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Overproduction of the β Subunit of DNA Polymerase III Holoenzyme Reduces UV Mutagenesis in *Escherichia coli*

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Overproduction of the β subunit of DNA polymerase III holoenzyme caused a 5- to 10-fold reduction of UV mutagenesis along with a slight increase in sensitivity to UV light in *Escherichia coli*. The same effects were observed in excision-deficient cells, excluding the possibility that they were mediated via changes in excision repair. In contrast, overproduction of the α subunit of the polymerase did not influence either UV mutagenesis or UV sensitivity. The presence of the mutagenesis proteins MucA and MucB expressed from a plasmid alleviated the effect of overproduced β on UV mutagenesis. We have previously suggested that DNA polymerase III holoenzyme can exist in two forms: β-rich form unable to bypass UV lesions and a β-poor form capable of bypassing UV lesions (O. Shavitt and Z. Livneh, J. Biol. Chem. 264:11275–11281, 1989). The β-poor form may be related to an SOS form of DNA polymerase III designed to perform translesion polymerization under SOS conditions and thereby generate mutations. On the basis of this model, we propose that the overproduced β subunit affects the relative abundance of the regular replicative β-rich polymerase and the SOS bypass-proficient polymerase by sequestering the polymerase molecules to the β-rich form and blocking the SOS form.

The SOS response in *Escherichia coli* is a stress response which is induced in cells in response to DNA damage and functions presumably to increase survival under adverse environmental conditions (41, 42). Mutagenesis induced by a variety of DNA-damaging agents such as UV radiation is associated with the SOS response and depends on specific genes, i.e., *umuD*, *umuC*, and *recA*, which are regulated by RecA and LexA, the activator and the repressor, respectively, of the SOS response (22, 41). The molecular mechanism of SOS mutagenesis targeted at DNA lesions is not clear. The current view is that it involves translesion polymerization by an SOS form of DNA polymerase III (PolIII) holoenzyme, the major replicative polymerase of *E. coli* (13, 41, 42). This view is based on genetic studies that demonstrated that SOS-targeted mutagenesis requires the *dnaE* (*polC*) gene, which encodes the α subunit of PolIII, in which the polymerizing active site resides (7–10, 15). The nature of the SOS alteration that PolIII undergoes is unknown.

DNA polymerase I, the major repair polymerase of *E. coli*, does not seem to be required for SOS-targeted mutagenesis, since strains with the *polA* gene (which encodes DNA polymerase I) deleted (2) and strains with a point mutation in *polA* (42) exhibit normal UV mutagenesis. The *polB* gene, which encodes DNA polymerase II, was found to be identical to *dinA* (6), a gene identified independently as being damage inducible (19). Strains with a *polB* point mutation (15) or with a *dinA* insertion mutation (19) were found to be UV mutable, thus arguing against the involvement of DNA polymerase II in UV mutagenesis.

PolIII holoenzyme is a complex composed of at least 10 subunits. Three subunits, α, ε, and θ, which are tightly bound to form an assembly termed DNA polymerase III, provide the catalytic core of PolIII holoenzyme (20, 28). The α subunit has the polymerase activity (25), and the ε subunit has the editing 3′→5′ exonuclease activity (35). The role of the θ subunit is unknown. The γ, δ, τ, and β subunits are auxiliary proteins whose function is to endow the polymerase with high catalytic activity and high processivity (27, 30). The β subunit plays an important role both in initiating polymerization and in determining the processivity of the polymerase (27, 28, 31). DNA polymerase III*, a subassembly of DNA polymerase III holoenzyme lacking the β subunit, has feeble synthetic activity on primed single-stranded DNA and has low processivity. It has been suggested that PolIII holoenzyme is an asymmetric dimeric particle with twin PolIII core active sites and two different sets of auxiliary units designed to enable simultaneous replication of the leading and lagging strands (26, 30).

Detailed biochemical analysis of the in vitro replication of damaged DNA with purified PolIII holoenzyme led us to focus on the processivity of the polymerase and on the stability of the polymerase-DNA association as the key factors governing bypass synthesis at lesions (16, 23, 24, 38, 39). Recently, we have found that the β subunit modulates bypass and termination at UV lesions during in vitro replication. Whereas at low β concentrations bypass synthesis was observed, at high β concentrations (which were similar to the intracellular concentration of β in noninduced cells) very little, if any, bypass of lesions occurred (37). This suggested a role for the β subunit of PolIII holoenzyme as a negative affector of UV mutagenesis. The present study addressed such a possible role for the β subunit in UV mutagenesis in vivo.

MATERIALS AND METHODS

Materials. Antibiotics and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside were purchased from Sigma, isopropyl-β-D-thiogalactopyranoside (IPTG) was from Boehringer Mannheim, and media for bacterial growth were from Difco.

Bacteria, plasmids, and culture growth. The strains used in this study are listed in Table 1. Cells were usually grown in Luria-Bertani (LB) medium supplemented, as required, with the following: ampicillin, 100 μg/ml; tetracycline, 12.5 μg/ml; kanamycin, 50 μg/ml; nalidixic acid, 50 μg/ml; IPTG, 0.5 mM; and glucose, 0.2%. F′*lacI*^{q} Tet^r was transferred from *E.*

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TABLE 1. Bacterial strains used in this study

Strain	Genotype	Source
AB1157	*argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rpsL31 supE44*	Laboratory stocks
AB1157XL	AB1157 F'::Tn*10 lacI*^{q} *lacZΔM15 proAB*	This study
AB1186	AB1157 *uvrA6*	Laboratory stocks
AB1186XL	AB1186 F'::Tn*10 lacI*^{q} *lacZΔM15 proAB*	This study
HC194	HfrC *pyrE metB thyA dnaN59*λ^r	A. Kornberg
MC4100	Δ(*argF-lac*)*205 araD139 rpsL150 thiA1 relA1 flb-5301 deoC1 ptsF25 rbsR*	C. Gross
MC4100XL	MC4100 F'::Tn*10 lacI*^{q} *lacZΔM15 proAB*	This study
TK603	*thr-1 leu-6 proA2 his-4 thi-1 lacY1 ilv-325 uvrA6*	T. Kato
TK603XL	TK603 F'::Tn*10 lacI*^{q} *lacZΔM15 proAB*	This study
TK610	TK603 *umuC36*	T. Kato
TK610XL	TK610 F'::Tn*10 lacI*^{q} *lacZΔM15 proAB*	This study
XL1-Blue	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* Δ(*pro-lac*)*5* F'::Tn*10 lacI*^{q} *lacZΔM15 proAB*	Laboratory stocks

coli XL1-Blue to other strains by conjugation as described elsewhere (29).

Construction of plasmids. Plasmid pUN234, which carries the *dnaN* gene under control of the *lac* promoter, was constructed by cloning the *Ssp*I (nucleotide [nt] 1366)-*Sty*I (nt 2881) (coordinates are according to reference 32]) 1.5-kbp DNA fragment from plasmid pSJS9 (4) into the *Sma*I site of plasmid pUC18. Plasmid pUN222 has the same *dnaN* DNA fragment cloned in the opposite orientation. As expected, these plasmids could complement the *dnaN59*(Ts) mutation in strain HC194 (34). Three plasmids carrying mutations in *dnaN* were derived from plasmid pUN234: pUN234B, in which the *Bst*XI (nt 2348)-*Bst*XI (nt 2857) C-terminal portion of *dnaN* was deleted; pUN234E, in which the *Eco*RV (nt 1591)-EcoRV (nt 1871) N-terminal portion of *dnaN* was deleted; and pUN234FS, in which a +4-bp insertion-frameshift mutation was introduced at the unique *Xho*I (nt 1785) site by cleavage with the restriction nuclease followed by filling in with the Klenow fragment of DNA polymerase I and self-ligation. Plasmid pSE992, a Δ*umuDC* derivative of plasmid pSE116 (14), was constructed by digestion with nuclease *Sal*I followed by self-ligation, resulting in the deletion of the entire *umuDC* operon. Plasmid pGWK1, which carries the *mucAB* and kanamycin resistance genes, was constructed by cloning the 1.43-kbp *Hae*II DNA fragment containing the kanamycin resistance gene from plasmid pACYC177 into the *Bam*HI site of plasmid pGW1700 (33).

Western blot (immunoblot) analysis. Proteins were extracted from culture samples containing the same number of cells by trichloroacetic acid precipitation. They were washed twice with 80% cold acetone and solubilized in sample buffer containing 160 mM Tris-HCl (pH 6.8), 4% sodium dodecyl sulfate (SDS), 200 mM dithiothreitol, 20% glycerol, and 0.1% bromphenol blue. The samples were fractionated on SDS–15% polyacrylamide gels by electrophoresis and then electroblotted onto nitrocellulose membrane filters. The blots were reacted with rabbit anti-β antibodies that were raised against purified β subunit. Next, the blots were treated with ^{125}I-protein A, and protein bands were visualized by autoradiography. The extent of overproduction was determined by densitometric tracing.

[^{35}S]methionine labeling of the α subunit of PolIII. Overproduction of the α subunit of PolIII was achieved by using plasmid pMJ201, a pUC19 derivative carrying the *dnaE* gene cloned under the *lac* promoter (a gift from H. Maki, Kyushu University, Fukuoka, Japan). Cells carrying plasmid pMJ201 were grown at 30°C to an optical density at 595 nm (OD_{595}) of 0.2 in M9 medium (14) containing glucose, ampicillin, and all amino acids except methionine. They were then washed in the same medium without glucose, divided into two parts, and grown in parallel with glucose or with IPTG at 37°C. At selected time points, 1-ml aliquots were withdrawn, labeled for 3 min with 50 μCi of [^{35}S]methionine, and chased for 10 min with unlabeled methionine. The samples were centrifuged and resuspended in 100 μl of loading buffer. Twenty-microliter samples were fractionated by electrophoresis on SDS–8% polyacrylamide gels, dried, and autoradiographed. Quantitation of the α subunit was done by densitometric scanning.

UV survival and mutagenesis. UV-induced mutations to rifampin resistance were assayed essentially as described previously (36). Cells harboring the various plasmids were grown at 30°C in LB medium containing 0.2% glucose and the appropriate antibiotics to an OD_{595} of 0.5. The cells were washed and then resuspended in 10 mM Tris-HCl (pH 7.5)–0.15 M NaCl, and 5-ml portions were UV irradiated with agitation at the UV doses indicated in the figures with a low-pressure mercury germicidal lamp (wavelength, 254 nm). The dose was 0.1 to 0.5 J m^{-2} s^{-1} as determined by a UV Products radiometer equipped with a UVX-25 sensor. Following irradiation, 0.1-ml portions were spread in parallel on LB plates containing ampicillin and tetracycline and either 0.2% glucose or 0.5 mM IPTG, and then they were covered with a layer of 3 ml of LB medium–0.6% agar. The plates were incubated for 3 h at 37°C to allow expression of mutations. Next, 3 ml of LB medium–0.6% agar containing rifampin (final concentration, 100 μg/ml in the plate) was added, and the plates were incubated at 37°C. UV-induced mutations conferring resistance to nalidixic acid were determined by a different procedure. After irradiation, equal numbers of survivors from each sample were diluted into two samples of 40 ml of LB medium each containing either 0.2% glucose or 0.5 mM IPTG and incubated for 3 h at 37°C to allow expression of mutations. The cells were then spread on LB plates containing ampicillin, tetracycline, nalidixic acid (50 μg/ml), and either glucose or IPTG and incubated at 37°C. Mutants were counted in each mutagenesis assay after 3 days. Survivors were counted after 24 h under the same conditions but without rifampin or nalidixic acid. Mutation frequencies were calculated by dividing the number of mutants by the overall number of colonies. In UV survival experiments, cells were diluted after irradiation and plated in parallel on LB plates with the appropriate antibiotics and either IPTG or glucose.

UV-induced mutations from histidine auxotrophy to prototrophy were assayed essentially as described elsewhere (14). AB1157XL cells harboring plasmid pUC18 or pUN234 were grown at 30°C in M9 medium supplemented with 0.2%

glycerol, 100 μg each of threonine, valine, proline, arginine, leucine, isoleucine, and histidine per ml, 3 μg of tetracycline per ml, and 50 μg of ampicillin per ml to an OD_{595} of 0.5. They were then harvested, resuspended in 10 mM Tris-HCl (pH 7.5)–0.15 M NaCl, and UV irradiated. Samples containing approximately 4×10^7 to 6×10^7 cells were then spread in parallel on M9 agar plates containing 0.2% glucose or 0.5 mM IPTG and incubated at 37°C. The plates were supplemented with the same nutrients as in the liquid growth medium (including glycerol), except that histidine was present in a limiting concentration of 2 μg/ml. Histidine-prototrophic colonies were counted after 4 days. AB1157XL (pUN234) cells grew more slowly on IPTG plates than on glucose plates; therefore, in this case, mutants were counted after 6 to 7 days. The number of surviving cells was determined by plating an appropriately diluted sample on the same plates.

RESULTS

Overproduction of the β subunit. Our in vitro analysis showed that when the β subunit was present in excess over the polymerase, bypass of UV lesions was reduced or even eliminated (37). If this result applied to the in vivo situation, one would expect that the overproduction of β in cells should decrease UV mutagenesis. In order to examine this possibility, we have cloned the *dnaN* gene, which encodes the β subunit, under the inducible *lac* promoter (*lacP*) in plasmid pUC18, yielding plasmid pUN234. The functionality of the *dnaN* gene in this construct was proven by the ability of pUN234 to complement the temperature sensitivity of *E. coli* HC194 *dnaN59*(Ts). To achieve efficient repression of *dnaN* carried on the multicopy plasmid, all strains contained an F′ episome with the *lacI*^{q} gene, which overproduces the LacI repressor. Western blot analysis of cells harboring plasmid pUN234 revealed that treatment with IPTG, a synthetic inducer of *lacP*, induced the synthesis of the β subunit (Fig. 1). Maximal repression was achieved by growing the cells at 30°C in the absence of inducer and in the presence of glucose, a catabolic repressor of the *lac* promoter. The amount of the β subunit increased 50-fold upon addition of IPTG. Control cells harboring the vector pUC18 did not show such IPTG induction, as expected (Fig. 1). The presence of plasmid pUN234 did not affect the growth rate of host cells in rich liquid medium, even in the presence of IPTG. However, when the cells were plated on LB plates containing IPTG, the overproduced β subunit led to the formation of small colonies for reasons which are not fully understood.

Overproduction of the β subunit reduces chromosomal UV mutagenesis. *E. coli* MC4100XL cells harboring plasmid pUN234 were UV irradiated and plated in parallel on LB plates containing IPTG or glucose. As shown in Fig. 2, the presence of plasmid pUN234 slightly sensitized the cells to killing by UV radiation when they were grown in the presence of IPTG. This sensitization was not observed with cells carrying vector pUC18 or plasmid pUN222, in which *dnaN* was cloned in the opposite orientation. Nor was it observed with plasmid pUN234XFS, a derivative of pUN234, into which a +4-bp insertion-frameshift was engineered. Thus, the sensitization was caused by the presence of increased levels of the β subunit.

Next, we examined the effect of overproduction of the β subunit on chromosomal UV mutagenesis. Three types of mutations were examined: resistance to rifampin, which is acquired by point mutations in the *rpoB* gene, which encodes

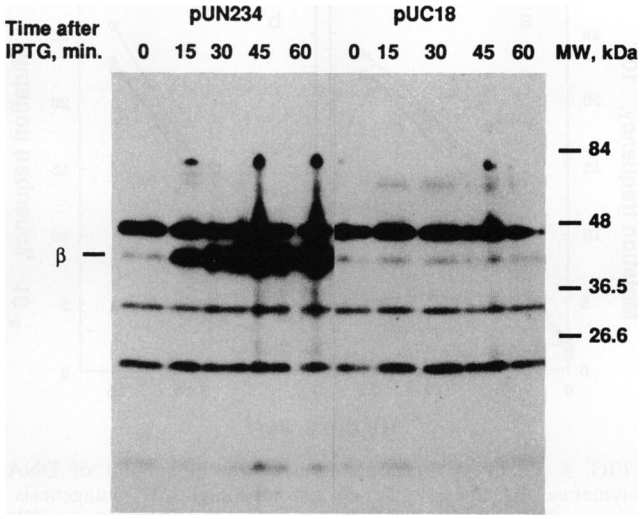

FIG. 1. Overproduction of the β subunit of DNA polymerase III holoenzyme in cells harboring plasmid pUN234. *E. coli* MC4100XL cells harboring various plasmids were treated with IPTG for the indicated periods. The amount of the β subunit of DNA polymerase III holoenzyme in the cells was analyzed as described in Materials and Methods by Western blot analysis of total protein extracts using anti-β subunit antibodies.

the β subunit of RNA polymerase; resistance to nalidixic acid, which is acquired usually by mutations in the *gyrA* gene, which encodes subunit A of DNA gyrase; and the reversion of a histidine auxotroph. As seen in Fig. 3, the presence of plasmid pUN234 caused a fivefold reduction in the frequency of UV-induced rifampin resistance (Rif^r) or nalidixic acid resistance (Nal^r) mutations when cells were plated in the presence of IPTG. In fact, the UV-induced Rif^r mutations were reduced even in the absence of IPTG and in the presence of glucose (Fig. 3a), consistent with the incomplete repression of *lacP* at 37°C. The effect of plasmid

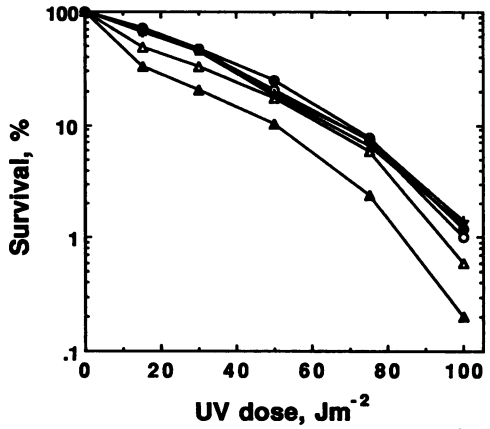

FIG. 2. Effect of overproduction of the β subunit of DNA polymerase III holoenzyme on survival of UV-irradiated cells. Cultures of *E. coli* MC4100XL harboring various plasmids were UV irradiated and assayed for survival as described in Materials and Methods. The cells contained plasmid pUC18 (circles) or pUN234 (*dnaN*$^+$) (triangles). Cells were grown in parallel on LB-glucose plates (empty symbols) and on LB-IPTG plates (filled symbols). Cells harboring plasmid pUN234FS (*dnaN*) were also examined on LB-glucose (+) or LB-IPTG (×) plates.

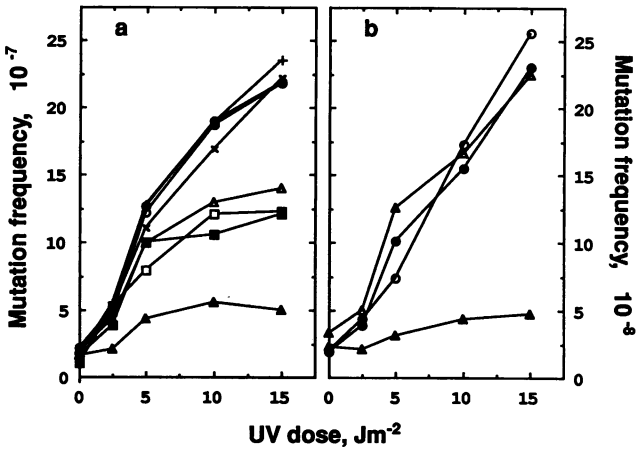

FIG. 3. Effect of overproduction of the β subunit of DNA polymerase III holoenzyme on chromosomal UV mutagenesis. Cultures of *E. coli* MC4100XL harboring various plasmids were UV irradiated and assayed for the production of Rif^r (a) or Nal^r (b) mutant colonies as described in Materials and Methods. Cells contained plasmid pUC18 (circles), pUN222 (*dnaN*$^+$) (squares), or pUN234 (*dnaN*$^+$) (triangles). Expression of mutations was examined in parallel on LB-glucose (empty symbols) and LB-IPTG (filled symbols) plates. (a) Cells harboring plasmid pUN234FS (*dnaN*) were examined for the production of Rif^r mutants on LB-glucose (+) or LB-IPTG (×) plates.

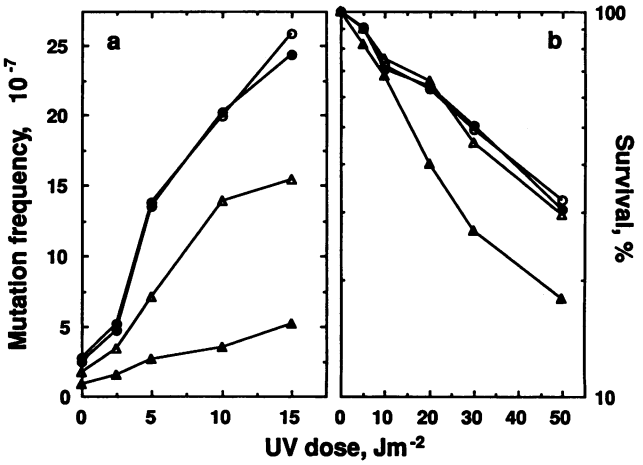

FIG. 4. Effect of overproduction of the β subunit of DNA polymerase III holoenzyme on UV survival and UV mutagenesis in *E. coli* AB1157XL. Cultures of cells harboring plasmid pUC18 (circles) or pUN234 (*dnaN*$^+$) (triangles) were UV irradiated and assayed for the production of UV-induced Rif^r mutants (a) and for UV survival (b) as described in Materials and Methods. Expression of mutations was examined in parallel on LB-glucose (empty symbols) and LB-IPTG (filled symbols) plates.

pUN222 was intermediate, and it was not influenced by IPTG treatment (Fig. 3a). It results probably from increased constitutive expression of β from the high-copy-number plasmid driven from its own promoter. The reduction in UV mutagenesis was not observed with vector pUC18 or with pUN234XFS, which carries a mutated *dnaN* gene (Fig. 3a). Nor was it observed with plasmid pUN234E or pUN234B, deletion derivatives of pUN234 which lack the N- or C-terminal portion of *dnaN*, respectively (data not shown). This rules out the possibility that the effect was mediated by *dnaN* mRNA or by sequences on plasmid pUN234 which titrated out a protein important for UV mutagenesis. Rather, the β subunit expressed from pUN234 was responsible for the reduction in UV mutagenesis. The same effects were observed with another strain, AB1157XL, indicating that they were not strain specific (Fig. 4). We also examined the effect of overproduction of the β subunit in a third mutagenesis system, assaying for the reversion of a histidine auxotroph. The effect of β on UV mutations in this system was even more pronounced than for the antibiotic resistance mutations, showing a 10-fold reduction in UV-induced His$^-$→His$^+$ mutations (Fig. 5).

Overproduction of the α subunit of PolIII holoenzyme does not affect UV mutagenesis. It has been previously reported that overproduction of the ε subunit of PolIII holoenzyme reduced UV mutagenesis (17). In order to examine an additional PolIII subunit, we have determined the effect of overproduction of the α subunit on UV mutagenesis. This was done by using cells harboring plasmid pMJ201, a pUC19 derivative that carries the *dnaE* (*polC*) gene encoding the α subunit of PolIII. Treatment of these cells with IPTG led to a 30-fold increase in the synthesis of the α subunit (Fig. 6). However, in contrast to the β subunit, overproduction of α had no effect on chromosomal UV mutagenesis (Fig. 7), nor did it affect UV survival (data not shown).

The effects of the β subunit are not mediated via excision repair. The β subunit may exert its effect indirectly by stimulating excision repair. This might reduce the amount of UV lesions, resulting in fewer mutations. This seems unlikely, since one would then expect that the overproduced β would increase UV survival, whereas the opposite was observed (Fig. 2). However, to test this possibility directly, the effect of overproduced β in a strain deficient in excision repair was examined. As seen in Fig. 8, overproduction of the β subunit resulted in UV sensitization and a reduction of UV mutagenesis similar to the effects observed with excision-proficient cells. Thus, the effects of β were not mediated via changes in excision repair.

A *mucAB* plasmid partially overcomes the inhibition of UV

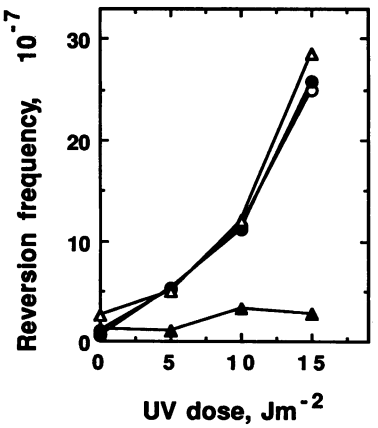

FIG. 5. Effect of overproduction of the β subunit of DNA polymerase III holoenzyme on UV-induced reversion of histidine auxotrophy. Cultures of AB1157XL harboring plasmid pUC18 (circles) or pUN234 (triangles) were UV irradiated and assayed for the production of His$^+$ revertants as described in Materials and Methods. Expression of mutations was examined in parallel on plates containing glucose (empty symbols) or IPTG (filled symbols).

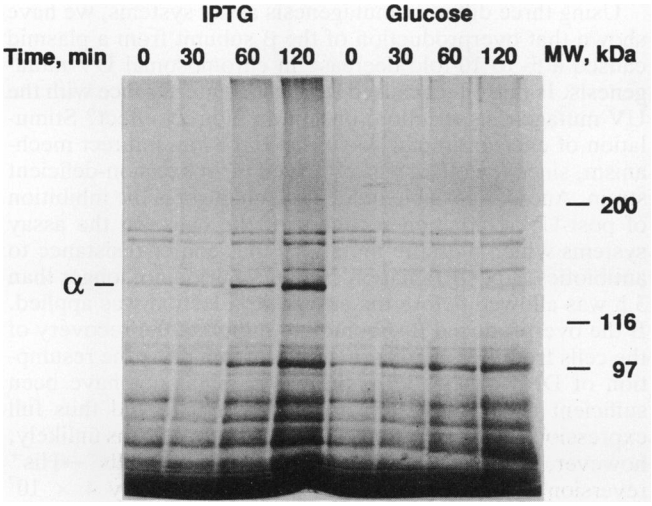

FIG. 6. Overproduction of the α subunit of DNA polymerase III holoenzyme in cells harboring plasmid pMJ201. *E. coli* MC4100XL cells harboring plasmid pMJ201 (*dnaE*$^+$) were treated with IPTG, and samples were pulse-labeled with [^{35}S]methionine at the indicated time points. Total protein extracts from these samples were fractionated by SDS-PAGE and radiolabeled proteins were visualized by autoradiography. The band corresponding to the α subunit is indicated.

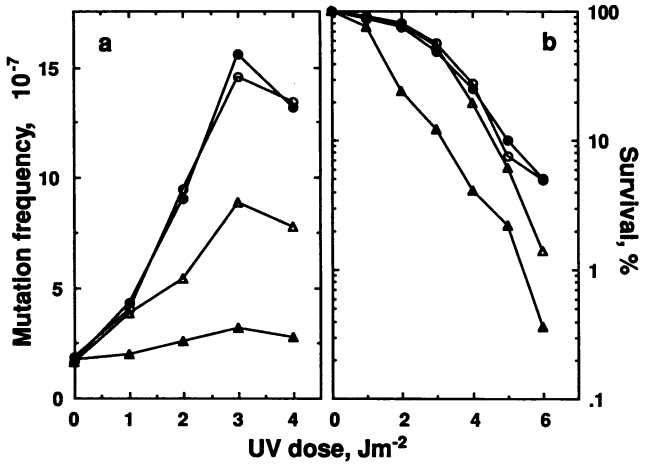

FIG. 8. Effect of overproduction of the β subunit of DNA polymerase III holoenzyme on UV survival and UV mutagenesis in an excision repair-deficient strain. *E. coli* AB1186XL (*uvrA6*) cells harboring plasmid pUC18 (circles) or pUN234 (*dnaN*$^+$) (triangles) were UV irradiated and assayed for the production of UV-induced Rif^r mutants (a) and for UV survival (b) as described in Materials and Methods. Expression of mutations was examined in parallel on LB-glucose (empty symbols) and LB-IPTG (filled symbols) plates.

mutagenesis caused by the overproduced β subunit. The UmuD and UmuC proteins are required for UV mutagenesis (41). If the effect of the β subunit was mediated by interfering with the *umu* pathway of UV mutagenesis, it is possible that increasing the amounts of UmuD and UmuC in the cell would reverse the effect of β. To test this possibility, we examined the UV mutability of cells harboring both plasmid pUN234 and plasmid pSE116, which carries the *umuDC* genes (14). As seen in Fig. 9a, the presence of pSE116 (together with the control plasmid pUC18) caused a slight increase in chromosomal UV mutagenesis (compare Fig. 9a and 3). It also caused a slight alleviation of the inhibition caused by plasmid pUN234. This is illustrated for the 10-J m^{-2} dose for cells plated in the presence of IPTG as follows (Fig. 9a). Cells with plasmids pUC18 and pSE116 showed a mutation frequency of 35 × 10^{-7}, compared with a frequency of 5 × 10^{-7} for cells harboring plasmid pUN234 and a control Δ*umuDC* deletion derivative of pSE116 (plasmid pSE992). This 7-fold inhibition of UV mutagenesis was

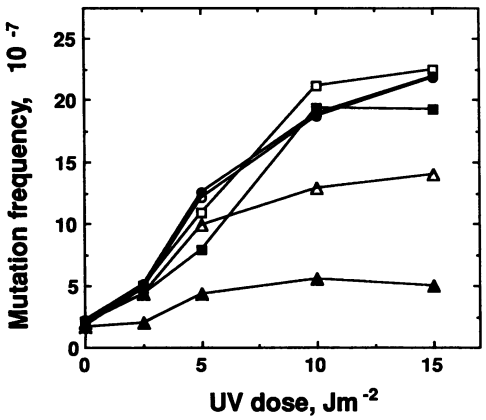

FIG. 7. Effect of overproduction of the α subunit of DNA polymerase III on chromosomal UV mutagenesis. *E. coli* MC4100XL cells harboring plasmid pUC18 (circles), pMJ201 (*dnaE*$^+$) (squares), or pUN234 (*dnaN*$^+$) (triangles) were UV irradiated and assayed for the production of UV-induced Rif^r mutants as described in Materials and Methods. Expression of mutations was examined in parallel on LB-glucose (empty symbols) and LB-IPTG (filled symbols) plates.

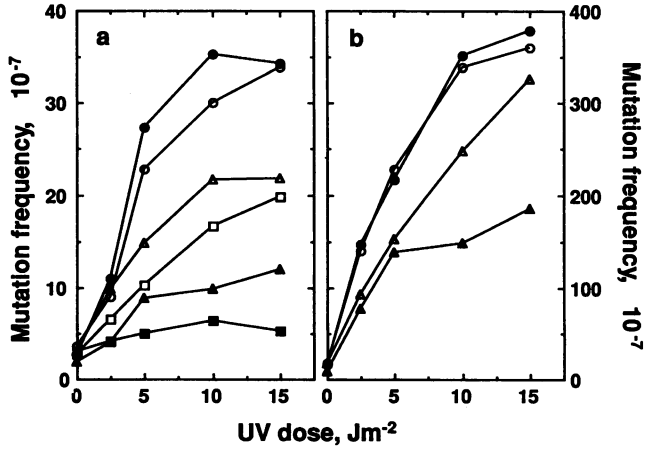

FIG. 9. Effect of *umuDC* and *mucAB* plasmids on chromosomal UV mutagenesis in cells that overexpress the β subunit of DNA polymerase III holoenzyme. Cultures of *E. coli* MC4100XL harboring the β subunit overproducer along with a *umuDC* plasmid (a) or a *mucAB* plasmid (b) were UV irradiated and assayed for the production of Rif^r mutant colonies as described in Materials and Methods. Cells containing the following plasmid combinations were assayed: pUC18 and pSE116 (*umuDC*$^+$) (circles), pUN234 (*dnaN*$^+$) and pSE992 (*umuDC*) (squares), and pUN234 (*dnaN*$^+$) and pSE116 (*umuDC*$^+$) (triangles) (a) and pUC18 and pGWK1 (*mucAB*$^+$) (circles) and pUN234 (*dnaN*$^+$) and pGWK1 (*mucAB*$^+$) (triangles) (b). Expression of mutations was examined in parallel on LB-glucose (empty symbols) and LB-IPTG (filled symbols) plates.

reduced to a 3.2-fold inhibition when plasmid pSE116 was present along with pUN234 (a mutation frequency of 11×10^{-7}). The reason that plasmid pSE116 was not very effective in alleviating the inhibition of UV mutagenesis might have resulted from insufficient quantities of the UmuDC proteins produced from this low-copy-number plasmid. We thus decided to try a plasmid carrying the *mucAB* genes (33), the plasmidic homologs of *umuDC*, whose products were found to be more effective in UV mutagenesis than UmuDC (5). The introduction of plasmid pGWK1, which carries the *mucAB* genes, into cells harboring control plasmid pUC18, led to a 10-fold stimulation of UV mutagenesis, consistent with previous reports on the activity of the MucAB proteins (5). When plasmid pGWK1 was introduced into cells harboring plasmid pUN234, the frequency of UV-induced Rif^r mutations in the presence of IPTG was only twofold lower than in the absence of pUN234 (Fig. 9b). Thus, in the presence of the *mucAB* genes on a plasmid, the inhibitory effect of overproduced β on UV mutagenesis was reduced, probably because of a strong countereffect of the MucAB proteins.

DISCUSSION

The β subunit of PolIII holoenzyme is present in excess over the polymerase molecules in *E. coli*. Thus, while a typical cell contains 15 to 20 molecules of DNA PolIII (20), the β subunit is present at 300 molecules per cell (11). The β subunit is loosely bound to the polymerase when the polymerase is free in solution; however, when the polymerase is complexed with primed single-stranded DNA, a stable polymerase-DNA complex, in which the β subunit is believed to serve as the clamp responsible for the tight enzyme-DNA association is formed (12, 21). On the basis of biochemical analysis of the replication of UV-irradiated DNA, we have previously suggested that the β subunit is not only a clamp but also serves as a "molecular sensor" which responds to DNA lesions by promoting dissociation of the polymerase from the DNA, thus causing termination of polymerization. At first glance, it seems that the clamp function should promote binding rather than dissociation at the sites of lesions, similar to its activity during replication of undamaged DNA. However, the clamp and sensor functions are not necessarily mutually exclusive. A possible model that incorporates the two functions is that of a conditional clamping activity of the β subunit that depends on an unperturbed primer-template structure. Any distortion caused by a DNA lesion loosens the clamp, causing dissociation.

Purified PolIII holoenzyme contains one β dimer per polymerase molecule. However, it has been shown that in the presence of excess β subunit, as in the in vivo situation, PolIII holoenzyme molecules with more than one β dimer are formed (21). It is not clear which form functions in vivo, and as far as catalytic efficiency is concerned, these forms are indistinguishable (21), implying similar clamp properties. The only difference reported so far is our finding that the β-poor PolIII holoenzyme is able to bypass UV lesions at a frequency of 20% during replication in vitro, whereas the β-rich polymerase is unable to do so (37). It thus appears that the β-rich polymerase has a stronger sensor activity which makes it stop at DNA lesions. On the basis of stoichiometry, it can be argued that the β-rich form prevails in vivo. This may be the reason why very little replicative bypass was observed in vivo when the SOS response was not induced (1).

Using three different mutagenesis assay systems, we have shown that overproduction of the β subunit from a plasmid caused a 5- to 10-fold decrease in chromosomal UV mutagenesis. Is this effect caused by a direct interference with the UV mutagenesis reaction, or is it an indirect effect? Stimulation of excision repair was ruled out as an indirect mechanism, since the effect was observed in an excision-deficient strain. Another possible indirect mechanism is the inhibition of post-UV irradiation recovery of the cells. In the assay systems which measure mutations that confer resistance to antibiotics, a postirradiation recovery period not longer than 3 h was allowed before the antibiotic selection was applied. If the overproduced β subunit had inhibited the recovery of the cells from UV irradiation (e.g., by inhibiting the resumption of DNA replication), these 3 h might not have been sufficient to allow full recovery of the cells and thus full expression of UV mutations. This possibility seems unlikely, however, on the basis of the results in the His$^-$→His$^+$ reversion system. In this system, approximately 4×10^7 cells are spread on a plate that contains a limited amount of histidine. This allows several cell divisions before the histidine is exhausted and the cells stop multiplying. From this point on, only mutants which acquired histidine prototrophy continue to grow and form colonies over the lawn of His$^-$ cells. In this assay system, the selection is applied not after a certain time but rather when the histidine in the plate is exhausted. For slowly growing cells, this occurs at a later time point than for rapidly growing cells, and in both cases the cells have a much longer postirradiation recovery period than in the antibiotic resistance systems. This argues strongly that the overproduced β subunit inhibits UV mutagenesis by directly interfering with the mutagenic process.

Consistent with current ideas about UV mutagenesis, we assume that under SOS conditions a specialized version of PolIII is formed. The composition of this putative "SOS polymerase" is not clear, but according to our model it must be different from the β-rich form of PolIII holoenzyme. It may be a derivative of the β-poor PolIII holoenzyme, and it may contain an SOS-inducible truncated form of β, recently discovered in our laboratory and termed β* (40). This SOS polymerase may interact with the mutagenesis-specific proteins UmuD and UmuC and with RecA, making it a specialized DNA polymerase complex designed to carry out bypass of lesions.

The equilibrium between free β and β complexed within PolIII holoenzyme provides rational grounds for experiments designed to analyze the effects of overproduction of β on UV survival and mutagenesis. The in vivo results presented above are consistent with the model derived from the in vitro studies. According to this model, artificial overproduction of β shifts the equilibrium towards the β-rich polymerase, thus sequestering most of the PolIII molecules in the replicative form and preventing or reducing the formation of the SOS polymerase form. This in turn reduces bypass synthesis, and thus UV mutagenesis is decreased. The function of the UmuDC or MucAB proteins is unknown, and thus the interpretation of experiments with plasmids expressing these proteins is not straightforward. They may be involved in the formation of the SOS polymerase (e.g., by direct complex formation), as indicated by their homology to the processivity factors of phage T4 DNA polymerase (3). In such a case, their ability to alleviate the inhibition imposed by excess β subunit can be interpreted as successful countercompetition which converts enough of the β-poor polymerase to the SOS form, thus enabling partial restoration of UV mutagenesis.

Why does overproduction of β reduce UV survival? The effect is not likely to be the result of inhibition of DNA excision repair, since the same effect was observed in excision-deficient cells. One possible explanation is based on the assumption that bypass synthesis contributes to the survival of UV-irradiated cells, as supported by the higher UV sensitivity of *umuC* strains (18). In such a case, overproduction of β, which reduces bypass synthesis, is expected to cause decreases in both UV mutagenesis and UV survival. Another possibility is that excess β interferes with post-UV irradiation resumption of DNA replication, but this point has to be investigated further.

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