I. GENE EXPRESSION AND REGULATION*

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Tamar Paz-Elizur, Rami Skaliter, Sara Blumenstein, and Zvi Livneh‡
From the Department of Biochemistry, Weizmann Institute of Science, Rehovot 76100, Israel

The 40.6-kDa β subunit of DNA polymerase III of Escherichia coli is a sliding DNA clamp responsible for tethering the polymerase to DNA and ending it with high processivity (Stukenberg, P. T., Studwell-Vaughan, P. S., and O’Donnell, M. (1991) J. Biol. Chem. 266, 11328-11334). UV irradiation of E. coli induces a smaller 26-kDa form of the β subunit, termed β*, that, when overproduced from a plasmid, increases UV resistance of E. coli (Skaliter, R., Paz-Elizur, T., and Livneh, Z. (1996) J. Biol. Chem. 271, 2478-2481). Here we show that this protein is synthesized from a UV-inducible internal gene, termed dnaN*, that is located in-frame inside the coding region of dnaN, encoding the β subunit. The initiation codon and the Shine-Dalgarno sequence of dnaN* were identified by site-directed mutagenesis. The dnaN* transcript was shown to be induced upon treatment with nalidixic acid, and transcriptional dnaN*-cat gene fusions were UV inducible, suggesting induction of dnaN* at the transcriptional level. Analysis of translational dnaN*-lacZ gene fusions revealed that UV irradiation was abolished in strains carrying the recA56, lexA3, or ΔrpoH mutations, indicating involvement of both SOS and heat shock stress responses in the induction process. Expression of dnaN* represents a strategy of producing several proteins with related functional domains from a single gene.

UV irradiation of Escherichia coli cells leads to the formation of both mutagenic and inactivating DNA lesions (1). The cells respond by an immediate arrest of DNA replication, followed by a period of extensive DNA repair, that operates to eliminate DNA damage in order to prevent replication obstacles (2). These processes are controlled primarily by the SOS stress regulon, which involves more than 20 genes that are commonly regulated by the LexA repressor and the RecA activator (3, 4). However, UV irradiation induces changes in heat shock genes (5) and other genes (6) which affect the post-UV physiology of the cell. We have previously found that the β subunit of DNA polymerase III holoenzyme, the major replicase of E. coli, is required for UV survival (7). This led us to examine whether the small β subunit from a plasmid caused a reduction in UV resistance and in UV mutagenesis of E. coli cells (9).

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. We found that upon UV irradiation a smaller form of the β subunit, termed β*, was induced. When overproduced from a plasmid under the inducible lac promoter, β* caused up to a 6-fold increase in UV resistance of E. coli cells, suggesting a role in recovery from UV damage, e.g. by involvement in DNA repair or reactivation of DNA replication (48).

Smaller derivatives of proteins that are found in cells are frequently generated by proteolysis, as in the case of the mutagenesis protein UmuD* that is formed from UmuD by specific cleavage promoted by the RecA protein (10). Alternatively, the proteolytic fragments may have different functions than the full-size proteins. Proteolysis of several E. coli proteins, for example LexA repressor, the LexA repressor and the β subunit of DNA polymerase III (11), and a variety of other SOS genes, results in a smaller truncated protein that is transferred to the chromosomal DNA (12). The truncated protein is then thought to function independently of the chromosomal DNA (13).

EXPERIMENTAL PROCEDURES

Materials—The sources for materials used in these studies were as follows: isopropyl β-D-thiogalactopyranoside (IPTG), rifampicin, nalidixic acid, p-nitrophenyl-β-D-galactoside, 5-bromo-4-chloro-3-indolyl-β-D-galactoside, ampicillin, spermidine, sodium deoxycholate, Tween 20, Nonident P-40, and n-butyryl CoA, Sigma; radiolabeled nucleotides, Amersham Corp.; [3H]chloramphenicol (30.7 Ci/mmol), DuPont NEN; uracil, ICN; nitrocellulose membrane, Schleicher & Schuell; M9 medium, minimal A medium, and LB were prepared as described by Miller (11).

Proteins—LexA repressor and the β subunit of DNA polymerase III were purified as described by Little (12) and Johanson et al. (13), respectively. β* was purified from an overproducer strain that was constructed in our laboratory (49). Anti-β antibodies were affinity-purified on β immobilized on nitrocellulose as previously described (48). αβ RNA polymerase was a gift from R. Burgess (University of Wisconsin). DNA polymerase I, T4 DNA ligase, alkaline phosphatase, T7 RNA polymerase, E. coli RNA polymerase, RNase T1, and RNase A, and bovine serum albumin were purchased from Boehringer Mannheim. Restriction endonucleases were purchased from New England Biolabs. Polynucleotide kinase was from U. S. Biochemical Corp. Proteinase K, chicken egg white lysozyme, and anti-β-galactosidase antibody were obtained from Sigma.

Bacterial Strains and Bacteriophages—The bacterial strains used in this study are listed in Table I. E. coli R40NL8 was obtained by removing the imm21 prophage from E. coli R40 (14) by superinfection with a heterocommune λ derivative, λbx2 (λ immunity). Phage λbx2 has a deletion at the att site so it cannot integrate into the chromosome; rather it enters the lytic pathway, and can supply the proteins needed for DNA replication and repair.

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‡ To whom all correspondence should be addressed. Tel.: 972-8-343203; Fax: 972-8-9344169; E-mail: BCLIVNEH@WEIZMANN.WEIZMANN.AC.IL.
for excision of the λ prophage from the chromosome. The reduced level of heat shock proteins (that are needed for the life cycle of λ) slows down the lytic infection by λb2. This raises the probability of obtaining colonies of λ-free E. coli R40 cells as a result of asymmetric segregation during cell division that has occurred after λ excision. E. coli R40NL8 was sensitive to all types of antibiotics except ampicillin, as expected from a non-lysogen, and resistant to chloramphenicol. The R40NL8 prophage from the chromosome. The reduced level of heat shock proteins (that are needed for the life cycle of λ) slows down the lytic infection by λb2. This raises the probability of obtaining colonies of λ-free E. coli R40 cells as a result of asymmetric segregation during cell division that has occurred after λ excision. E. coli R40NL8 was sensitive to all types of antibiotics except ampicillin, as expected from a non-lysogen, and resistant to chloramphenicol.

**Construction of Plasmids—** The plasmids used in this study are presented in Table I. Plasmid pRPfH11 that served as a template for the synthesis of the dnaN* riboprobe is a pBluescript SK− derivative in which the HpaI-(1942)–FspI(2142) dnaN DNA fragment was cloned in an orientation opposite to the T7 RNA polymerase promoter. Plasmid pCAT was derived from plasmid pCM4, a pBR327 derivative containing the coding sequence of the cat gene downstream to the tet promoter (Fig. 1). The BamHI site located at the 3′ end of the cat gene was eliminated by a partial digestion of plasmid pCM4 to full-length linear DNA, followed by filling-in of the termini and self-ligation to yield plasmid pCM5. The T7 promoter of E. coli was isolated from plasmid pBS15 (15) by digesting with restriction endonucleases HindIII and MboI, to produce a 326-bp fragment, followed by further digestion with restriction endonucleases AhalI to generate the 247-bp HindIII-AhalI fragment. It was cloned into the Aall site of pCM5, to yield plasmid pCAT. Plasmid pCAT was the parent for the various mutants described in this work, which was obtained on 1.5- or 2-kb fragments from the M13 clones by cleavage with MboI and HindIII, and cloned into the Smal site of plasmid pUC18 under the control of the lacUV5 promoter. This yielded plasmids pCM13, pCM2, pCR1, and pCRM that carry the wild-type, Met1, Met2, SD-5, and SD-4 sequences, respectively. The same fragments were also used to produce plasmids and cloned into the EcorV site in plasmid pBluescript SK− in two orientations, yielding two series of plasmids: pBSW7 (wild-type), pBSM11 (Met1), pBSM21 (Met2), pBSR51 (SD-5), and pBSR51(MD) contained the dnaN* fragments from the phage T7 promoter, whereas plasmids pBSOW1 (wild-type), pBSO11 (Met1), pBSM22 (Met2), pBSR51 (SD-5), and pBSR51(MD) contained the same fragments as the opposite orientation expressed from the lac promoter. The BamHI-(719)–HaeIII(1130) segments carrying the lac promoter were deleted from plasmids pBSW7, pBSM11, pBSM21, pBSR51, and pBSR51, yielding plasmids pNLW11, pNLW11, pNLW51, pNLRS11, and pNLR51, respectively.

**Bacterial strains—** The bacterial strains used in this study are presented in Table I. The strains used in this study were obtained from the American Type Culture Collection (Manassas, VA), the Center for Genetic Analysis (Madison, WI), and the laboratory of one of the authors. The laboratory of one of the authors. The coordinates of the cat gene and cloning it into the Smal site of pCM1853 (SD-5) to yield A*CAA*G CAA CGA TG-3′ of the lacZ gene was eliminated by a partial digestion of plasmid pCM1853 to full-length linear DNA, followed by filling-in of the termini and self-ligation to yield plasmid pCM1853. The T7 promoter of E. coli was isolated from plasmid pBS15 (15) by digesting with restriction endonucleases HindIII and MboI, to produce a 326-bp fragment, followed by further digestion with restriction endonucleases AhalI to generate the 247-bp HindIII-AhalI fragment. It was cloned into the Aall site of pCM5, to yield plasmid pCAT. Plasmid pCAT was the parent for the various mutants described in this work, which was obtained on 1.5- or 2-kb fragments from the M13 clones by cleavage with MboI and HindIII, and cloned into the Smal site of plasmid pUC18 under the control of the lacUV5 promoter. This yielded plasmids pCM13, pCM2, pCR1, and pCRM that carry the wild-type, Met1, Met2, SD-5, and SD-4 sequences, respectively. The same fragments were also used to produce plasmids and cloned into the EcorV site in plasmid pBluescript SK− in two orientations, yielding two series of plasmids: pBSW7 (wild-type), pBSM11 (Met1), pBSM21 (Met2), pBSR51 (SD-5), and pBSR51(MD) contained the dnaN* fragments from the phage T7 promoter, whereas plasmids pBSOW1 (wild-type), pBSO11 (Met1), pBSM22 (Met2), pBSR51 (SD-5), and pBSR51(MD) contained the same fragments in the opposite orientation expressed from the lac promoter. The BamHI-(719)–HaeIII(1130) segments carrying the lac promoter were deleted from plasmids pBSW7, pBSM11, pBSM21, pBSR51, and pBSR51, yielding plasmids pNLW11, pNLW11, pNLW51, pNLRS11, and pNLR51, respectively.

**dnaN* Gene Expression and Regulation—** The kinetics of degradation of dnaN* transcripts was monitored by Northern blot analysis, as described previously (44). The kinetics of degradation of dnaN* transcripts was monitored by Northern blot analysis, as described previously (44). The kinetics of degradation of dnaN* transcripts was monitored by Northern blot analysis, as described previously (44).
pCAT was derived from pCM4 by eliminating the side of NaOH according to Bradford (21). Aliquots containing 5 μm promoter were grown in LB medium supplemented with ampicillin (100 μg/ml) and glucose (0.2%) at 30 °C to OD₅₉₅ = 0.5. The cells were induced with IPTG (0.5 mM) for 1.5 h at 30°C, after which they were harvested by centrifugation, washed once with Tris-HCl, pH 7.5, and resuspended in liquid nitrogen. Total RNA was isolated as described elsewhere (22) and analyzed by RNA gel electrophoresis. Analysis of transcription initiation sites was performed by the RNase protection assay as described (22). A hundred μg of RNA were hybridized with the riboprobe at 45°C for 12 h to allow annealing of the riboprobe to the specific RNA. The mixture was heated at 85°C for 5 min, and then it was incubated at 45°C for 12 h to allow annealing of the riboprobe to the specific RNA. The mixture was then boiled to remove unhybridized riboprobe.

### Table II

| Name     | Vector        | Description                        | Source       |
|----------|---------------|------------------------------------|--------------|
| pCW3     | pUC18         | dnaN under lacP                     | This study   |
| pCM2     | pUC18         | dnaN (Met2) under lacP              | This study   |
| pCM13    | pUC18         | dnaN (Met1) under lacP              | This study   |
| pCRM     | pUC18         | dnaN (SD-M) under lacP              | This study   |
| pCR5     | pUC18         | dnaN (SD-S) under lacP              | This study   |
| pBSW7    | pBluescript SK⁺ | dnaN⁺ under T7 promoter            | This study   |
| pBSM11   | pBluescript SK⁺ | dnaN⁺ (Met1) under T7 promoter     | This study   |
| pBSM21   | pBluescript SK⁺ | dnaN⁺ (Met2) under T7 promoter     | This study   |
| pBSR1    | pBluescript SK⁻ | dnaN⁻ under T7 promoter            | This study   |
| pNLM1    | pBluescript SK⁺ | dnaN⁺ under T7 promoter; ΔlacP      | This study   |
| pNLM2    | pBluescript SK⁺ | dnaN⁺ (Met1) under T7 promoter; ΔlacP | This study   |
| pNLM3    | pBluescript SK⁺ | dnaN⁺ (Met2) under T7 promoter; ΔlacP | This study   |
| pNLS1    | pBluescript SK⁺ | dnaN⁺ (SD-M) under T7 promoter; ΔlacP | This study   |
| pNLS2    | pBluescript SK⁺ | dnaN⁺ (SD-S) under T7 promoter; ΔlacP | This study   |
| pCM4     | pBR327        | cat under tet promoter              | Pharmacia    |
| pCMB     | pBR327        | 3' cat BamHI site in pCM4 eliminated | This study   |
| pCAT     | pCMB          | Terminator upstream tet promoter    | This study   |
| pNCB17   | pCAT          | dnaN-cat fusion                     | This study   |
| pNCH6    | pCAT          | dnaN⁺-cat fusion                    | This study   |
| pNC14    | pCAT          | dnaN⁻-cat fusion                    | This study   |
| pPC1     | pCAT          | P₅-cat fusion                       | This study   |
| pRC5     | pCAT          | recA-cat fusion                     | This study   |
| pMC1403  | pMC1403       | Parent for lacZ fusions             | M. Casadaban (16) |
| pH5A2    | pMC1403       | dnaA-lacZ translational fusion      | This study   |
| pH5C6    | pMC1403       | dnaA-lacZ translational fusion      | This study   |
| pNB3     | pMC1403       | dnaN⁺-lacZ translational fusion     | This study   |
| pSB6     | pMC1403       | dnaN⁻-lacZ translational fusion     | This study   |
| pTEN5    | pMC1403       | dnaN⁻-lacZ translational fusion     | This study   |
| pACYC184 | pACYC184      | Tet⁴ cap⁻ ori p15A                   | S. Cohen (20) |
| pCM1     | pBR322        | Chloramphenicol derivative of pFN97 | This study   |
| pFN97    | pBR322        | Carries rpoH and bla                | F. Neidhard (19) |
| pJL59    | pUC18         | Overproducer of LexA                | J. Little (47) |
| pPHF11   | pBluescript SK⁺ | Template of riboprobe for dnaN⁺     | This study   |
| pUN121   | pBluescript SK⁺ | Source for phage λ cl gene          | A. Zamir (18) |

### Fig. 1

**Vectors used to construct cat gene fusions.** Plasmid pCAT was derived from pCM4 by eliminating the BamHI site at the 3' side of cat, and by inserting the T₇-rmb transcription terminator into the AattI site upstream to cat. Transcriptional gene fusions to cat were constructed by replacing the tet promoter (P) located on the EcoRV-ClaI fragment with the promoter to be studied. See “Experimental Procedures” for details.
was UV-irradiated and returned for further growth at 37 °C. Usually a UV dose of 30 J m⁻² was used. Samples of 1.5-6 ml were withdrawn at various time points, sedimented, and resuspended in an extraction buffer (50 mM Tris HCl, pH 7.5, 30 mM dithiothreitol). The cell suspension was then frozen in liquid nitrogen, thawed, and sonicated for 30 s. The mixture was centrifuged and the concentration of the soluble protein fraction was measured according Bradford (21). CAT activity was assayed essentially as previously described (23), based on the ability of CAT to transfer a butyryl residue from butyryl-CoA to one or two hydroxyl residues on the chloramphenicol molecule, thus causing its inactivation. Butyrylation of [³H]chloramphenicol was followed by extraction of [³H]chloramphenicol (preextracted twice with xylene), 25 μl of 2:1 tetramethylpentadecane:xylene, and the mixture was centrifuged and the concentration of the soluble protein fraction was then measured according Bradford. CAT activity was then determined by liquid scintillation counting.

RESULTS

Identification of the ATG Initiation Codon and the Shine-Dalgarno Sequence of dnaN*—The DNA sequence of the begin- ning of the dnaN* gene is shown in Fig. 2, including the Shine-Dalgarno sequence of dnaN* gene. In addition, the two recF promoters (P1 and P2) and the Pα promoter located inside dnaN/dnaN* and several cleavage sites of restriction nucleases are shown.

2043ATG serves as the initiation codon of dnaN*. In order to examine this possibility we have mutated each of the ATG codons into a CTG codon by site-directed mutagenesis. The mutated dnaN* genes were cloned under the strong phage T7 promoter in plasmid pBluescript SK⁺. E. coli BL21(ADE3) cells harboring plasmid pNLW1 (wild-type dnaN*), pNLW11 (2043C*GT mutation, Met1), or pNLW12 (2076C*GT mutation, Met2) were grown to OD₅₉₅ = 0.4 in minimal medium supplemented with ampicillin, MgSO₄ and glucose. Expression of dnaN* was turned-on by the addition of IPTG, followed by the addition of rifampicin to inhibit transcription by the host RNA polymerase. Newly synthesized proteins were pulse-labeled with [³S]methionine and analyzed by SDS-PAGE followed by fluorography. Lower panel, Qualification of the fluorogram shown in A was done by scanning with a Molecular Dynamics 300A computing densitometer. Full squares, Wt; empty squares, Met2; circles, Met1.

Fig. 2. The 5'-region of the dnaN* gene. The dnaN* promoter, its Shine-Dalgarno sequence (SD), its 2043ATG initiation codon, and codon 2076ATG are indicated. In addition, the two recF promoters (P1 and P2) and the Pα promoter located inside dnaN/dnaN* and several cleavage sites of restriction nucleases are shown.
nine. From the decay in the amount of radiolabeled βn*, its half-life is approximately 40 min (Fig. 4), much higher than the time scale used to estimate metabolic rates of synthesis. Thus, βn* is relatively a stable protein, and its degradation is not expected to affect significantly the measurements of its synthesis.

Mutating 2076ATG (Met2) had essentially no effect on the rate of synthesis of βn* (Fig. 3). In contrast, mutating 2043ATG (Met1) caused a 3-fold reduction in the rate of synthesis of βn* (Fig. 3), suggesting that 2043ATG is the initiation codon of dnaN*

To further support this conclusion, we have constructed another set of plasmids, in which the dnaN* gene was cloned under the lac promoter in plasmid pUC18. In this case we detected βn* by Western blot analysis of cell extracts, using affinity-purified anti-β antibodies. As can be seen in Fig. 5 (lanes 7–12), when the lac promoter was repressed by glucose, βn* was not produced. Upon induction by IPTG, the dnaN* plasmid yielded two products, a major product that comigrated with a sample of βn* purified from an overproducing cell and a minor product that migrated slightly faster (Fig. 5, lanes 7–12), consistent with the 5′ termini of the βn* transcript. Thus, induction of dnaN* is controlled directly by LexA, the global SOS repressor.

The assignment of 2043ATG as the initiation codon of dnaN* pointed to the GCAGG sequence as a likely Shine-Dalgarno sequence involved in ribosome binding. In order to examine this possibility we prepared two Shine-Dalgarno double mutants: GCA GG → ACA AG (SD-S) and GCA GG → GCA AA (SD-M). As can be seen in Fig. 5 (lanes 5 and 6), both mutants exhibited reduced expression of dnaN*, consistent with the suggested role of the GCAGG sequence in ribosome binding.

The dnaN* Transcript Is Induced by Nalidixic Acid—Total RNA was isolated from E. coli cells, and the 5′ termini of mRNAs initiating at the promoter region of dnaN* were analyzed using the RNase protection techniques. Several transcription initiation sites could be detected in the region analyzed, including the major recF transcript initiating at promoter P1, and a fully protected RNA probe, which represents the overlapping dnaN mRNA (Fig. 6, Pn). In the dnaN* promoter region, a band of approximately 130 bases was detected, suggesting that transcription of dnaN* starts near position 2013 (Fig. 2).

The promoter region of the dnaN* gene contains the sequence 5′-CTCTTTATCACATCCGACGG-3′ (positions 1960–1979; Fig. 2), resembling the consensus sequence of the binding site of the LexA repressor, 5′-NNCTGTNTncnNCAGNN-3′ (3). The most conserved 8 nucleotides are present in the dnaN* box-like sequence, including the inverted repeat CGCTGTCTACCCTGCCAGCG of which in our case is part of a pentanucleotide inverted repeat, CGCTGTCTACCCTGCCAGCG. If indeed this sequence binds LexA, it is expected that the gene will be inducible by agents that induce the SOS regulon. In agreement with such a prediction, UV irradiation of E. coli cells was found to cause induction of βn*, the dnaN* gene product (48). As can be seen in Fig. 6, treatment of cells with nalidixic acid, a potent inducer of the SOS and the heat shock responses, caused a 4–5-fold induction in the dnaN* transcript. Thus, induction of dnaN* expression is regulated, at least in part, at the transcriptional level.

In order to examine whether dnaN* is controlled directly by LexA, the global SOS repressor, we studied the binding of purified LexA repressor to the promoter region of dnaN*, using the gel mobility shift assay (22). Binding of LexA to the promoter region of recA has been demonstrated by this technique (24). Indeed, the LexA protein caused specific retardation of a 148-bp MspI restriction DNA fragment carrying the recA promoter which served as a positive control (data not shown). However, we could not to detect any specific binding of LexA to the dnaN* promoter region under a variety of condition (data not shown). This suggests that the inducibility of the dnaN* gene is not regulated directly by LexA.
were digested with RNase A and RNase T1, then treated with protein-labeled RNA probe transcribed from plasmid pRPHF11. The hybrids of cellular RNA was extracted from MC4100 wild-type cells at the indicated time points after irradiation as described under “Experimental Procedures.” The inducing UV doses were 0 (open circles); 2 J m⁻² (black squares); 5 J m⁻² (white triangles); 15 J m⁻² (black circles); 30 J m⁻² (white squares).

Procedures."

The region of the recF promoters located inside the dnaN* gene contains an antisense promoter, termed Pᵣ (25) (Fig. 2). The transcript directed by this promoter is complementary to mRNAs initiating inside the dnaN* gene.

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DNA\(^\text{A}\)–lacZ Translational Gene Fusions Are UV-inducible in strains KY700, KY703, and KY705, respectively. The inducing UV doses were 60, 5, and 1 J m\(^{-2}\) for strains KY700, KY703, and KY705, respectively.

The control fusion of the DNA* gene did show UV induction (Fig. 8). Plasmid pTEN5 is the only DNA* gene fusion plasmid that did not contain the two recF promoters that are located inside DNA*. Thus, in plasmid pPC1 cat is transcribed from the antisense promoter P\(_x\) and in the control plasmid pNCH20 there is no known promoter to transcribe cat.

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indeed due to the absence of the establishment that the loss of UV-inducibility in strain R40NL8 was type parent. As seen in Fig. 12 lacZ was observed before (28). This may be the result of the fact that the active structure of β-galactosidase is a tetramer (29), and that oligomerization of the fused β-galactosidase molecules might be slow, particularly when their concentration is low. Indeed, when the induction of the β*-β-galactosidase protein was examined at the protein level, by Western blot analysis using polyclonal antibodies against β-galactosidase, maximal induction of β-galactosidase occurred approximately 60–90 min after UV irradiation (Fig. 11). This time period is close to the time of induction of the β* protein (48). This result shows that the UV-induced increase in the activity of β-galactosidase was indeed due to an increase in the synthesis of the enzyme, and it is consistent with the suggestion that the slower kinetics of induction of the activity of the fused enzyme was due to the slow rate of assembly of the active tetrameric structure.

dnaN+ is under the Control of the Heat Shock Response—UV irradiation and nalidixic acid induce in E. coli both the SOS and heat shock responses (5). In order to test the involvement of the heat shock response in UV induction of dnaN we assayed a dnaN*-lacZ fusion gene in cells which lack the heat shock σ32 subunit. E. coli R40NL8 is a derivative of E. coli MC4100 that has a deletion in rpoH, the gene encoding σ32. Because σ32 is essential for growth at temperatures above 20°C, R40NL8 (like its parent R40) (14) contains a suppressor mutation that causes overproduction of groE, and thus enables it to grow at 37°C (but not at 42.5°C). The kinetics of β-galactosidase induction from the dnaN*-lacZ gene fusion was determined after UV-irradiation of the ΔrpoH30 mutant and its isogenic wild-type parent. As seen in Fig. 12B, there was no UV induction of β-galactosidase activity from pTEN5 in cells which lack the heat shock σ32 subunit, whereas a 10-fold increase in activity was found in the isogenic wild-type cells (Fig. 12A). Interestingly, the basal level of β-galactosidase from plasmid pTEN5 was the same in the wild-type and ΔrpoH30 strains. In order to establish that the loss of UV inducibility in strain R40NL8 was indeed due to the absence of the σ32 subunit, we supplied it in trans from plasmid pCM+1. This plasmid did not affect the UV inducibility of the dnaN*-lacZ fusion from pTEN5 in the wild-type parent MC4100 (Fig. 12A). However, introduction of pCM+1 into R40NL8 harboring pTEN5, rendered the dnaN*-lacZ fusion UV-inducible again (Fig. 12B). The extent of UV-induction of the dnaN*-lacZ fusion was 3-fold lower than in the wild-type strain. This may be due to the fact that the strains are not fully isogenic (i.e. R40NL8 but not MC4100 overproduce groE). In any case, it is clear that introducing the plasmid that expressed σ32 made the dnaN*-lacZ gene fusion UV-inducible again, suggesting that dnaN+ is controlled by the heat shock activator, the RNA polymerase σ32 subunit.

We attempted transcribe dnaN+ in vitro using purified RNA polymerase. We were unable to detect any in vitro initiation of transcription from the dnaN+ promoter using either the regular σ70 RNA polymerase, or the heat shock-specific σ32 RNA polymerase, although the recA transcripts were observed (data not shown). Thus, it seems that transcription of dnaN+ requires additional factors, or possibly another σ subunit. Possible candidates are σ24, which is specific for some heat-induced genes (30, 31), and σ54, which transcribes stationary phase genes (32, 33). Consistent with such a possibility we found a higher amount of β* in stationary phase cells (48).

DISCUSSION

We have previously shown that a smaller form of the β subunit of DNA polymerase III holoenzyme is induced in E. coli by UV irradiation (48). Such a protein can be generated by proteolytic processing, like the mutagensis protein UmuD*, that is formed from UmuD by specific proteolysis promoted by the RecA protein (10). Alternatively, the protein can be translated from the dnaN mRNA by a de novo translational start, or it can be expressed from an internal in-frame gene.

The data presented here suggests that β* is expressed from an internal in-frame gene termed dnaN+. This is based on the following observations. 1) The ATG initiation codon of dnaN+ and its Shine-Dalgarno sequence were identified by site-directed mutagenesis. 2) A transcription initiation site was mapped inside dnaN, upstream to a Shine-Dalgarno sequence. 3) Plasmids carrying the dnaN+ gene expressed β*. 4) When cloned into a plasmid, the promoter region of dnaN* directed the expression of a promoter-less cat gene. 5) When the control region of dnaN*, including the beginning of its coding region, was fused in-frame to a portion of the lacZ gene lacking all transcriptional and translational control elements as well as its first 8 codons, it directed the synthesis of a fused β*-β-galactosidase protein.

The expression of dnaN+ is complex and is likely to be regulated via several mechanisms. Transcription of dnaN+ was not observed in vitro using either σ70 or σ32 RNA polymerase, suggesting that another transcription factor is required. Internal initiation of translation at the dnaN+ ATG initiation codon on the intact dnaN mRNA seems to be very inefficient. This is
indicated by the fact that overexpressing dnaN mRNA from the lac promoter on a plasmid did not yield any detectable βp. Only after introducing a frameshift mutation into dnaN, upstream to dnaN*, that eliminated overproduction of the β subunit, expression of βp was observed from dnaN mRNA (48). Thus, it seems that, under normal conditions, synthesis of βp from dnaN mRNA is strongly inhibited, e.g. due to its engagement in translation of the β subunit or due to direct inhibition by the β subunit. The antisense transcript originating from Pm may also be involved in the down-regulation of the expression of dnaN*.

UV induction of dnaN* is regulated at the transcriptional level, and subjected to control by both the SOS and heat shock responses, as indicated by the dependence of UV induction of dnaN*-lacZ gene fusions on rea, lexA, and rpoH. However, this dual regulation is indirect, since dnaN* did not bind LexA, and it was not transcribed by α32 RNA polymerase. Thus, another factor(s) that is controlled by these major stress responses, is responsible for the UV induction of dnaN*.

The role of the SOS box-like sequence in the promoter region of dnaN* is puzzling. It may represent a degenerated LexA binding site, or it may be a coincidental homology of no functional role, especially since its 5’-half was found to be dispensable for UV induction of dnaN*-lacZ gene fusions. It should be noted that if the sequence 5’-TACTGTATATATATACAGTA-3’ is taken as the consensus LexA binding site, then based of the differences between it and the dnaN* SOS box-like sequence (34), the latter is predicted to have no specific binding to LexA. Similar SOS box-like sequences, that did not bind LexA, were found in the phr gene, encoding DNA photolyase (35), and in the uvrC gene, encoding a subunit of the UvrABC repair excinuclease (36); however, their significance remains unclear. In addition to dnaN* at least three other genes are inducible by DNA-damaging agents in a rea- and lexA-independent pathway, but are not directly regulated by LexA: The phr gene mentioned above (35), the dnaQ gene encoding the proofreading ε subunit of DNA polymerase III (37), and the dnaN gene (37, 38). The mechanism of this regulation is unknown yet, representing another layer of complexity of the SOS regulatory network. It may be performed by a factor which is by itself repressed directly by LexA.

Genes whose coding sequences overlap are not rare; however, extensively overlapping genes, or genes nested within other genes, are not common in the chromosome (39). A well-documented case is the phage T7 gene gp4, encoding a helicase-primease. The gene encodes two proteins of 63 and 56 kDa, the latter generated by an internal in-frame start site (40). The dnaN gene encodes two subunits of DNA polymerase III holoenzyme: γ and γ′. They both start at the same site, but γ is terminated before γ′ by a mechanism of ribosomal frameshifting, leading to the production of proteins of 47.5 and 71 kDa (7). The expression of the internal dnaN* gene produces a protein that lacks precisely one of the three repeating domains of the β subunit. In this respect, it belongs to a family of mechanisms such as alternative splicing, that produce from a single gene more than one protein, differing by one or more defined functional domains. Such mechanisms generate a protein (or more) with a subset of the properties of the parental intact protein. They might be required to fulfill biochemically similar reactions under different conditions, or with conjunction with different counterpart proteins. Such are the cases of the dnaN gene and the T7 gp4 genes. The intact β subunit forms a β2 ring-shaped sliding DNA clamp, that confers high processivity on DNA polymerase III holoenzyme by tethering it to the DNA (41, 42). As shown in a companion study (49), βp forms an alternative DNA clamp for DNA polymerase III that may have a specialized function connected to DNA synthesis in the UV-irradiated cell. The increase in UV resistance caused by overproducing βp is consistent with such a model (48).
β*, a UV-inducible Smaller Form of the β Subunit Sliding Clamp of DNA Polymerase III of *Escherichia coli*: I. GENE EXPRESSION AND REGULATION

Tamar Paz-Elizur, Rami Skaliter, Sara Blumenstein and Zvi Livneh

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