The β subunit of DNA polymerase III holoenzyme of Escherichia coli is a 40.6-kDa protein that functions as a sliding DNA clamp (Stuckenberg, P. T., Studwell-Vaughan, P. S., and O'Donnell, M. (1993) J. Biol. Chem. 268, 11328–11334). It is responsible for tethering the polymerase to DNA and endowing it with the high processivity required for DNA replication. Here and in a companion study (Paz-Elizur, T., Skalker, R., Blumenstein, S., and Livneh, Z. (1996) J. Biol. Chem. 271, 2482–2490) we report that the dnaN gene, encoding the β subunit, contains an internal in-frame gene, termed dnaN*, that encodes a smaller form of the β subunit. The novel 26-kDa protein, termed β*, is UV-inducible, and when overexpressed from a plasmid under an inducible promoter, it increases up to 6-fold the UV resistance of E. coli cells. These findings suggest that the β* protein functions in a reaction associated with DNA repair or recovery of DNA replication in UV-irradiated cells.

UV irradiation of Escherichia coli cells produces in DNA primarily cyclobutyl pyrimidine dimers and pyrimidine-pyrimidone (6–4) adducts that are responsible for most of the mutagenic and inactivating effects of UV irradiation (1). The immediate cellular response to UV irradiation is an arrest of chromosome replication in order to allow a period of elimination of DNA damage by DNA repair mechanisms (2). Many of the genes known to be involved in these processes, such as uvrA, uvrB, recA, umuD, and umuC are regulated by the SOS regulatory network, whose primary function is to help the cell in coping with DNA damage (3, 4). However, UV irradiation induces also heat shock genes (5) and other genes (6) which affect the post-UV physiology of the cell.

We have previously examined in detail the replication of UV-irradiated DNA with purified proteins (7–10). These studies revealed that the β subunit of DNA polymerase III holoenzyme, the major replicase of the E. coli chromosome (11), limits the ability of the purified polymerase to bypass UV lesions during in vitro replication of UV-irradiated single-stranded DNA (10). Consistent with this result, overproduction of the β subunit from a plasmid caused a reduction in UV resistance and in UV mutagenesis of E. coli cells (12). This involvement of the β subunit in UV irradiation effects prompted us to examine whether it may be present in a different form in UV-irradiated cells. Here and in two companion studies (27, 28), we report that UV irradiation induces a shorter form of the β subunit that functions in vitro as an alternative DNA polymerase clamp. We suggest that this protein functions in DNA synthesis associated with post-UV recovery in E. coli.

**MATERIALS AND METHODS**

**Bacterial Strains**—The following E. coli K-12 strains were used in this study: MC4100(argF-lac205 araD139 rpsL150 thiA1 relA1 lacIq lacZD47K-12); MC4100XL, same as MC4100 but also F⁻: Tn10 laddc lacZAM15 proAB; AB1157XL (argE3 his4 leuB6 proA2 thr1 ara14 galK2 lacY1 mtl1 yhi1 tsx33 rpsL31 supE44 F−); MC4100(lacZAM15 proAB). Plasmids—Plasmid pUN234 carries the dnaN gene cloned under the lac promoter in plasmid pUC18 (12). Plasmid pUN234FS2 is a derivative of pUN234 containing a +4 insertion/frameshift mutation in the dnaN upstream of dnaN* (12). Plasmid pBSOW1 contains dnaN* expressed under the lac promoter in plasmid pBluescript SK− and, plasmid pBSW7 is similar to pBSOW1 except that dnaN was cloned in the opposite orientation. These plasmids were constructed by cloning, in two orientations, the EcoRV-(1871)–BanII-(2832) DNA fragment from plasmid pUN234 into the EcoRV site in plasmid pBluescript SK−.

**Proteins and Chemicals**—The β subunit of DNA polymerase III was purified as previously described (13). It was then fractionated by 10% SDS-PAGE, and a gel slice containing 250–500 μg of the protein was ground, mixed with complete Freund’s adjuvant, and injected into young female rabbits. The anti-β antibodies obtained were affinity-purified on purified β that was fixed onto a nitrocellulose membrane (Schleicher & Schuell, 0.2 μm) as described elsewhere (14). Purified β was prepared from an overproducing cell (28). Restriction nuclease were purchased from Pharmacia and from New England Biolabs. T4 DNA ligase was the product of Stratagene, and DNA polymerase I and calf alkaline phosphatase were from U. S. Biochemical Corp.

**UV Irradiation**—Cultures were UV-irradiated at 254 nm using a low pressure mercury germicidal lamp. The dose rate was 0.1–0.5 J m⁻² s⁻¹ as determined by a UV products radiometer equipped with a UVX-25 sensor.

**Analysis of β Expression in Cells Harboring dnaN and dnaN**—E. coli MC4100XL cells harboring plasmids with the dnaN or the dnaN* genes cloned under the lac promoter were treated with 0.5 mM IPTG to induce expression from the lac promoter. Total cellular proteins were fractionated by SDS-PAGE, transferred to a nitrocellulose membrane, and detected with the affinity-purified anti-β antibodies using enhanced chemiluminescence for detection (ECL, Amersham Corp.). The following plasmids were tested: pUN234, pUN234FS2, pUC18, pBSOW1, and pBSW7.

**Identification of the Cellular β**—MC4100 cells were grown in a M9 medium supplemented with 0.2% glucose, vitamin B₃, and the amino acids histidine, arginine, proline, threonine, and isoleucine (20 mg/liter each). The protein extracts from cells grown at the late logarithmic phase (OD₅₉₅ = 1.5) were fractionated by two-dimensional gel electrophoresis according to O’Farrell (15), after which they were transferred onto a nitrocellulose membrane and probed with anti-β antibodies using enhanced chemiluminescence for detection (ECL, Amersham Corp.).

**Induction of β**—UV Irradiation—E. coli MC4100 were grown to OD₅₉₅ = 0.3 in a minimal A medium supplemented with vitamin B₃, 0.2% glucose, and histidine, arginine, proline, threonine, and isoleucine.
sequence homologies raised the possibility of a UV-inducible, internal in-frame gene that expresses a shorter form the β subunit sliding DNA clamp.

A Plasmid Carrying the Intact dnaN Gene Does Not Express β* Unless the Expression of the β Subunit Is Inactivated—We examined whether plasmids carrying the entire dnaN gene direct the synthesis of β*. First we used plasmid pUN234 (12), which carries the intact dnaN gene cloned under the lac promoter. Since dnaN* is included within dnaN, we expected that β* would be expressed from this plasmid in addition to the β subunit. Cells harboring pUN234 were grown in parallel in medium containing either glucose, a catabolic repressor of the lac operon, or IPTG, a synthetic inducer of the lac operon. Total cellular protein was then fractionated by SDS-PAGE and immunoblotted with polyclonal antibodies against the β subunit. Since β* is identical to the C-terminal two-thirds of the β subunit, we expected a good cross-reactivity with β*. Purified β* that was prepared from an overproducing cell (28) served both as an electrophoretic marker and for the affinity purification of the polyclonal antibodies.

As can be seen in Fig. 2 (lane 4), the β subunit was overproduced; however, no protein of 26 kDa was detected. Puzzled by the inability of this plasmid to express β*, we examined its expression from plasmid pUN234F52, which contains a +4 insertion/frameshift mutation inside the dnaN gene upstream to the beginning of the dnaN* gene. As can be seen (Fig. 2, lane 6), the mutation essentially eliminated the synthesis of the β subunit from the plasmid, and at the same time a band of 26 kDa could be detected. This represents most likely β* synthesized from the mutated plasmid. A possible explanation is that the β subunit itself is a negative regulator of β* expression, and when present in large amounts, it inhibits the expression of β*.

This experiment also suggests that the 26-kDa protein was not formed by proteolysis of the β subunit either in vivo or during extract preparation and handling, otherwise it would have been expected to appear in lane 4. Notice that a 26-kDa protein was not observed also in lanes 2 or 3 in Fig. 2 suggesting that...
Its ability to direct the synthesis of cellular DNA polymerase III subunit which is needed constantly during the stationary phase of growth.

The beta subunit protein is expressed from a plasmid carrying the dnaN* gene cloned under the lac promoter. Total cellular proteins were fractionated by SDS-PAGE, transferred to a nitrocellulose membrane, and detected with affinity purified anti-beta antibodies using enhanced chemiluminescence as described under “Materials and Methods.” The detailed procedures are described under “Materials and Methods.” Lane M contains purified beta subunit and beta* as markers. Lane E contains an extract fractionated by one-dimensional SDS-PAGE. Arrows 1 and 2 mark beta* and the beta subunit, respectively.

The beta* protein is expressed from a plasmid carrying the dnaN* gene. We next cloned the dnaN* gene under the inducible lac promoter, to yield plasmid pBSOW1, and examined its ability to direct the synthesis of beta*. As can be seen in Fig. 3, extracts prepared from cells grown with IPTG (but not in its absence) contained a protein of 26 kDa that reacted with anti-beta antibodies. In contrast, extracts from cells with plasmid pBSW7, in which dnaN* was cloned in the opposite orientation to the lac promoter, did not contain beta*. Thus, the dnaN* gene directs the synthesis of beta* when present on a plasmid.

Identification of the Cellular beta*—In order to identify the cellular beta* protein, we probed Western blot analysis of a protein extract prepared from late logarithmic/early stationary phase E. coli MC4100 using affinity-purified antibodies. As can be seen in Fig. 4, a protein of 26 kDa reacted with the anti-beta antibodies, and exhibited an electrophoretic mobility identical to the overproduced and purified beta*. Extracts obtained from early log phase cells gave a weaker beta band (see Fig. 5), indicating that it is induced in the stationary phase. In order to verify that this is the cellular beta*, we analyzed it by immunoblotting. The detailed procedure is presented under “Materials and Methods.”

UV irradiation induces beta*—The induction of beta* was examined directly by immunoblot analysis using purified anti-beta antibodies. As can be seen in Fig. 5, UV irradiation caused an increase in the level of beta*, peaking at 1–1.5 h after irradiation. Based on densitometric tracing of the blot, the extent of induction was 7-fold, similar to the induction observed with dnaN*::lacZ translational gene fusions (27).

Overproduction of beta* increases UV resistance—What is the physiological role of beta*? The beta* protein is not a general substitute for the beta subunit which is needed constantly during the
Overproduction of β* increases UV resistance

Overnight cultures of AB1157XL or MC4100XL cells harboring the various plasmids were washed and grown in parallel to OD 595-0.5 in fresh medium under conditions in which the lac promoter was repressed (30°C in the presence of glucose) or fully induced (37°C in the presence of IPTG). The cells were then washed, resuspended in 10 mM Tris-HCl, pH 7.5, 0.15 mM NaCl, and UV-irradiated with agitation at the indicated UV doses. Following irradiation the cultures were diluted and spread on LB plates containing ampicillin and either 0.2% glucose or 0.5 mM IPTG, according to their growth in culture. Cells without plasmids were treated by the same procedure, except that ampicillin was omitted.

| Strain          | Survival at UV dose (J m⁻²) of | %     |
|-----------------|--------------------------------|-------|
|                 | 0                              | 50    | 75    | 100   | 125   |
| AB1157XL(pBSW1) | +IPTG                          | 100   | 33    | 13    | 2.0   | 0.18  |
|                 | +Glucose                       | 100   | 22    | 4.1   | 0.4   | 0.03  |
|                 | Survival increase, -fold       | 1     | 1.5   | 3.2   | 5.0   | 6.0   |
| AB1157XL(pBSW7) | +IPTG                          | 100   | 25    | 4.5   | 0.6   | 0.05  |
|                 | +Glucose                       | 100   | 16    | 3.0   | 0.45  | 0.03  |
|                 | Survival increase, -fold       | 1     | 1.6   | 1.5   | 1.3   | 1.6   |
| MC4100XL(pBROW1) | +IPTG                         | 100   | 31    | 5.5   | 0.8   | 0.08  |
|                 | +Glucose                       | 100   | 28    | 4.3   | 0.7   | 0.08  |
|                 | Survival increase, -fold       | 1     | 1.1   | 1.3   | 1.1   | 1     |
| MC4100XL(pBWSW7)| +IPTG                          | 100   | 33    | 12    | 1.3   | 0.09  |
|                 | +Glucose                       | 100   | 26    | 5.0   | 0.3   | 0.02  |
|                 | Survival increase, -fold       | 1     | 1.3   | 2.4   | 4.3   | 6.5   |
| MC4100XL        | +IPTG                          | 100   | 28    | 6     | 0.5   | 0.02  |
|                 | +Glucose                       | 100   | 38    | 9     | 0.5   | 0.02  |
|                 | Survival increase, -fold       | 1     | 0.7   | 0.7   | 1     | 1     |
| MC4100XL        | +Glucose                       | 100   | 26    | 6.4   | 0.6   | 0.02  |
|                 | Survival increase, -fold       | 1     | 0.8   | 0.8   | 0.9   | 0.7   |

UV-induced Shorter Form of β Subunit of DNA Polymerase III

* participants in a recovery process in the cell, which is limited by the amount of β*. Overproduction of β* would be expected to facilitate this recovery reaction, and thus increase UV resistance. Possible pathways to be affected are DNA repair or the reactivation of DNA replication (2).

It was recently shown by x-ray crystallography that the β subunit is composed of three structurally similar domains, and it dimerizes to form a hexagon-like ring (20). β* contains precisely none of the three domains of the β subunit, raising the possibility that it forms a trimeric alternative clamp that functions in the UV-irradiated cell with one of the DNA polymerases. Such a clamp activity is demonstrated in our companion study (28). The function of the β subunit is carried out in eukaryotes by the proliferating cell nuclear antigen (PCNA), which serves as the processivity clamp of DNA polymerase α (21). PCNA and the β subunit are structurally very similar, forming nearly identical hexagonal rings (22, 23). In contrast to the dimeric structure of the 40.6 kDa β subunit, PCNA is 29 kDa and forms a trimer (25). Thus β* bears resemblance to PCNA with a possible evolutionary link. In this context it is interesting to note that PCNA is the target for several regulatory mechanisms that coordinate the response of mammalian cells to UV irradiation (24, 25).

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