturer's recommendation. Plasmid DNAs were isolated using the QIA-Spin Prep kit (Qiagen) as per the manufacturer's recommendation.

Genetic assay for the selection of novel *dnaN* alleles. A derivative of AB1157 bearing the plasmid pGY9738, which constitutively expresses elevated levels of the *umuD′C* gene products (51), grows well at 30°C. However, if AB1157(pGY9738) also carries the compatible plasmid pJRC210, which overexpresses the β processivity clamp of Pol III from the P_{tac} promoter, its growth is cold sensitive (Table 2). To select for *dnaN* alleles unable to confer the cold-sensitive phenotype, we selected for derivatives of AB1157 bearing both the *umuD′C*-expressing plasmid and the β-expressing plasmid that were able to grow at 30°C. In this analysis, we used a derivative of the β-expressing plasmid (pJRC210) that had been chemically mutagenized in vitro with hydroxylamine as described previously (31) before transformation into AB1157(pGY9738). To minimize the isolation of siblings, transformation reaction mixtures were aliquoted prior to their outgrowth; automated DNA sequence analysis revealed that all of the *dnaN* missense alleles identified by this approach arose independently. Following a 60-min outgrowth period, a portion of each aliquot was plated at 42°C (the permissive temperature) to permit a calculation of the total number of transformants obtained. The remainder of each transformation reaction mixture was plated directly at 30°C to select for plasmid-encoded *dnaN* alleles that allowed growth at this otherwise nonpermissive temperature. To confirm that growth at 30°C was attributable to the plasmid-encoded *dnaN* allele, plasmid DNAs were purified and subsequently retransformed back into AB1157 bearing the *umuD′C*-expressing plasmid (pGY9738). One half of each transformation reaction mixture was plated at 30°C, and the other half was plated at 42°C. Plates were scored after incubation overnight (~20 h).

It should be stressed that high-level overproduction of β by addition of IPTG (isopropyl-β-D-thiogalactopyranoside) was not necessary for expression of the synthetic lethal phenotype. The noninduced level of β expressed from the P_{tac} promoter of pJRC210 was sufficient. Consequently, all of the experiments described in this report designed to measure the phenotypes of the wild-type and mutant *dnaN* alleles were performed in the absence of added IPTG, unless otherwise stated.

Nucleotide sequence analysis of *dnaN* alleles. The promoter region and entire coding sequence for each plasmid-encoded *dnaN* allele were determined by automated DNA sequence analysis using at least four of the following six dif-

TABLE 2. Overexpression of β exacerbates the cold sensitivity conferred by elevated levels of the *umuDC* gene products[a]

Plasmid	*umuD* and *umuC* alleles	Plating efficiency (30°C/42°C) prior to transformation with pBR322 or pJRC210[b]	AB1157 transformants/ml (30°C/42°C)[c] with:	
			pBR322 (control)	pJRC210 (P_{tac} *dnaN*)
pGB2	None	1.08	0.99	0.87
pSE115	o^+umuD^+ $umuC^+$	ND[d]	1.11	0.81
pGY9739	$o^c_1umuD^+$ $umuC^+$	4.5×10^{-3}	3.5×10^{-3}	5.1×10^{-4}
pGYDΔC	$o^c_1umuD^+$ $\Delta umuC$	0.70	0.92	0.92
pGY9738	o^c_1umuD' $umuC^+$	0.94	0.98	2.6×10^{-4}
pGYD'ΔC	o^c_1umuD' $\Delta umuC$	ND	0.77	0.85

[a] *E. coli* AB1157 and plasmid DNAs are described in Table 1.

[b] Plating efficiency is expressed as the ratio of the CFU obtained at 30°C to those obtained at 42°C after overnight incubation. These values were reported previously (59) and are included here only for comparison to the values obtained following transformation with either pBR322 or pJRC210.

[c] AB1157 bearing the indicated *umuDC*-expressing plasmid was transformed with either pBR322 or pJRC210. The ratio of the CFU obtained at 30°C to those obtained at 42°C was measured after overnight incubation at each temperature. The *dnaN* gene encodes the β processivity clamp of Pol III (4). The strains expressing only elevated levels of β formed colonies that were slightly smaller at 30°C than those formed by strains not expressing β. Transformation efficiencies at 42°C for all strains were on the order of ~10^5 to ~10^6 CFU per μg of plasmid DNA, indicating that overexpression of either the *umuDC* or *umuD'C* gene products with or without simultaneous overexpression of β is not lethal at 42°C under these conditions.

[d] ND, not determined.

ferent oligonucleotide primers (GIBCO-BRL) corresponding to the indicated nucleotide positions (with position 1 corresponding to the first base of the coding sequence of *dnaN*): BETA-R1, positions 292 to 331 of the noncoding strand; BETA-F2, positions 236 to 258 of the coding strand; BETA-F3, positions 815 to 836 of the coding strand; BETA-R4, positions 879 to 901 of the noncoding strand; BETA-F5, positions 97 to 120 of the coding strand; and BETA-R6, positions 394 to 415 of the noncoding strand. Analyses of the nucleotide sequences were performed using the Lasergene software package (version 3.6.0), and mutations were identified by comparison of the sequence data files to the wild-type *dnaN* sequence deposited in GenBank (accession number J01602).

RESULTS

Overexpression of the β processivity clamp of Pol III exacerbates *umuDC*-mediated cold sensitivity. We have previously suggested that interaction of UmuD with the β processivity clamp of Pol III is important for the checkpoint role of UmuD$_2$C (57). We have also suggested that the cold sensitivity conferred by elevated levels of the *umuDC* gene products is due to the inappropriate expression of UmuD$_2$C functions involved in this checkpoint (59). To explore this hypothesis further, we used the previously described quantitative transformation assay (38) to investigate whether or not overexpression of the β clamp would affect the extent of the cold sensitivity conferred by elevated levels of the *umuDC* gene products. In these experiments, elevated levels of the *umuDC* gene products were supplied from a pSC101 derivative named pGY9739 (Table 1) (52) that contains a single base change in the operator site near the *umuDC* promoter (the o^c_1 mutation). This mutation eliminates much of the repression normally conferred by the LexA repressor (52). As controls, we used either pGB2, the parent to pGY9739 which lacks a *umuDC* operon, as well as a comparable pSC101-based plasmid named pSE115, which expresses the *umuDC* gene products from their wild-type, LexA-regulated promoter (8). Thus, in AB1157, expression of the *umuDC* gene products from

pSE115 is efficiently blocked by LexA protein, while expression of the *umuDC* gene products from the o^c_1umuDC allele contained in pGY9739 is not.

In this analysis, competent cells of AB1157 bearing either pGY9739, pGB2, or pSE115 were prepared following their growth at 42°C. Consistent with our previous observations that overexpression of *umuDC* causes cold sensitivity (59), AB1157 bearing pGY9739 does not grow well at 30°C (Table 2). We then transformed these two AB1157 derivatives with either pBR322 (as a control) or a pBR322 derivative that expresses the β processivity clamp of Pol III from the P_{tac} promoter (pJRC210); no IPTG was added to induce β expression in these experiments. Following transformation and a 60-min period of outgrowth at 42°C, the reaction mixtures were split and aliquots were plated at both 30 and 42°C. As expected, the extent of the growth defect at 30°C of the AB1157 derivative bearing pGY9739, which expresses elevated levels of the *umuDC* gene products, was unaffected by transformation with pBR322; it still plated nearly 300-fold less efficiently at 30°C than at 42°C (Table 2). The lack of a cold-sensitive growth phenotype after transformation with pBR322 for AB1157 bearing either pGB2, which does not encode a *umuDC* operon, or pSE115, which does not express detectable levels of the *umuDC* gene products in AB1157 due to the presence of the LexA repressor (data not shown), confirms that the cold sensitivity conferred by pGY9739 was due to the elevated levels of UmuD$_2$C.

However, when the *umuDC*-expressing strain was transformed with pJRC210, the pBR322 derivative that overexpresses β from the P_{tac} promoter, it became more cold sensitive for growth (i.e., from a plating efficiency ratio at 30°C/42°C of 3.5×10^{-3} with pBR322 to 5.1×10^{-4} with pJRC210 [Table 2]), despite the fact that the P_{tac} promoter was not induced. This enhancement in *umuDC*-mediated cold sensitivity was independent of the order in which the two plasmids were introduced into AB1157; comparable plating efficiencies were observed whether the β-expressing plasmid was transformed into the *umuDC*-expressing strain or vice versa (data not shown). Despite the enhanced cold-sensitive growth phenotype of the strain overexpressing the *umuDC* gene products following its transformation with the β-expressing plasmid, its transformation efficiency with the same plasmid at 42°C was roughly 10^5 CFU per μg of plasmid DNA (see Table 2, footnote c). This efficiency is similar to that observed with the same strain with pBR322 in place of pJRC210, indicating that growth of the strain expressing elevated levels of both β and the *umuDC* gene products was essentially unaffected at 42°C. The control strain bearing pSE115, which did not express elevated levels of UmuD$_2$C due to the presence of the LexA repressor, did not become cold sensitive when transformed with pJRC210. This indicated that the exacerbation of the cold sensitivity of the *umuDC*-expressing strain was due to the combination of elevated levels of UmuD$_2$C together with elevated levels of β.

These results, indicating that the β processivity clamp of Pol III might be involved in *umuDC*-mediated cold sensitivity, prompted us to evaluate the effect of overexpression of β on a strain that expressed elevated levels of the *umuD'C* gene products. In this analysis, we used a plasmid named pGY9738 that is similar to the one described above containing the o^c_1 mutation except that it results in constitutive expression of the

umuD'C gene products instead of the *umuDC* gene products. Such a strain is not normally cold sensitive (59), presumably due to the inability of UmuD′₂C to function in the UmuD₂C-dependent checkpoint control (37). Consistent with our previous results (59), AB1157 expressing elevated levels of the *umuD'C* gene products was not cold sensitive when transformed with pBR322 (Table 2). However, transformation of this *umuD'C*-expressing strain with the β-expressing plasmid (pJRC210) resulted in a severe cold-sensitive growth phenotype, as indicated by the nearly 4,000-fold reduction in its plating efficiency at 30°C (Table 2). This plating efficiency is similar to that which we observed for the *umuDC*-expressing strain following its transformation with the β-expressing plasmid (pJRC210). However, one important difference between the *umuDC*- and *umuD'C*-expressing strains is that the latter is cold sensitive only following its transformation with the β-expressing plasmid (Table 2), while the former is cold sensitive regardless of whether or not it bears the β-expressing plasmid and becomes more cold sensitive after transformation with the β-expressing plasmid.

We have previously reported that *umuDC*-mediated cold sensitivity requires the *umuC* gene product but not its catalytic proficiency as a DNA polymerase (59). Likewise, we found that overexpression of β together with elevated levels of UmuD₂ or UmuD′₂ alone did not confer cold sensitivity; the *umuC* gene product was absolutely required for cold sensitivity (Table 2). Finally, the extent of cold sensitivity conferred upon AB1157 expressing elevated levels of the *umuDC* or *umuD'C* gene products by the β-expressing plasmid (pJRC210) was unaffected by addition of IPTG, which induces high-level overproduction of β (data not shown); the noninduced level of β expressed from pJRC210 was sufficient (Table 2). Thus, it is important to stress that all the experiments described in this report designed to measure the phenotype of the pJRC210-encoded *dnaN* allele were performed in the absence of IPTG. Finally, although we have not yet carefully quantitated the level of expression of β from pJRC210 in the absence of IPTG, it is clear that strains bearing this plasmid and grown in rich medium, such as LB medium, express at least ∼10- to 100-fold more β from the plasmid than they do from their chromosomal allele (data not shown).

The cold sensitivity conferred by the overexpression of β together with elevated levels of the *umuDC* gene products is more severe than that conferred by β together with *umuD'C*. The quantitative plating assay that we typically use to gauge the extent of cold sensitivity measures the ability of a strain to form a colony on selective medium after overnight incubation (38). Consequently, it is a relatively insensitive method for characterizing small differences between strains, such as growth rates. In addition, we have previously found that depending upon the selectable antibiotic marker being used, the upper limit with respect to the extent of cold sensitivity that can be observed may vary. For example, using the same *E. coli* strain (GW8018) and the same *umuDC*-expressing plasmid (pSE117), which confers resistance to both ampicillin and kanamycin, we observed a 659-fold range in the extent of the cold sensitivity depending on whether we selected for growth using solid medium supplemented with ampicillin (32) or kanamycin (38). Therefore, to establish whether the *umuDC*- and *umuD'C*-expressing strains were similarly affected by the si-
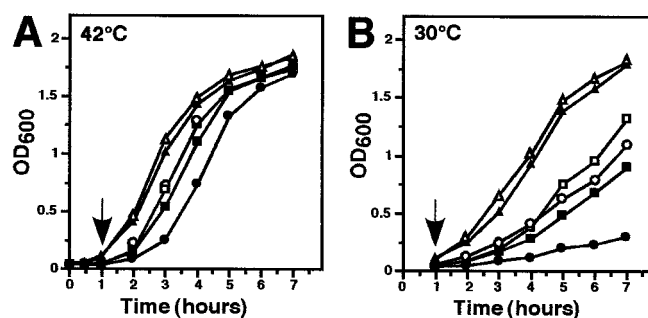

FIG. 1. The cold sensitivity conferred upon a *umuDC*-expressing strain by overexpression of β is more severe than that conferred upon a *umuD'C*-expressing strain. Growth curve analyses were performed as described in the text. The arrow indicates the time at which cultures were spilt into two equal parts and one of each pair was shifted to 30°C (B) while the other was maintained at 42°C (A). Symbols: □, AB1157 (pGY9738)(pBR322); ■, AB1157(pGY9738)(pJRC210); ○, AB1157 (pGY9739)(pBR322); ●, AB1157(pGY9739)(pJRC210); △, AB1157 (pSE115)(pBR322); ▲, AB1157(pSE115)(pJRC210). *E. coli* AB1157 and plasmid DNAs are described in Table 1. OD₆₀₀, optical density at 600 nm.

multaneous overproduction of β, we further characterized the growth defects at 30°C relative to those at 42°C of some of the same strains described in Table 2 by monitoring their growth rates in liquid culture. Our focus in these experiments was on comparing the effects of elevated levels of *umuDC* to elevated levels of *umuD'C* with or without simultaneous expression of the β clamp. The AB1157 derivative bearing pSE115, which did not express detectable levels of UmuD₂C (due to the presence of the LexA repressor protein), with or without the β-expressing plasmid, served as the control. Briefly, overnight cultures of each strain grown at 42°C in M9 minimal medium supplemented with 0.5% Casamino Acids, 0.2% glucose, and the appropriate antibiotics were subcultured 1:125 into the same prewarmed medium and grown at 42°C for 60 min. Cultures were then split in half, and one of each pair was shifted to 30°C while its partner was maintained at 42°C. One-milliliter aliquots of each culture were then removed at various times for determination of their optical density at 600 nm (Fig. 1).

The growth rates of AB1157 bearing pBR322 and expressing elevated levels of either the *umuDC* (pGY9739) or the *umuD'C* (pGY9738) gene products were slightly retarded at 42°C relative to that of the control strain bearing the *umuDC* genes under control of their native, LexA-regulated promoter (pSE115), which grew well at both temperatures (Fig. 1). Consistent with previous reports (38), the growth defect of the *umuDC*-expressing strain was more pronounced at 30°C, while the growth rate of the strain expressing elevated levels of the *umuD'C* gene products was somewhat less affected (Fig. 1, compare panels A and B).

In contrast to these findings, the growth rates of the strains expressing elevated levels of the *umuDC* or *umuD'C* gene products together with elevated levels of β were significantly more retarded at both 30 and 42°C compared to the same strains bearing pBR322 in place of the β-expressing plasmid. Interestingly, the growth rate of the strain expressing *umuDC* together with β was considerably more retarded at both 30 and 42°C than was that of the strain expressing *umuD'C* together with β (Fig. 1). The control strain bearing pSE115, which does

not express detectable levels of the *umuDC* gene products because of the LexA repressor, together with the β-expressing plasmid was only slightly cold sensitive compared to the same strain bearing pBR322 in place of the β-expressing plasmid.

Taken together, these results (Fig. 1) confirm that exacerbation of the cold sensitivity requires elevated levels of both β and the *umuDC* gene products and indicate that the cold sensitivity conferred by the combination of elevated levels of UmuD₂C together with elevated levels of β is more severe than that conferred by elevated levels of UmuD′₂C together with β. Our inability to detect this difference in our plating assay is presumably due to its insensitivity with respect to small differences in growth rate (see above).

Direct selection for novel *dnaN* alleles deficient in conferring a cold-sensitive phenotype upon a *umuD′C*-expressing *E. coli* strain. The ability of the β-expressing plasmid (pJRC210) to confer cold sensitivity upon AB1157 expressing elevated levels of the *umuD′C* gene products provided us with an extremely powerful means of identifying *dnaN* (which encodes β) alleles deficient in conferring this cold sensitivity. Based on our findings that (i) the *umuD* gene products interact with β in vitro and (ii) UmuD has a greater affinity for β than does UmuD′ (57) (an observation that correlates well with their respective degrees of cold sensitivity [Fig. 1]), we reasoned that the exacerbation of *umuDC*-mediated cold sensitivity conferred by elevated levels of β was in fact a manifestation of these physical interactions. Thus, we hoped that by selecting for *dnaN* alleles unable to exacerbate *umuD′C*-mediated cold sensitivity, we would be able to identify missense mutant β proteins deficient in physical interactions with the *umuDC* gene products. We therefore used hydroxylamine to chemically mutagenize the β-expressing plasmid in vitro and then transformed this chemically mutagenized plasmid into AB1157 bearing pGY9738, which constitutively expresses the *umuD′C* gene products. Transformation reaction mixtures were plated at the normally nonpermissive temperature of 30°C to select for plasmid-encoded *dnaN* alleles deficient in conferring cold sensitivity. To minimize selecting for siblings, transformation reaction mixtures were aliquoted prior to their outgrowth; nucleotide sequence analysis (see below) confirmed that none of the missense *dnaN* alleles we identified were siblings.

By this approach, we identified 76 strains whose plating efficiencies at 30 and 42°C were similar. Although we had selected these clones by directly plating the transformation reaction mixtures at 30°C, we had also plated an aliquot of each reaction mixture at the permissive temperature of 42°C to allow for a determination of the total number of viable transformants. This analysis indicated that these 76 putative mutants were selected from a total pool of ∼60,000 independent transformants. To confirm that the observed phenotypes of these 76 presumed mutant *dnaN* alleles were conferred by the respective pJRC210 derivatives and not by a mutation mapping to the host chromosome, we isolated the plasmid DNA from each of the 76 clones and transformed it back into AB1157 bearing the *umuD′C*-expressing plasmid. Of the 76 pJRC210 plasmids, 75 were unable to confer cold sensitivity upon AB1157 expressing elevated levels of the *umuD′C* gene products, indicating that they carried the mutation responsible for the observed phenotype.

To characterize these 75 plasmid-encoded *dnaN* alleles de-

ficient in conferring the cold-sensitive phenotype further, we assessed their respective abilities to overproduce β in response to addition of IPTG; the *dnaN* gene in pJRC210 is under the control of the IPTG-inducible P_{tac} promoter. The purpose of this analysis was to differentiate between those pJRC210 derivatives unable to confer cold sensitivity because of an expression defect (i.e., a mutation affecting the expression of the *dnaN* gene product) or a nonsense mutation (which was not suppressed by the *supE44* allele of AB1157 and would result in expression of a truncated form of β) from those expressing a full-length β protein that is expressed at a level comparable to that observed for the *dnaN*⁺ allele. The latter class represented the type of allele we were interested in and presumably corresponds to those containing one or more missense mutations that affect the ability of the β derivatives they encode to interact physically with the *umuD′C* gene products.

pJRC210, the parent plasmid, overexpresses β to very high levels in response to IPTG (>20% total soluble protein [data not shown]). Thus, we were able to easily distinguish those pJRC210 derivatives able to overexpress the β clamp from those unable to do so by Coomassie blue staining of sodium dodecyl sulfate-polyacrylamide gel electrophoresis-fractionated whole-cell lysates prepared from cultures of AB1157 derivatives bearing the respective β-expressing plasmids after their treatment with IPTG (data not shown). This analysis indicated that of the 75 plasmids identified by our genetic assay, 13 were capable of overproducing a full-length β protein (data not shown). Thus, only 13 of the ∼60,000 transformants carrying a hydroxylamine-treated pJRC210 derivative possessed the desired phenotypes of (i) expressing a full-length β protein and (ii) being unable to confer a cold-sensitive phenotype upon a *umuD′C*-expressing strain.

Nucleotide sequence determination of novel *dnaN* alleles. To determine the specific nucleotide alterations in each of the 13 *dnaN* alleles presumed to contain a missense mutation, we sequenced the promoter region and entire coding sequence of each using at least four different oligonucleotide primers (see Materials and Methods). In addition, we also sequenced 14 alleles that we suspected, based on analysis of whole-cell lysates (see above), to be either expression mutations (i.e., mutations mapping to the promoter region that affect expression) or alleles that expressed a truncated form of the β clamp (i.e., nonsense mutations). This analysis, summarized in both Table 3 and Fig. 2, confirmed our hypothesis regarding the alleles we suspected to contain either one or more mutations in their promoter region, thus affecting expression of *dnaN*, or a nonsense mutation resulting in expression of a truncated form of the β protein. In addition, it indicated that the 13 alleles presumed to contain a missense mutation(s) actually represent eight unique and novel missense *dnaN* alleles. These eight alleles identify a total of eight different amino acid residues that, when changed, affect the ability of β to confer cold sensitivity upon the *umuD′C*-expressing strain. Of the 35 mutations identified by nucleotide sequence analysis (see the legend to Fig. 2), only four could not be accounted for by the known propensity of in vitro hydroxylamine treatment to cause C-to-T transitions (31). Finally, this analysis indicated that, with the exceptions of pJRCHA-5.1 (S107L) and pJRCHA-6.2 (P363S), all eight alleles contained only a single missense mutation; the

TABLE 3. Summary of nucleotide sequence analysis of novel *dnaN* alleles containing missense mutations[a]

Plasmid	*dnaN* allele (deduced amino acid substitution)[b]	Nucleotide substitution[c]	No. of occurrences (degree of conservation)[d]
pJRCHA-4.1	Q61K	^{181}CAG→AAG	1 (2/22)
pJRCHA-5.1	S107L	^{319}TCG→TTG ^{685}GAC→CAT (silent)	1 (3/22) NA[e]
pJRCHA-8.1	D150N	^{448}GAC→AAC	2 (13/22)
pJRCHA-5G11	G157S	^{469}GGT→AGT	5 (16/22)
pJRCHA-8I11	V170M	^{508}GTG→ATG	1 (16/22)
pJRCHA-7.1	E202K	^{604}GAA→AAA	1 (12/22)
pJRCHA-6F11	M204K	^{610}ATG→AAG	1 (10/22)
pJRCHA-6.2		^{223}TTC→TTT (silent)	NA
	P363S	^{1087}CCA→TCA	1 (21/22)

[a] See Fig. 2 for a complete summary of all nucleotide alterations in *dnaN* identified in this study.
[b] The name for each *dnaN* allele is based on its deduced amino acid substitution in one-letter code and its respective position within the primary structure of the β protein. For example, Q61K represents the *dnaN* allele bearing a Q-to-K substitution at position 61.
[c] The mutation(s) resulting in each allele is indicated in the context of its respective codon(s). The numbering is relative to the start of each codon, with position 1 corresponding to the A in the ATG of the first codon of *dnaN*. Missense mutations that did not affect the amino acid inserted, due to the degeneracy of the genetic code, are termed silent.
[d] The number of times that each respective *dnaN* allele was identified (occurrences) in the complete collection of 27 alleles subjected to automated DNA sequence analysis is indicated (see Fig. 2 for a complete summary of the DNA sequence analysis). The degree of conservation for each position among the 22 *dnaN* homologs whose sequence is in the National Center for Biotechnology Information database (as of 4 December 2000) is indicated in parentheses. This *dnaN* alignment was performed by the COGS application of the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov/COG/).
[e] NA, not applicable.

two exceptions contained, in addition, one silent mutation each (Table 3).

All eight amino acid substitutions identified by our genetic assay correspond to residues located at or very close to the surface of the molecule (Fig. 3) based on the crystal structure of the β clamp reported by Kong et al. (21), with the majority of them affecting the face of β that is believed to interact with DNA Pol III (Fig. 3B) (23, 24, 41, 55). Incidentally, some of the native residues that were mutated are not easily visible in the surface representations of the β clamp (Fig. 3C to G). This is because their position either is located just below the surface or is obstructed by other surface features of the β clamp in the views shown.

The *dnaN* alleles described in this report likely represent a majority of the total number of *dnaN* alleles possible with this phenotype following treatment of the plasmid-encoded *dnaN*⁺ gene with hydroxylamine in vitro. This conclusion is based on the fact that the D150N and G157S alleles were identified two and five independent times, respectively, indicating that our selection-screen was partially saturating (Table 3). The large number of mutations mapping to the same small number of residues within the promoter region, including the ribosome binding site (a region of 8 bp [$^{-16}$TAGGAGGT^{-9}] [Fig. 2]), combined with the fact that 48 more alleles unable to overpro-

duce *dnaN* to a detectable level were identified but not sequenced (see above), indicates that the target size for amino acid substitutions in β that can affect its ability to confer cold sensitivity must be very small.

Finally, it is interesting that each of the eight *dnaN* alleles encodes a mutant β protein with a comparatively larger residue in place of the smaller, native residue (e.g., G157S). In addition, some also radically affect the charge characteristics of the native residue (e.g., E202K), and many of the deduced amino acid substitutions identified affect residues well conserved among other prokaryotic β homologs (Table 3). It is also important to point out that mutations were identified in all three structural domains of β; each β protomer contains threefold structural similarity (21) (Fig. 2). In addition, two (Q61K and S107L) of the eight missense mutations are located in the first structural domain of β (domain I), which is lacking in an alternative form of the β clamp referred to as β* (see Fig. 2 and Discussion for further details) (39, 50).

Steady-state levels of mutant β proteins. Prior to their nucleotide sequencing, we had screened each *dnaN* allele for its ability to overexpress a full-length β protein. Their ability to do so suggested that their promoter regions did not contain mutations that affected their respective abilities to express their gene products, an assumption corroborated by their nucleotide sequence analysis (see above). To confirm that the mutant β proteins were present at comparable levels, relative both to each other and to the wild-type control (pJRC210), in the absence of their induced expression by IPTG, we measured their respective steady-levels by qualitative Western blot analysis using a polyclonal antibody specific to β. The steady-state level of each of the eight mutant β proteins was found to be similar to that observed for the wild-type control in the absence of added IPTG (Fig. 4), indicating that their deduced amino acid substitutions do not significantly affect their overall stability in vivo. This result confirms that the respective deficiencies of these *dnaN* alleles to confer cold sensitivity in our genetic assay are due to their deduced amino acid substitutions and not to a large change in their abundance. Finally, it is worthwhile emphasizing that the level of β expressed from pJRC210 (as well as the levels of the mutants expressed from the pJRC210 derivatives) in the absence of IPTG is far greater than the level expressed from the chromosomally carried *dnaN*⁺ or *dnaN59*(Ts) allele (Fig. 4). Initial attempts to quantitate the level of expression from pJRC210 suggest that it is at least 10- to 100-fold higher than that observed from the chromosomal *dnaN*⁺ allele (data not shown).

Novel *dnaN* alleles are deficient in exacerbating *umuDC*-mediated cold sensitivity. As discussed in the report of our previous in vitro analysis (57), UmuD interacts more strongly with β than does UmuD′. Two possible explanations for this difference are (i) UmuD and UmuD′ require similar structural features of β for their interactions, but the extra N-terminal 24 amino acids in UmuD enable it to make additional contacts with β, thus accounting for its stronger interaction (57), or (ii) UmuD and UmuD′ each interact with different structural features of β, and hence their different affinities are due to the different natures of their interactions. To begin to differentiate between these two possibilities, we investigated whether or not the eight *dnaN* alleles identified by virtue of their inability to interact genetically with the *umuD′C* gene products were sim-
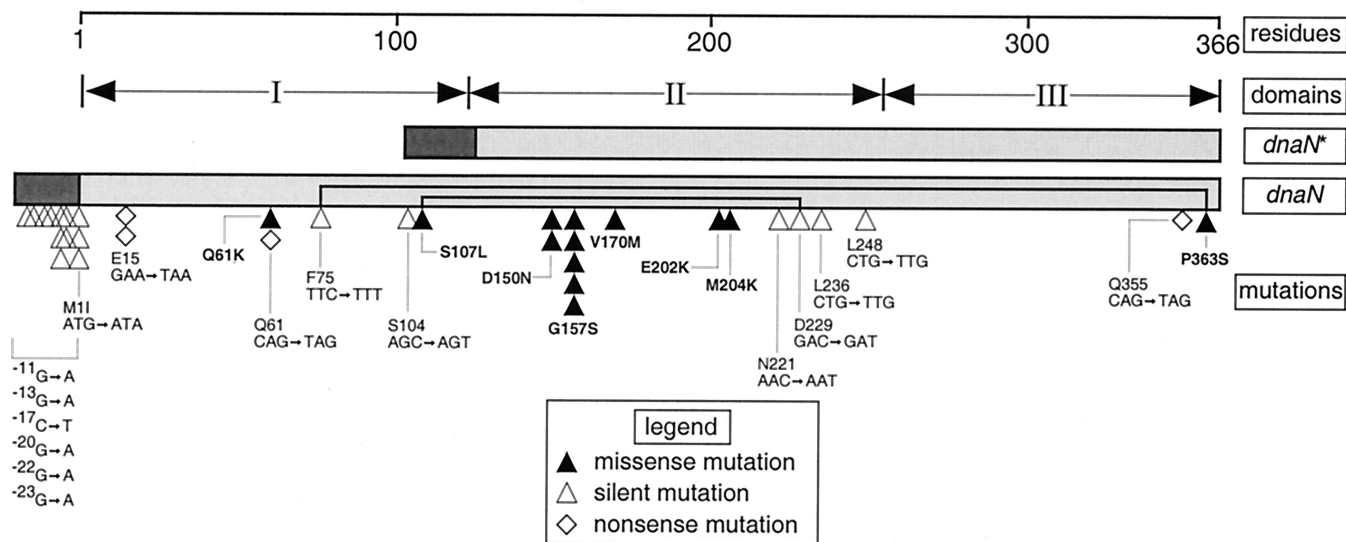
FIG. 2. Stick representation of the *dnaN* gene, summarizing the relative positions of all mutations identified in this study. The positions of all nucleotide alterations identified in the 27 *dnaN* alleles subjected to automated DNA sequence analysis are shown. Approximate positions of amino acid residues in β and the approximate delineation of each of the three structurally similar domains (I, II, and III) comprising each β monomer (21) are indicated. The light-shaded rectangles represent the coding sequences of the *dnaN* and *dnaN** (see text) genes, while the dark-shaded rectangles represent their respective promoters. Approximate positions of those mutations resulting in deduced amino acid substitutions and described in Table 3 are indicated by filled triangles; approximate positions of silent mutations, including those which map to the promoter region, are indicated by open triangles; and approximate positions of nonsense mutations are indicated by open diamonds. Except for the F75 and D229 silent mutations that were identified together with the P363S and S107L mutations, respectively (indicated by the lines connecting these respective positions), the alleles containing silent or nonsense mutations are not described in Table 3. The silent mutation L236 results in formation of a rare Leu codon leading to a significantly reduced steady-state level of the expressed β protein (data not shown). The silent mutations S104, N221, and L248 were identified in combination with at least one other mutation mapping in the vicinity of the ribosome binding site (which maps to nucleotide positions −16 to −9) and appear to also express significantly lower levels of β (data not shown). The four mutations that cannot be accounted for by hydroxylamine treatment (which promotes C→T transitions when used in vitro [31]) are ^{181}C→A (Q61K) (Table 3), ^{611}T→A (M204K) (Table 3), ^{685}G→C (the silent mutation in S107L) (Table 3), and ^{45}G→T (resulting in the E15→nonsense mutation).

ilarly affected with respect to their interactions with the *umuDC* gene products.

If UmuD and UmuD′ interact with different structural features of β, we might have expected that most, if not all, of the *dnaN* alleles that we isolated by virtue of their inability to confer cold sensitivity for growth upon a *umuD′C*-expressing strain would be proficient in causing the β-dependent exacerbation of the cold sensitivity of a *umuDC*-expressing strain. However, as shown in Table 4, all eight *dnaN* alleles that were deficient in conferring cold sensitivity upon AB1157 expressing elevated levels of *umuD′C* were also deficient in their ability to exacerbate the cold sensitivity conferred by elevated levels of the *umuDC* gene products. These observations strongly suggest that the interactions between UmuD′ and β are a subset of those between UmuD and β. Thus, we suggest that UmuD has a higher affinity for β than does UmuD′ because of additional, important interactions of β with the N-terminal 24 residues of UmuD, which are missing from UmuD′. Strikingly, some of the *dnaN* alleles (i.e., G157S, M204K, and, to a lesser extent, E202K) not only were deficient in exacerbation of the cold sensitivity but actually suppressed the inherent cold sensitivity conferred by elevated levels of the *umuDC* gene products in the absence of elevated levels of β (Table 4). This phenotype is interpreted more thoroughly in Discussion.

Novel *dnaN* alleles deficient in exacerbating *umuDC*-mediated cold sensitivity are proficient for DNA replication in vivo when overexpressed. To determine whether or not the novel

dnaN alleles that we identified by our genetic assay were able to function in chromosomal DNA replication, we attempted to cross them onto the *E. coli* chromosome using the pKO3-based system devised by Link et al. (25). This plasmid was specifically designed for moving in-frame deletions and mutations onto the bacterial chromosome. Our inability to homogenize the five *dnaN* alleles we tested (Q61K, D150N, G157S, E202K, and M204K) (data not shown) suggests that they are unable to function in chromosomal replication at a level adequate for viability when expressed at the normal, physiological level.

In light of this, we chose to measure the respective abilities of these *dnaN* alleles to function in chromosomal DNA replication when they were present at an elevated gene dosage by virtue of residing on a multicopy plasmid. For this, we used the same plasmids that we initially identified by our genetic screen and asked whether they could complement the temperature-sensitive phenotype of a *dnaN59*(Ts) strain. *E. coli* strains bearing the *dnaN59*(Ts) allele are temperature sensitive due to the poor stability of the mutant β protein (DnaN59) at the elevated temperature (4). Using the previously described quantitative transformation assay (38), we found that all eight *dnaN* alleles were similar to the plasmid-encoded *dnaN*⁺ allele with respect to their abilities to complement the temperature-sensitive phenotype of the *dnaN59*(Ts) allele (Table 5). In contrast, pBR322 was unable to substitute for pJRC210 in suppression of the temperature-sensitive phenotype of the *dnaN59*(Ts) allele. These results indicate that each of the novel
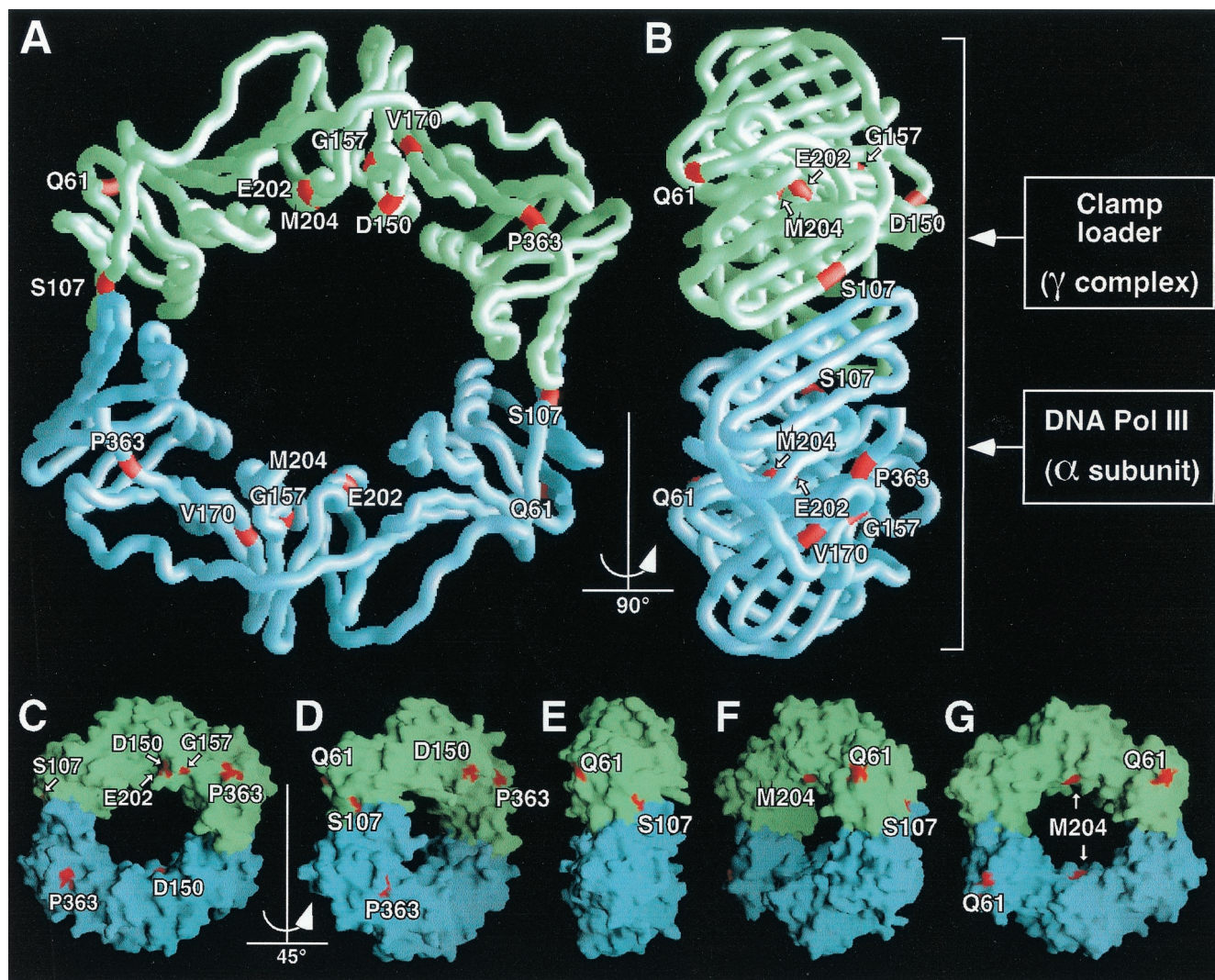
FIG. 3. Relative position of each deduced amino acid substitution in the β crystal. (A and B) Worm representation of the β clamp showing the positions of missense mutations as viewed from the face bearing the extruding C-terminal tails of each protomer (A), and the side (B), corresponding to a 90° rotation of that shown in panel A. The crystal structure of the homodimeric β clamp, solved by Kong et al. (21), is shown with one β protomer in green and the other in blue. Positions of deduced amino acid substitutions are in red. The γ complex clamp loader and the α subunit of Pol III are both believed to interact with the face of β bearing the extruding C-terminal tails (B). See the text for further details. (C through G) Surface representations of the β crystal in successive rotations of 45° each (i.e., 45°, 90°, 135°, and 180°, respectively) relative to the angle shown in panel A. This view bears the extruding C-terminal tails and is arbitrarily defined as 0°. Again, one β protomer is in green and the other is in blue, and positions of deduced amino acid substitutions are in red. Note that not all of the missense mutations (for example, E204) are easily visible in the surface representation. This is because their position either is located just below the surface or is obstructed by other surface features of the β clamp in the views shown. This figure was generated with GRASP (Molecular Simulations, Inc.) using the atom coordinates of the β crystal (2POL) from the Protein Data Bank and a Silicon Graphics R10000 workstation.

dnaN alleles described in this report retains at least some biological activity with respect to DNA replication in vivo. However, their apparent inability to function in chromosomal replication at a level adequate for viability when expressed at the normal, physiological level suggests that it is difficult to genetically separate the replication function of β from its ability to interact with the *umuDC* gene products.

DISCUSSION

Interactions of UmuD$_2$C with components of Pol III help to enable a DNA damage checkpoint control. The cold sensitivity conferred by elevated levels of UmuD$_2$C, a phenomenon that

apparently is due to the inappropriate expression of their DNA damage checkpoint function (59), is exacerbated by overexpression of the β processivity clamp (Table 2). This observation, taken together with our previous findings that overexpression of the ε proofreading subunit of Pol III or deletion of its structural gene (*dnaQ*) suppresses *umuDC*-mediated cold sensitivity whereas overexpression of each of the other eight Pol III subunits (α, θ, τ, γ, δ, δ′, χ, and ψ) does not (56), suggests that UmuD$_2$C associates with the replisome via direct interactions with ε and β and that these interactions serve to antagonize the DNA polymerase activity of Pol III, thereby arresting DNA synthesis (30, 37).
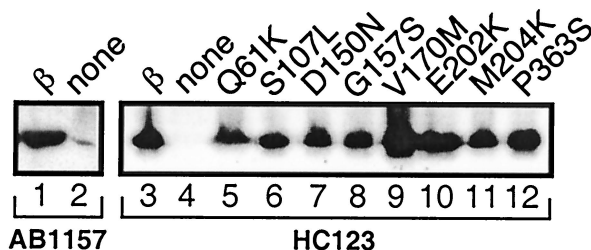
FIG. 4. Steady-state levels of mutant β proteins in the absence of IPTG. The steady-state level of each mutant β protein was measured in the absence of added IPTG by Western blot analysis using polyclonal antibodies specific to β and chemiluminescent detection (Tropix) as described previously (36, 57). Approximately 10^8 cells of each strain grown in LB medium at 30°C to mid-exponential phase (optical density at 600 nm, ~0.5) were electrophoresed in a sodium dodecyl sulfate–15% polyacrylamide gel and then transferred to a polyvinylidene difluoride membrane as described previously (36, 57). Lane 1, AB1157(pJRC210); lane 2, AB1157(pBR322); lane 3, HC123(pJRC210); lane 4, HC123(pBR322); lane 5, HC123(pJRCHA-4.1); lane 6, HC123(pJRCHA-5.1); lane 7, HC123(pJRCHA-8.1); lane 8, HC123(pJRCHA-5G11); lane 9, HC123(pJRCHA-8I11); lane 10, HC123(pJRCHA-7.1); lane 11, HC123(pJRCHA-6F11); and lane 12, HC123(pJRCHA-6.2). *E. coli* AB1157 and HC123 are described in Table 1; plasmids are described in Tables 1 and 3. Note that under the conditions used in this analysis, endogenous levels of β expressed from the chromosomal *dnaN*+ allele (AB1157) (lane 2) were only barely detectable, while those expressed from the *dnaN59*(Ts) allele (HC123) (lane 4) were not detectable.

Overexpression of β also conferred a severe cold-sensitive growth phenotype upon a strain expressing moderately elevated levels of the *umuD'C* gene products. Such a strain is not otherwise normally cold sensitive (59), although higher-level overexpression of UmuD'$_2$C does confer a cold sensitivity (38). We have previously suggested that the cold sensitivity conferred by higher-level overexpression of *umuD'C* is a result of the ability of higher levels of UmuD'$_2$C to mimic the

TABLE 4. Novel *dnaN* alleles deficient in conferring cold sensitivity upon a *umuD'C*-expressing strain are unable to exacerbate the cold sensitivity of a *umuDC*-expressing strain

Plasmid	*dnaN* allele	AB1157 transformants/ml (30°C/42°C)[a] with:	
		pGY9738 (o^c ₁*umuD'C*)	pGY9739 (o^c ₁*umuDC*)
pBR322	None	0.98[b]	3.5×10^{-3b}
pJRC210	*dnaN*+	2.6×10^{-4b}	5.1×10^{-4b}
pJRCHA-4.1	Q61K	0.71	1.7×10^{-3}
pJRCHA-5.1	S107L	0.37	1.5×10^{-3}
pJRCHA-8.1	D150N	0.11	1.7×10^{-3}
pJRCHA-5G11	G157S	0.83	0.25
pJRCHA-8I11	V170M	0.43	1.2×10^{-3}
pJRCHA-7.1	E202K	0.47	2.7×10^{-2}
pJRCHA-6F11	M204K	0.98	0.15
pJRCHA-6.2	P363S	0.30	3.5×10^{-3}

[a] AB1157 bearing either pGY9738 or pGY9739 was transformed with either pBR322 or the indicated pJRC210 derivative. The ratio of CFU obtained at 30°C relative to those obtained at 42°C was measured as described in Table 2, footnote c. Transformation efficiencies at 42°C for all strains were on the order of ~10^5 to ~10^6 CFU per μg of plasmid DNA. These results indicate that overexpression of either the *umuD* or *umuD'C* gene products with or without simultaneous overexpression of β is not lethal at 42°C under these conditions.
[b] This value is the same as that reported in Table 2 and is included here for comparison to the eight novel *dnaN* alleles.

TABLE 5. Abilities of novel *dnaN* alleles to complement the temperature sensitivity of the *dnaN59*(Ts) *E. coli* strain HC123

Plasmid	*dnaN* allele	HC123 transformants/ml (42°C/30°C)[a]
pBR322	None	$<8.6 \times 10^{-4}$
pJRC210	*dnaN*+	1.11
pJRCHA-4.1	Q61K	0.71[b]
pJRCHA-5.1	S107L	1.02
pJRCHA-8.1	D150N	1.10
pJRCHA-5G11	G157S	0.86[b]
pJRCHA-8I11	V170M	0.95
pJRCHA-7.1	E202K	1.01
pJRCHA-6F11	M204K	0.92
pJRCHA-6.2	P363S	0.95

[a] The ratio of CFU obtained at 42°C to those obtained at 30°C was measured as described in Table 2, footnote c. Transformation efficiencies were on the order of ~10^5 to ~10^6 CFU per μg of plasmid DNA.
[b] This strain formed small colonies at 42°C, suggesting that the plasmid was slightly less efficient than pJRC210 at suppressing the temperature sensitivity of *E. coli* HC123.

UmuD$_2$C-dependent DNA damage checkpoint control (59). Thus, we now suggest that the β-expressing plasmid confers cold sensitivity upon an *E. coli* strain expressing moderate levels of *umuD'C* (a strain that is not normally cold sensitive [59]) by facilitating the ability of moderate levels of *umuD'C* to mimic the DNA damage checkpoint function of UmuD$_2$C, which presumably involves, at least in part, interaction of UmuD$_2$C with β (57, 59).

We exploited the ability of the β-expressing plasmid (pJRC210) to confer cold sensitivity upon a *umuD'C*-expressing strain to identify eight unique and novel missense *dnaN* mutations. The fact that these plasmid-borne mutant *dnaN* alleles could complement the temperature sensitivity of a *dnaN59*(Ts) mutant (Table 5) suggests that when overexpressed, these mutant β proteins retain a partial ability to participate in normal DNA replication. Some of the *dnaN* alleles described in this report (i.e., G157S, M204K, and, to a lesser extent, E202K) were able to suppress the inherent cold sensitivity conferred by elevated levels of the *umuDC* gene products in the absence of elevated levels of β (Table 4), suggesting that their presence in the replisome confers upon it an immunity to the replication block normally imposed by elevated levels of UmuD$_2$C. However, a definitive answer to the question of whether or not these mutant β proteins are deficient in interaction with UmuD$_2$C must await their biochemical characterization.

The eight mutant β proteins described in this report bear substitutions of amino acid residues located at or very close to the surface of the molecule (Fig. 3C to G), based on the crystal structure of the β clamp reported by Kong et al. (21). Some of the native residues that were mutated are not easily visible in the surface representations of the β clamp. This is because their position either is located just below the surface or is obstructed by other surface features of the β clamp in the views shown. With respect to the fact that all of the mutations were located at or very close to the surface of the molecule, it is worthwhile emphasizing the fact that all of the mutations correspond to substitutions of a comparatively larger residue in place of the smaller, native residue (e.g., G157S). Thus, all of the mutant β proteins described in this report are expected to have a slightly altered surface relative to that of the wild-type

β protein due to the presence of their respective amino acid substitutions, consistent with the mutant β proteins being affected for interaction with the *umuDC* gene products.

With the exception of Q61K, all of the substitutions reside on the same face of the β clamp (Fig. 3B). Interestingly, this face is the same as that which has been previously suggested to interact with both the α catalytic subunit (23, 24, 55) and the five-subunit clamp loader, or γ complex, of Pol III (23, 24) (Fig. 3B). This finding suggests that interaction of the *umuDC* gene products with β may eliminate the ability of β to subsequently interact with the α catalytic subunit and/or γ complex of Pol III. Furthermore, our recent observation that the *umuD* gene products interact with the same structural domain of ε that has been previously shown to interact with α suggests that a similar situation is true for the UmuD-ε interaction; i.e., UmuD and α associate with ε in a mutually exclusive fashion (56).

If the UmuD$_2$C complex were to interact with both the ε and the β subunits of Pol III in such a way as to preclude their subsequent association with α, then the *umuDC*-dependent checkpoint could not be a manifestation of interactions involving UmuD$_2$C and the Pol III multiprotein complex. Rather, these observations suggest that UmuD$_2$C exerts its checkpoint role by competing with α for binding to ε and β. Viewed in this way, the UmuD$_2$C-dependent checkpoint control has certain aspects in common with the eukaryotic S-phase checkpoint control. One component of this surveillance mechanism is the p21 protein, which acts not only to regulate cyclin–cyclin-dependent kinase complexes (15, 16) but also to regulate the availability of the eukaryotic PCNA processivity clamp (66). By associating directly with the same face of PCNA as does Pol δ, p21 may preclude binding of Pol δ to PCNA, thereby helping to regulate DNA replication in response to DNA damage (66).

The important findings discussed above, taken together with previous observations (37, 38, 56, 57, 59), suggest at least four different models for how interactions of UmuD$_2$C with the ε and the β subunits of Pol III could enable a DNA damage checkpoint control (Fig. 5). With respect to these four models, the absolute requirement of *umuC* for exacerbation of the cold sensitivity (30, 38, 59) suggests that UmuC may also interact directly with ε and β, perhaps serving to impose a directionality upon the interactions of the symmetrical UmuD$_2$ homodimer (in the form of the UmuD$_2$C complex) with ε and β.

In the first model (Fig. 5A), UmuD$_2$C, ε, and β interact, forming a single, multiprotein complex. Since associations of both ε (53) and β (4, 54) with α significantly enhance its DNA polymerase activity, titration of either or both away from the replisome by UmuD$_2$C would be expected to antagonize the catalytic DNA polymerase activity of Pol III. Overexpression of β may exacerbate *umuDC*-mediated cold sensitivity by stabilizing the multiprotein complex, thereby effectively removing ε from the replisome.

A variation of this model is suggested by our findings that deletion of the structural gene encoding ε (*dnaQ*) results in only a partial suppression of *umuDC*-mediated cold sensitivity (56), while overexpression of β strongly exacerbates *umuDC*-mediated cold sensitivity. These findings suggest that ε may play a comparatively less important role in *umuDC*-mediated cold sensitivity than does β. In light of this possibility, it could have been argued that if *umuDC*-mediated cold sensitivity was pri-
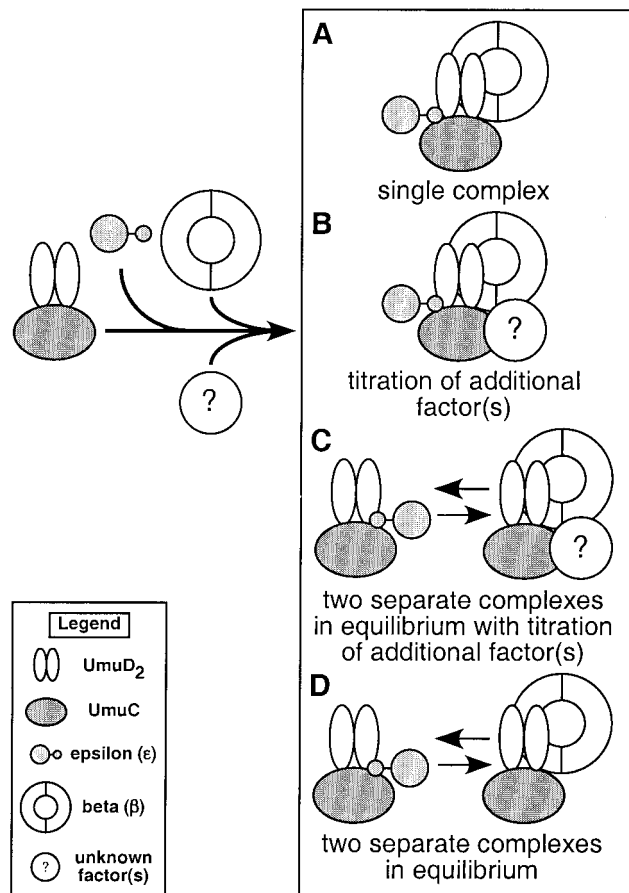

FIG. 5. Four possible models to describe the involvement of the ε and the β subunits of Pol III in UmuD$_2$C-dependent cold sensitivity and in the UmuD$_2$C-dependent checkpoint control. Our results discussed here, taken together with our previous findings (37, 38, 56, 57, 59), suggest at least four different models to describe the involvement of the ε and the β subunits of Pol III in UmuD$_2$C-dependent cold sensitivity, which presumably is a manifestation of functions of UmuD$_2$C involved in the DNA damage checkpoint control (37, 59). Previously described genetic interactions between *umuC* and ε (encoded by *dnaQ*) (10, 11, 17, 19, 35, 56), together with our findings discussed in this report regarding genetic interactions of β with both *umuDC* and *umuD'C*, collectively suggest that UmuC might interact directly with both ε and β. Therefore, for the purposes of these models, we have assumed that interaction of UmuC with ε or β imparts an asymmetry on the UmuD$_2$ homodimer that is important for defining its subsequent interaction (when in the form of the UmuD$_2$C complex) with ε or β. The two proposed structural domains of ε (40, 61) are represented here as circles; the N-terminal domain is represented by the large circle, while the C-terminal domain is represented by the small circle. It has previously been demonstrated that the small C-terminal domain of ε interacts with both the α subunit of Pol III (40, 61) and the *umuD* gene products (56). Interaction of the unknown factor(s) with UmuC and β (B) is arbitrary and is intended only to imply the possible involvement of additional, as-yet-unidentified components of the UmuD$_2$C-dependent checkpoint control pathway. See Discussion for further details regarding these four different models.

marily due to UmuD$_2$C (or possibly a UmuD$_2$C-ε complex) sequestering β away from the replisome, then overexpression of β would be expected to suppress much of the cold sensitivity, the opposite to what we observed. However, if the unusually high levels of β were acting to stabilize a UmuD$_2$C-β-ε complex that

recruits an additional factor(s) which serves an essential role(s) (for example, the α subunit of Pol III), its sequestration into the multiprotein complex could cause the exacerbation of cold sensitivity that we have observed (Fig. 5B).

Two further models to describe how interactions of UmuD$_2$C with the ε and β subunits of Pol III might serve to regulate DNA replication in response to DNA damage take into account the possibility that ε and β may interact with UmuD$_2$C in mutually exclusive fashions, such that two separate complexes are formed. In such a case, one could imagine either that the UmuD$_2$C-β complex acts to recruit an additional factor(s) (Fig. 5C), as described above for the UmuD$_2$C-ε-β complex, or that the UmuD$_2$C-ε and UmuD$_2$C-β complexes simply exist in equilibrium, with neither recruiting additional factors (Fig. 5D). In both cases, one might predict that overexpression of ε would suppress *umuDC*-mediated cold sensitivity (as we have previously reported [56]) by favoring formation of the UmuD$_2$C-ε complex, which may be less efficient than UmuD$_2$C-β at conferring cold sensitivity (see above). This would have the effect of shifting the equilibrium away from formation of the UmuD$_2$C-β complex, which may be recruiting an additional factor(s). In addition, overexpression of ε would replenish the levels of ε initially sequestered away from Pol III by elevated levels of UmuD$_2$C. Likewise, both of these models (Fig. 5C and D) would predict that overexpression of β would act to shift the equilibrium toward formation of the UmuD$_2$C-β (or UmuD$_2$C-β-unknown factor[s]) complex, thus exacerbating the extent of cold sensitivity.

Although all four of the models discussed above share some common aspects, they have important differences as well. Experiments are under way to distinguish which of these four models most closely resembles the events that occur in response to DNA damage in vivo.

Does the UmuD′$_2$C-β interaction serve an important role in the living cell? Despite the finding that UmuD′$_2$C can carry out efficient lesion bypass in vitro in the presence of RecA and ssDNA binding protein (SSB) but in the absence of Pol III (43, 63), various genetic and biochemical observations suggest that some form of DNA Pol III plays a role in determining when and where UmuD′$_2$C-dependent TLS takes place (57; recently reviewed in references 2 and 58). These findings include the following: (i) some *E. coli dnaE* alleles (*dnaE* encodes the α subunit of Pol III) appeared to increase UV-induced *umuDC*-dependent SOS mutagenesis in vivo (45), (ii) the DNA polymerase activity of the temperature-sensitive DnaE1026 mutant α protein was stabilized by the UmuD′$_2$C complex in vitro (63), and (iii) in vitro UmuD′$_2$C-dependent TLS was significantly enhanced by small amounts of either the mutant DnaE1026 protein or Pol III core (63).

In contrast to the role of the α subunit of Pol III in TLS, the role, if any, of the β processivity clamp is less clear. Although Reuven et al., who have successfully reconstituted UmuD′$_2$C-dependent lesion bypass in vitro, found it to be completely independent of the β clamp of Pol III (43), Tang et al., using a slightly different approach to reconstitute UmuD′$_2$C-dependent TLS in vitro (63), reported a strict requirement of both the β clamp and the five-subunit clamp loader (γ complex) of Pol III for lesion bypass. This difference with respect to the requirement of the β clamp for TLS in vitro has been attributed to differences in the template DNAs and the levels of

RecA protein used by these two groups (62, 67), although it could also be due to the maltose binding protein-UmuC fusion protein utilized by Reuven et al. (67).

Our results regarding the cold sensitivity conferred upon the *umuD′C*-expressing strain by the β-expressing plasmid could be the result of the previous proposal that the requirement for the β clamp and γ complex of Pol III for UmuD′$_2$C-dependent TLS in vitro may not be physiologically relevant but rather may be due to the fact that UmuD$_2$C interacts with β to enable a DNA damage checkpoint control (37, 57), and because of this, UmuD′$_2$C retains a comparatively limited yet extraneous ability to also interact with β (13, 44). Alternatively, it is possible that interactions of β with the *umuDC* gene products are important for both the DNA damage checkpoint control (57) and TLS (62, 63) and that self-cleavage of UmuD to yield UmuD′ serves to modulate the interactions of UmuD$_2$C and UmuD′$_2$C with β to effect the release of the checkpoint control and enable TLS (57). In any event, a definitive answer regarding the role of the β clamp in TLS in the living cell will likely require a combined biochemical and genetic approach. Further characterization of the mutant β proteins described in this report will help to establish whether or not β serves an important role in TLS.

Relationship of the eight novel *dnaN* alleles to *dnaN**. Following exposure of *E. coli* to various DNA-damaging agents, the level of transcription of the *dnaN* gene increases (42, 60). In addition, expression of a truncated form of β corresponding almost exactly to the C-terminal two-thirds of the protein is also induced (39, 50). This protein, termed β*, is expressed, in a *recA*- and *lexA*-dependent fashion, from a promoter located within the coding region of the *dnaN* gene (Fig. 2) (39, 50). The gene for β*, which is contained entirely within the *dnaN*$^+$ gene, has been termed *dnaN** (39, 50). Biochemical characterization of β* indicates that it can form a trimer in solution (49). Furthermore, in vitro studies have demonstrated that β* can modestly enhance the processivity of Pol III*, a form of Pol III containing all subunits except the β clamp (49). However, despite these detailed genetic and biochemical analyses, the physiological role of β* is presently unknown. Interestingly, it has recently been suggested that several eukaryotic proteins originally identified because of their roles in cell cycle checkpoints act by serving as an alternative to PCNA, the normal processivity clamp (33, 64, 65), or as an alternate subunit of the five-subunit replication factor C complex (65), the normal PCNA clamp loader.

Each β protomer has threefold structural similarity (21) (Fig. 2). Because each β* protomer corresponds almost exactly to the C-terminal two-thirds of the *dnaN* gene product (39, 50), it retains the C-terminal two structural domains but is missing the N-terminal domain I. It is interesting that two of the mutations we identified that reduce interactions with the *umuDC* gene products, Q61K and S107L, both reside within the N-terminal domain (domain I), which is lacking in β*. Thus, relative to intact β, β* may also be deficient for interactions with the *umuDC* gene products. These results raise the interesting possibility that a clamp composed of a trimer of β* might constitute an efficient mechanism for conferring processivity upon Pol III, as well as possibly other DNA polymerases, including Pol II (1), that is insensitive to the checkpoint function(s) of UmuD$_2$C. Such a mechanism might be particularly

important during replication restart (7) (or induced replisome reactivation [20]) or following TLS, a time in which the cell is presumably involved in reestablishing a replisome wherein processive and accurate DNA synthesis is carried out primarily by Pol III (6, 14, 58). However, further genetic and biochemical characterization of β* will be required in order to establish its physiological role(s).

REFERENCES

1. **Bonner, C. A., P. T. Stukenberg, M. Rajagopalan, R. Eritja, M. O'Donnell, K. McEntee, H. Echols, and M. F. Goodman.** 1992. Processive DNA synthesis by DNA polymerase II mediated by DNA polymerase III accessory proteins. J. Biol. Chem. **267:**11431–11438.
2. **Bridges, B.** 2000. DNA polymerases and SOS mutagenesis: can one reconcile the biochemical and genetic data? Bioessays **22:**933–937.
3. **Burckhardt, S. E., R. Woodgate, R. H. Scheuermann, and H. Echols.** 1988. UmuD mutagenesis protein of *Escherichia coli:* overproduction, purification, and cleavage by RecA. Proc. Natl. Acad. Sci. USA **85:**1811–1815.
4. **Burgers, P. M., A. Kornberg, and Y. Sakakibara.** 1981. The *dnaN* gene codes for the beta subunit of DNA polymerase III holoenzyme of *Escherichia coli.* Proc. Natl. Acad. Sci. USA **78:**5391–5395.
5. **Churchward, G., D. Belin, and Y. Nagamine.** 1984. A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors. Gene **31:**165–171.
6. **Cox, M. M., M. F. Goodman, K. N. Kreuzer, D. J. Sherratt, S. J. Sandler, and K. J. Marians.** 2000. The importance of repairing stalled replication forks. Nature **404:**37–41.
7. **Echols, H., and M. F. Goodman.** 1991. Fidelity mechanisms in DNA replication. Annu. Rev. Biochem. **60:**477–511.
8. **Elledge, S. J., and G. C. Walker.** 1983. Proteins required for ultraviolet light and chemical mutagenesis. Identification of the products of the *umuC* locus of *Escherichia coli.* J. Mol. Biol. **164:**175–192.
9. **Fernandez De Henestrosa, A. R., T. Ogi, S. Aoyagi, D. Chafin, J. J. Hayes, H. Ohmori, and R. Woodgate.** 2000. Identification of additional genes belonging to the LexA regulon in *Escherichia coli.* Mol. Microbiol. **35:**1560–1572.
10. **Foster, P. L., and A. D. Sullivan.** 1988. Interactions between epsilon, the proofreading subunit of DNA polymerase III, and proteins involved in the SOS response of *Escherichia coli.* Mol. Gen. Genet. **214:**467–473.
11. **Foster, P. L., A. D. Sullivan, and S. B. Franklin.** 1989. Presence of the *dnaQ-rnh* divergent transcriptional unit on a multicopy plasmid inhibits induced mutagenesis in *Escherichia coli.* J. Bacteriol. **171:**3144–3151.
12. **Friedberg, E. C., G. C. Walker, and W. Siede.** 1995. DNA repair and mutagenesis. ASM Press, Washington, D.C.
13. **Goldsmith, M., L. Sarov-Blat, and Z. Livneh.** 2000. Plasmid-encoded MucB protein is a DNA polymerase (pol RI) specialized for lesion bypass in the presence of MucA′, RecA, and SSB. Proc. Natl. Acad. Sci. USA **97:**11227–11231.
14. **Goodman, M. F.** 2000. Coping with replication 'train wrecks' in *Escherichia coli* using Pol V, Pol II and RecA proteins. Trends Biochem. Sci. **25:**189–195.
15. **Harper, J. W., G. R. Adami, N. Wei, K. Keyomarsi, and S. J. Elledge.** 1993. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell **75:**805–816.
16. **Harper, J. W., S. J. Elledge, K. Keyomarsi, B. Dynlacht, L. H. Tsai, P. Zhang, S. Dobrowolski, C. Bai, L. Connell-Crowley, E. Swindell, et al.** 1995. Inhibition of cyclin-dependent kinases by p21. Mol. Biol. Cell **6:**387–400.
17. **Jonczyk, P., I. Fijalkowska, and Z. Ciesla.** 1988. Overproduction of the epsilon subunit of DNA polymerase III counteracts the SOS mutagenic response of *Escherichia coli.* Proc. Natl. Acad. Sci. USA **85:**9124–9127.
18. **Jonczyk, P., and A. Nowicka.** 1996. Specific in vivo protein-protein interactions between *Escherichia coli* SOS mutagenesis proteins. J. Bacteriol. **178:**2580–2585.
19. **Kanabus, M., A. Nowicka, E. Sledziewska-Gojska, P. Jonczyk, and Z. Ciesla.** 1995. The antimutagenic effect of a truncated subunit of DNA polymerase III in *Escherichia coli* cells irradiated with UV light. Mol. Gen. Genet. **247:**216–221.
20. **Khidhir, A. M., S. Casaregola, and I. B. Holland.** 1985. Mechanism of transient inhibition of DNA synthesis in ultraviolet-irradiated *E. coli:* inhibition is independent of *recA* whilst recovery requires RecA protein itself and an additional, inducible SOS function. Mol. Gen. Genet. **199:**133–140.
21. **Kong, X. P., R. Onrust, M. O'Donnell, and J. Kuriyan.** 1992. Three-dimensional structure of the beta subunit of *E. coli* DNA polymerase III holoenzyme: a sliding DNA clamp. Cell **69:**425–437.
22. **Kowalczykowski, S. C., D. A. Dixon, A. K. Eggleston, S. D. Lauder, and W. M. Rehrauer.** 1994. Biochemistry of homologous recombination in *Escherichia coli.* Microbiol. Rev. **58:**401–465.
23. **Latham, G. J., D. J. Bacheller, P. Pietroni, and P. H. von Hippel.** 1997. Structural analyses of gp45 sliding clamp interactions during assembly of the bacteriophage T4 DNA polymerase holoenzyme. II. The Gp44/62 clamp loader interacts with a single defined face of the sliding clamp ring. J. Biol. Chem. **272:**31677–31684.
24. **Latham, G. J., D. J. Bacheller, P. Pietroni, and P. H. von Hippel.** 1997. Structural analyses of gp45 sliding clamp interactions during assembly of the bacteriophage T4 DNA polymerase holoenzyme. III. The Gp43 DNA polymerase binds to the same face of the sliding clamp as the clamp loader. J. Biol. Chem. **272:**31685–31692.
25. **Link, A. J., D. Phillips, and G. M. Church.** 1997. Methods for generating precise deletions and insertions in the genome of wild-type *Escherichia coli:* application to open reading frame characterization. J. Bacteriol. **179:**6228–6237.
26. **Little, J. W., S. H. Edmiston, L. Z. Pacelli, and D. W. Mount.** 1980. Cleavage of the *Escherichia coli* lexA protein by the recA protease. Proc. Natl. Acad. Sci. USA **77:**3225–3229.
27. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli.* Cell **29:**11–22.
28. **Little, J. W., D. W. Mount, and C. R. Yanisch-Perron.** 1981. Purified lexA protein is a repressor of the *recA* and *lexA* genes. Proc. Natl. Acad. Sci. USA **78:**4199–4203.
29. **Maor-Shoshani, A., N. B. Reuven, G. Tomer, and Z. Livneh.** 2000. Highly mutagenic replication by DNA polymerase V (UmuC) provides a mechanistic basis for SOS untargeted mutagenesis. Proc. Natl. Acad. Sci. USA **97:**565–570.
30. **Marsh, L., and G. C. Walker.** 1985. Cold sensitivity induced by overproduction of UmuDC in *Escherichia coli.* J. Bacteriol. **162:**155–161.
31. **Miller, J. H.** 1992. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
32. **Murli, S., T. Opperman, B. T. Smith, and G. C. Walker.** 2000. A role for the *umuDC* gene products of *Escherichia coli* in increasing resistance to DNA damage in stationary phase by inhibiting the transition to exponential growth. J. Bacteriol. **182:**1127–1135.
33. **Neuwald, A. F., and A. Poleksic.** 2000. PSI-BLAST searches using hidden Markov models of structural repeats: prediction of an unusual sliding DNA clamp and of beta-propellers in UV-damaged DNA-binding protein. Nucleic Acids Res. **28:**3570–3580.
34. **Nohmi, T., J. R. Battista, L. A. Dodson, and G. C. Walker.** 1988. RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and posttranslational activation. Proc. Natl. Acad. Sci. USA **85:**1816–1820.
35. **Nowicka, A., M. Kanabus, E. Sledziewska-Gojska, and Z. Ciesla.** 1994. Different UmuC requirements for generation of different kinds of UV-induced mutations in *Escherichia coli.* Mol. Gen. Genet. **243:**584–592.
36. **Ohta, T., M. D. Sutton, A. Guzzo, S. Cole, A. E. Ferentz, and G. C. Walker.** 1999. Mutations affecting the ability of the *Escherichia coli* UmuD′ protein to participate in SOS mutagenesis. J. Bacteriol. **181:**177–185.
37. **Opperman, T., S. Murli, B. T. Smith, and G. C. Walker.** 1999. A model for a *umuDC*-dependent prokaryotic DNA damage checkpoint. Proc. Natl. Acad. Sci. USA **96:**9218–9223.
38. **Opperman, T., S. Murli, and G. C. Walker.** 1996. The genetic requirements for UmuDC-mediated cold sensitivity are distinct from those for SOS mutagenesis. J. Bacteriol. **178:**4400–4411.
39. **Paz-Elizur, T., R. Skaliter, S. Blumenstein, and Z. Livneh.** 1996. Beta*, a UV-inducible smaller form of the beta subunit sliding clamp of DNA polymerase III of *Escherichia coli.* I. Gene expression and regulation. J. Biol. Chem. **271:**2482–2490.
40. **Perrino, F. W., S. Harvey, and S. M. McNeill.** 1999. Two functional domains of the epsilon subunit of DNA polymerase III. Biochemistry **38:**16001–16009.
41. **Pietroni, P., M. C. Young, G. J. Latham, and P. H. von Hippel.** 1997. Structural analyses of gp45 sliding clamp interactions during assembly of the bacteriophage T4 DNA polymerase holoenzyme. I. Conformational changes

within the gp44/62-gp45-ATP complex during clamp loading. J. Biol. Chem. **272:**31666–31676.

42. **Quinones, A., J. Kaasch, M. Kaasch, and W. Messer.** 1989. Induction of *dnaN* and *dnaQ* gene expression in *Escherichia coli* by alkylation damage to DNA. EMBO J. **8:**587–593.

43. **Reuven, N. B., G. Arad, A. Maor-Shoshani, and Z. Livneh.** 1999. The mutagenesis protein UmuC is a DNA polymerase activated by UmuD′, RecA, and SSB and is specialized for translesion replication. J. Biol. Chem. **274:**31763–31766.

44. **Reuven, N. B., G. Arad, A. Z. Stasiak, A. Stasiak, and Z. Livneh.** 2001. Lesion bypass by the *Escherichia coli* DNA polymerase V requires assembly of a RecA nucleoprotein filament. J. Biol. Chem. **276:**5511–5517.

45. **Ruiz-Rubio, M., and B. A. Bridges.** 1987. Mutagenic DNA repair in *Escherichia coli*. XIV. Influence of two DNA polymerase III mutator alleles on spontaneous and UV mutagenesis. Mol. Gen. Genet. **208:**542–548.

46. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

47. **Sassanfar, M., and J. W. Roberts.** 1990. Nature of the SOS-inducing signal in *Escherichia coli*. The involvement of DNA replication. J. Mol. Biol. **212:**79–96.

48. **Shinagawa, H., H. Iwasaki, T. Kato, and A. Nakata.** 1988. RecA protein-dependent cleavage of UmuD protein and SOS mutagenesis. Proc. Natl. Acad. Sci. USA **85:**1806–1810.

49. **Skaliter, R., M. Bergstein, and Z. Livneh.** 1996. Beta*, a UV-inducible shorter form of the beta subunit of DNA polymerase III of *Escherichia coli*. II. Overproduction, purification, and activity as a polymerase processivity clamp. J. Biol. Chem. **271:**2491–2496.

50. **Skaliter, R., T. Paz-Elizur, and Z. Livneh.** 1996. A smaller form of the sliding clamp subunit of DNA polymerase III is induced by UV irradiation in *Escherichia coli*. J. Biol. Chem. **271:**2478–2481.

51. **Sommer, S., F. Boudsocq, R. Devoret, and A. Bailone.** 1998. Specific RecA amino acid changes affect RecA-UmuD′C interaction. Mol. Microbiol. **28:**281–291.

52. **Sommer, S., J. Knezevic, A. Bailone, and R. Devoret.** 1993. Induction of only one SOS operon, *umuDC*, is required for SOS mutagenesis in *Escherichia coli*. Mol. Gen. Genet. **239:**137–144.

53. **Studwell, P. S., and M. O'Donnell.** 1990. Processive replication is contingent on the exonuclease subunit of DNA polymerase III holoenzyme. J. Biol. Chem. **265:**1171–1178.

54. **Stukenberg, P. T., P. S. Studwell-Vaughan, and M. O'Donnell.** 1991. Mechanism of the sliding beta-clamp of DNA polymerase III holoenzyme. J. Biol. Chem. **266:**11328–11334.

55. **Stukenberg, P. T., J. Turner, and M. O'Donnell.** 1994. An explanation for lagging strand replication: polymerase hopping among DNA sliding clamps. Cell **78:**877–887.

56. **Sutton, M. D., S. Murli, T. Opperman, C. Klein, and G. C. Walker.** 2001. *umuDC-dnaQ* interaction and its implications for cell cycle regulation and SOS mutagenesis in *Escherichia coli*. J. Bacteriol. **183:**1085–1089.

57. **Sutton, M. D., T. Opperman, and G. C. Walker.** 1999. The *Escherichia coli* SOS mutagenesis proteins UmuD and UmuD′ interact physically with the replicative DNA polymerase. Proc. Natl. Acad. Sci. USA **96:**12373–12378.

58. **Sutton, M. D., B. T. Smith, V. G. Godoy, and G. C. Walker.** 2000. The SOS response: recent insights into *umuDC*-dependent DNA damage tolerance. Annu. Rev. Genet. **34:**479–497.

59. **Sutton, M. D., and G. C. Walker.** 2001. *umuDC*-mediated cold sensitivity is a manifestation of functions of the UmuD₂C complex involved in a DNA damage checkpoint control. J. Bacteriol. **183:**1215–1224.

60. **Tadmor, Y., M. Bergstein, R. Skaliter, H. Shwartz, and Z. Livneh.** 1994. Beta subunit of DNA polymerase III holoenzyme is induced upon ultraviolet irradiation or nalidixic acid treatment of *Escherichia coli*. Mutat. Res. **308:**53–64.

61. **Taft-Benz, S. A., and R. M. Schaaper.** 1999. The C-terminal domain of *dnaQ* contains the polymerase binding site. J. Bacteriol. **181:**2963–2965.

62. **Tang, M., P. Pham, X. Shen, J.-S. Taylor, M. O'Donnell, M. Woodgate, and M. F. Goodman.** 2000. Roles of *E. coli* DNA polymerase IV and V in lesion-targeted and untargeted SOS mutagenesis. Nature **404:**1014–1018.

63. **Tang, M., X. Shen, E. G. Frank, M. O'Donnell, R. Woodgate, and M. F. Goodman.** 1999. UmuD′(2)C is an error-prone DNA polymerase. *Escherichia coli* pol V. Proc. Natl. Acad. Sci. USA **96:**8919–8924.

64. **Thelen, P. M., C. Venclovas, and K. Fidelis.** 1999. A sliding clamp model for the Rad1 family of cell cycle checkpoint proteins. Cell **96:**769–770.

65. **Venclovas, C., and M. P. Thelen.** 2000. Structure-based predictions of Rad1, Rad9, Hus1 and Rad17 participation in sliding clamp and clamp-loading complexes. Nucleic Acids Res. **28:**2481–2493.

66. **Waga, S., G. J. Hannon, D. Beach, and B. Stillman.** 1994. The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. Nature **369:**574–578.

67. **Walker, G. C.** 1998. Skiing the black diamond slope: progress on the biochemistry of translesion DNA synthesis. Proc. Natl. Acad. Sci. USA **95:**10348–10350.

68. **Woodgate, R., M. Rajagopalan, C. Lu, and H. Echols.** 1989. UmuC mutagenesis protein of *Escherichia coli*: purification and interaction with UmuD and UmuD′. Proc. Natl. Acad. Sci. USA **86:**7301–7305.