

DNA polymerase II is encoded by the DNA damage-inducible *dinA* gene of *Escherichia coli*

(LexA operator/*araBAD* operon/conserved domains/*polB* mutation)

CYNTHIA A. BONNER*, SHARON HAYS*, KEVIN MCENTEE†, AND MYRON F. GOODMAN*

*Department of Biological Sciences, Molecular Biology Section, University of Southern California, Los Angeles, CA 90089-1340; and †Department of Biological Chemistry and the Molecular Biology Institute, University of California at Los Angeles School of Medicine, Los Angeles, CA 90024

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ABSTRACT The structural gene for DNA polymerase II was cloned by using a synthetic inosine-containing oligonucleotide probe corresponding to 11 amino acids, which were determined by sequencing the amino terminus of the purified protein. The labeled oligonucleotide hybridized specifically to the λ clone 7H9 from the Kohara collection as well as to plasmid pGW511 containing the SOS-regulated *dinA* gene. Approximately 1400 base pairs of *dinA* sequence were determined. The predicted amino-terminal sequence of *dinA* demonstrated that this gene encoded DNA polymerase II. Sequence analysis of the upstream region localized a LexA binding site overlapping the –35 region of the *dinA* promoter, and this promoter element was found to be only two nucleotides downstream from the 3' end of the *araD* gene. These results demonstrate that the gene order is *thr-dinA* (pol II)-*ara-leu* on the *Escherichia coli* chromosome and that the DNA polymerase II structural gene is transcribed in the same direction as the *araBAD* operon. Based on the analysis of the predicted protein, we have identified a sequence motif Asp-Xaa-Xaa-Ser-Leu-Tyr-Pro-Ser in DNA polymerase II that is highly conserved among a diverse group of DNA polymerases, which include those from humans, yeast, Herpes and vaccinia viruses, and phages T4 and PRD1. The demonstration that DNA polymerase II is a component of the SOS response in *E. coli* suggests that it plays an important role in DNA repair and/or mutagenesis.

Of the three distinct DNA polymerizing activities purified from the bacterium *Escherichia coli*, the role of DNA polymerase II (pol II) is the least understood. The function of DNA polymerase I in replication and repair has been well documented (1), and the DNA polymerase III holoenzyme constitutes the replicative polymerase in this organism (2). Remarkably, however, the biological role of pol II has not been determined, and no phenotype has been identified for mutants (*polB*) deficient in this activity (3, 4).

Recently, we reported that purified pol II catalyzed the insertion of nucleotides opposite defined abasic sites in model templates (5). This insertion and the subsequent extension steps are thought to be critical features of "lesion bypass," which likely accounts for targeted mutagenesis in prokaryotes (6, 7). That pol II could incorporate a nucleotide (preferably dAMP) opposite a noncoding site in DNA was consistent with a role for this activity in mutagenesis. Of equal significance was our observation (5) that the levels of pol II increased in cells exposed to agents that block replication (nalidixate) and that this apparent increase in pol II activity was regulated by the *lexA* gene, which controls expression of the SOS response in *E. coli* (8). Induced mutagenesis in bacteria requires induction of components of the SOS regulon, including the *umuC*, *umuD*, and *recA* gene products (6,

7). Taken together, these results suggested that pol II performs a role in induced mutagenesis in *E. coli*.

Additional insight into the function of pol II in cell growth and mutagenesis requires a detailed characterization of the structural gene and its regulation. In this report we describe the cloning and partial sequence determination of the gene coding for pol II[‡]. During the course of this work, we discovered that pol II was encoded by the *dinA* gene, which had been identified previously as a DNA damage-inducible Mud(Ap^R, *lac*) gene fusion of unknown function (9). DNA sequence analysis of the *dinA* (pol II) upstream region provides additional information on the regulation of this gene and has localized it on the *E. coli* chromosome adjacent to *araD*. Sequence analysis of the pol II structural gene indicates that it shares remarkable similarity with a group of DNA polymerases from both prokaryotic and eukaryotic organisms. These molecular studies confirm and extend our initial biochemical investigation of pol II and strongly implicate this enzyme in the processes of DNA repair and mutagenesis.

EXPERIMENTAL PROCEDURES

Strains. The *E. coli* strains used were GW1002 [*lac* Δ (U169), *recA441* (*tif-1*), *sfiA11*/pGW511 (P *dinA*)], kindly provided by G. Walker (Massachusetts Institute of Technology) (10); JE22606/pLC26-6 from the *E. coli* Genetics Stock Center (Yale University) (11); CJ229 [F⁺, Δ (*gal-bio*), *thi-1*, *relA1*, *spoT1*, Δ *polA*, Km^r/pCJ102 (F' 5'ExoCm^r)], kindly provided by C. Joyce (Yale University) (12); and NM522 [*hsd5*, Δ (*lac-pro*), [F', *pro*⁺, *lacI*^qZ Δ M15]], from Pharmacia (13). The Kohara λ phage clones used (8D2, 8H11, 7H9, 15B8, 6F3, and 6C1) have been described (14) and were kindly provided by F. Blattner (University of Wisconsin).

Enzymes. *E. coli* pol II was purified through fraction III from strain CJ229 as described (5). Restriction enzymes were from Pharmacia LKB. T4 polynucleotide kinase and Sequenase version 2.0 were from United States Biochemical. T4 DNA ligase was purchased from New England Biolabs.

Microsequencing the Amino Terminus of pol II. Purified *E. coli* pol II was electrophoresed through an SDS/11% polyacrylamide gel. Staining a portion of the gel with Coomassie brilliant blue indicated that this fraction contained only two proteins, an 84-kDa band identified as pol II and a smaller protein (\approx 45 kDa). The proteins were transferred to a poly(vinylidene difluoride) membrane, and the amino acid sequence of each was determined with an Applied Biosystems 475A peptide microsequencer with an on-line HPLC analyzer. Protein sequencing was performed by Audree Fowler (University of California at Los Angeles Protein Microsequencing Facility).

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Abbreviation: pol II, DNA polymerase II.

[‡]The sequence reported in this paper has been deposited in the GenBank data base (accession no. M37727).

Degenerate Oligonucleotide Probe Synthesis. The degenerate oligonucleotide 5'-CA(A/G)TGGCGIGA(T/C)ACIC-CICA(A/G)GGIACIGA(A/G)GT-3', corresponding to residues 10 (Gln) to 20 (Val) of the peptide sequence, was synthesized on a DuPont generator DNA synthesizer by Dohn Glitz (Department of Biological Chemistry, University of California at Los Angeles).

Hybridization of pol II Probe to *dinA*. Labeling of probe. The degenerate oligonucleotide was 5' end-labeled with T4 polynucleotide kinase and [γ - 32 P]ATP (15) and was separated from unincorporated nucleotide as described (16).

DNA purification. Plasmids pLC26-6 and pGW511 were purified as described (17). DNA from the Kohara λ phage clones was purified by using a Qiagen purification kit according to the manufacturer's recommendations.

Dot blot. DNA (1 μ g of each) from plasmids pGW511 and pLC26-6 and Kohara λ phage clones 8D2, 8H11, 7H9, 15B8, 6F3, and 6C1 was heat-denatured, spotted onto Schleicher & Schuell BA85 nitrocellulose paper, and hybridized to labeled oligonucleotide as described (15). The filter was dried and placed under Kodak GPB film overnight.

Southern blot. DNA (1 μ g of each) from pGW511 and Kohara λ phage clone 7H9 was digested with *Bgl* II and *Hinf* I, electrophoresed through 0.8% agarose, transferred to nitrocellulose paper, and hybridized according to the protocol of Davis *et al.* (15).

Subcloning *dinA*. The *dinA* promoter and amino-terminal region contained within a 2.8-kilobase (kb) *Bam*HI/*Hind*III fragment on plasmid pGW511 was subcloned into phagemid vector pT7T3 18U (Pharmacia) to generate phagemid pCB100. The 1.78-kb *Bgl* II fragment was subcloned in both orientations into pT7T3 18U to generate pCB101 and pCB102; a 1.2-kb *Cla* I fragment was subcloned into pT7T3 19U to yield pCB103. All subcloning was performed as described (17).

Sequencing *dinA*. *E. coli* strain NM522 was transformed with recombinant phagemids pCB100, pCB101, pCB102, and pCB103 and grown overnight in LB broth containing ampicillin with helper phage M13KO7 (Pharmacia) added at a multiplicity of infection of four. Single-stranded DNA was isolated from the cells as described (17). M13 universal sequencing primer (Pharmacia) was initially used to sequence *dinA*. Additional primers complementary to a region near the 3' end of the preceding sequence were synthesized by using an Applied Biosystems 381 DNA synthesizer.

DNA sequencing (18) was performed by using Sequenase 2.0 and adenosine 5'-[γ - 35 S]thio]triphosphate.

RESULTS

Partial Amino Acid Sequence of pol II. Highly purified pol II was electrophoresed in a polyacrylamide gel and transferred to poly(vinylidene difluoride) membrane by electroblotting. Approximately 30 pmol of the 84-kDa protein was used for solid-phase peptide sequencing, which identified the first 27 amino-terminal residues. The sequence obtained was Asp(Ala)-Gln-Ala-Gly-Phe-Ile-Leu-Thr-Xaa-Gln-Trp-Arg-Asp-Thr-Pro-Gln-Gly-Thr-Glu-Val-His-Phe-Xaa-Leu-Ala-Thr-Tyr, where Xaa represents an unidentified residue.

Based upon this sequence information, a degenerate inosine-containing oligonucleotide was prepared corresponding to the sequence encoding residues Gln-10 to Val-20. The sequence of the 32-mer is given in *Experimental Procedures*. The oligonucleotide was labeled at the 5' end by using T4 polynucleotide kinase and [γ - 32 P]ATP and hybridized to the six clones from the Kohara collection, which covered the chromosomal interval between minutes 1 and 3 on the *E. coli* map, where the *polB* gene had been mapped previously (4, 19). Additionally, plasmids pLC26-6 and pGW511 were included in the hybridizations. The former plasmid contains DNA between *leuA* and *murEF* (11, 20), whereas the latter plasmid contains a portion of the

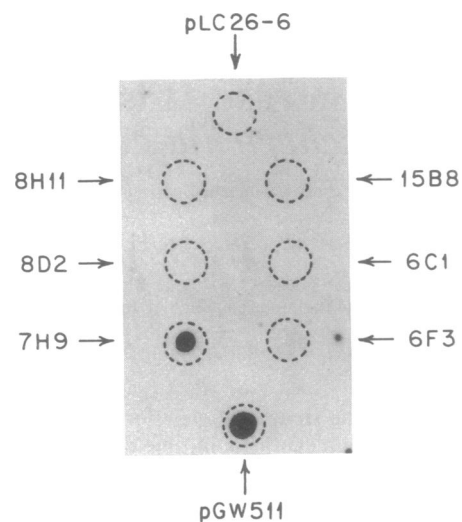


FIG. 1. DNA dot blot showing hybridization of a radiolabeled oligonucleotide probe derived from the amino terminus of pol II to pGW511 (*dinA*) and λ phage clone 7H9. DNA (1 μ g of each) from pGW511 and pLC26-6 and Kohara λ phage clones 7H9, 8D2, 8H11, 15B8, 6C1, and 6F3 were applied where indicated by dashed circles.

dinA gene and 5' flanking region. The original *dinA-lacZ* fusion from which this plasmid was derived was 50% linked to *leuA* in P1 transductional crosses (9).

The results of this dot blot hybridization are shown in Fig. 1. The 32 P-labeled oligonucleotide probe hybridized specifically to the Kohara clone 7H9 as well as to plasmid pGW511. No hybridization was detected to partially overlapping clones 8D2 and 8H11. This result allowed us to narrow the location of the pol II structural gene to a region of ≈ 4.5 kb. Moreover, the strong hybridization to plasmid pGW511 suggested that the gene encoding pol II was either the *dinA* gene or one extremely close to this locus.

The hybridization results shown in Fig. 1 are easily explained if the inserts in clone 7H9 and in plasmid pGW511 contained an overlapping region of DNA. Alternatively, it remained a formal possibility that the degenerate probe used in the hybridization was annealing to two related but distinct DNA sequences. Kenyon *et al.* (10) had located the promoter

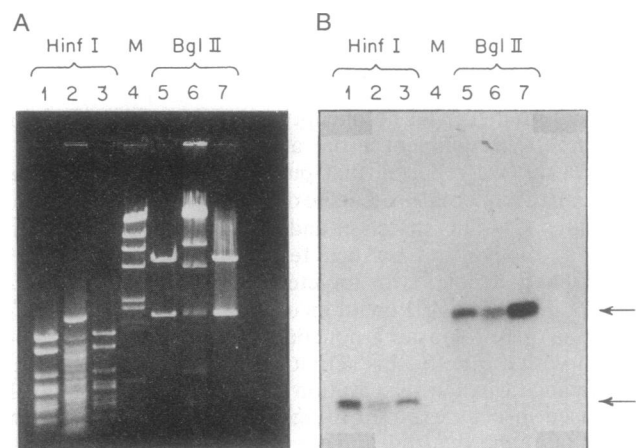


FIG. 2. Radiolabeled probe derived from the amino terminus of DNA pol II hybridizes to the same restriction fragments in pGW511 (*dinA*) and λ phage clone 7H9. Lanes: 1, pGW511 (1 μ g) digested with *Hinf* I; 2, 7H9 (1 μ g) digested with *Hinf* I; 3, pGW511 (0.2 μ g) digested with *Hinf* I; 4, λ /*Hind*III molecular size markers; 5, pGW511 (0.2 μ g) digested with *Bgl* II; 6, 7H9 (1 μ g) digested with *Bgl* II; 7, pGW511 (1 μ g) digested with *Bgl* II. (A) Ethidium-stained gel. (B) Autoradiograph of gel shown in A.

and amino-terminal coding region of the *dinA* gene on a 540-base-pair (bp) *HinfI* fragment in plasmid pGW511. This *HinfI* fragment was contained within a 1.78-kb *Bgl* II restriction fragment. DNA from phage clone 7H9 and plasmid pGW511 was digested separately with either *HinfI* or *Bgl* II, and the fragments were separated by electrophoresis in 0.8% agarose. The stained gel showed that clone 7H9 and plasmid pGW511 DNAs contained a 1.78 kb *Bgl* II fragment and a 540-bp *HinfI* product. These fragments hybridized to the labeled oligonucleotide, indicating that these clones contained an overlapping region of DNA (Fig. 2).

DNA Sequence of the *dinA* Region. To determine the primary sequence of the *dinA* gene, the *Bgl* II restriction fragment from plasmid pGW511 was subcloned into phagemid vector pT7T3 18U, a pUC18-derived vector containing an *f1* origin of replication. Single-stranded DNA was prepared and sequenced by the dideoxyribonucleotide chain termination method as described in *Experimental Procedures*. The *Bgl* II fragment was subcloned in both orientations, and the sequences of both strands were determined. The sequencing strategies are shown in Fig. 3A.

The sequence of ≈ 1400 nucleotides of the *dinA* region are

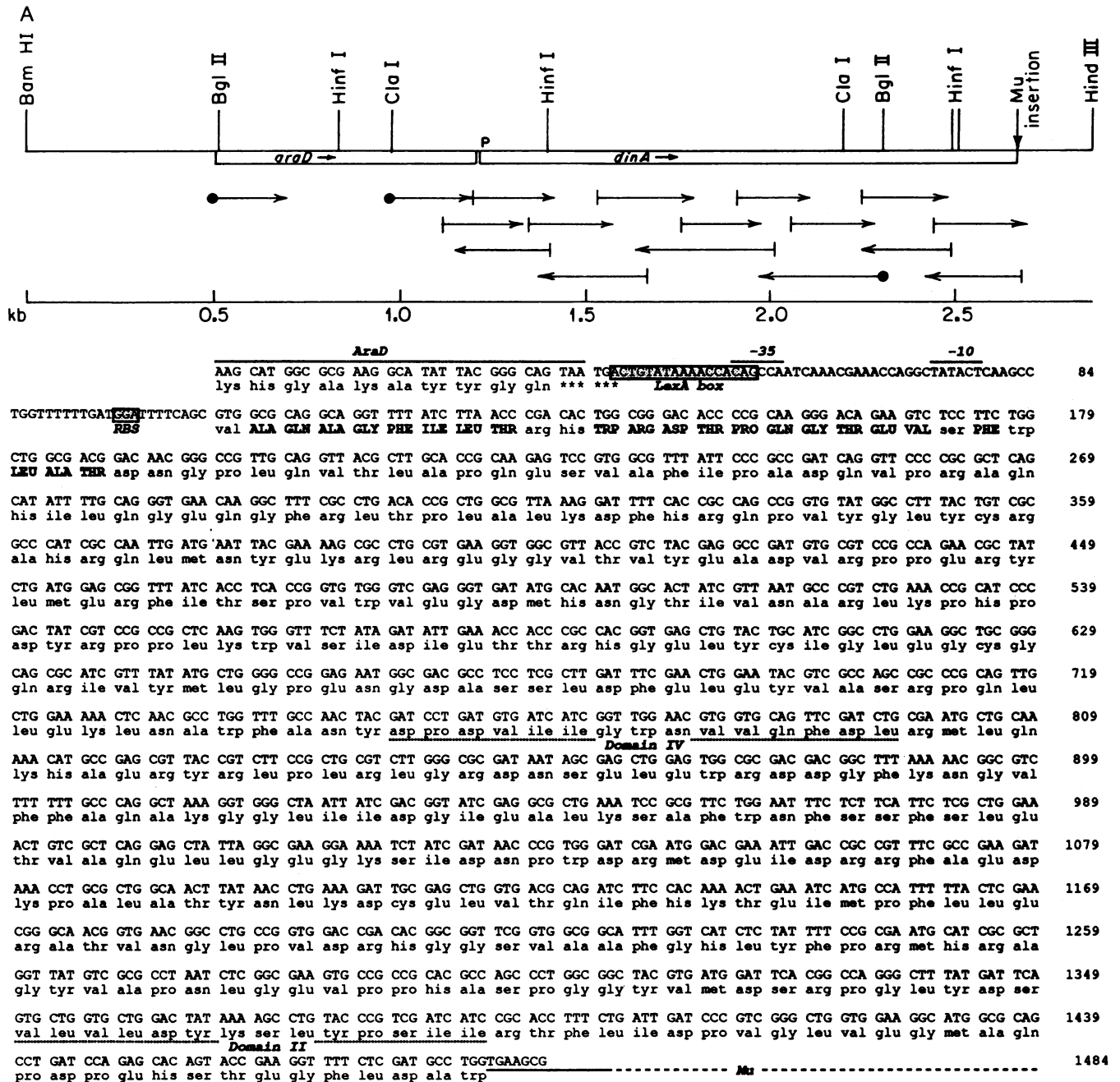


FIG. 3. (A) Strategy for sequencing the promoter and amino-terminal region of *dinA* (pol II). Arrows with solid circles represent regions sequenced with a universal primer complementary to vector sequences adjacent to *dinA* inserts; the other arrows represent regions sequenced with synthetic oligonucleotide primers complementary to sequence near the 3' end of the preceding sequence. (B) DNA sequence of promoter and amino-terminal portion of the *dinA* (pol II) gene. The amino acid sequence of the *dinA* (pol II) open reading frame is shown below the DNA sequence. A portion of the 3' terminus of *araD*, located immediately upstream of *dinA* (pol II), is shown (nucleotides 1–33) as is the insertion point (nucleotide 1478) of Mud(Ap^R, *lac*). The putative LexA binding site and ribosome binding site (RBS) are indicated by stippled boxes; the –35 and –10 regions are labeled. Amino acids in boldface correspond to those that match the microsequence analysis of purified pol II protein. Two regions of similarity to other polymerases (domain IV and domain II) are labeled.

shown in Fig. 3B. Nucleotides 108–1478 correspond to an open reading frame in which translation is initiated with a GTG codon. The sequence of the first 27 predicted amino acids in the *dinA* protein were in excellent agreement with the sequence obtained directly from purified pol II protein. The sequences matched at 22 of these 27 residues and demonstrated that pol II is encoded by the *dinA* gene.

The sequence of the *dinA* gene predicts a relatively weak ribosome binding site containing a GGA triplet of the Shine-Dalgarno sequence (Fig. 3B). This potential ribosome binding site is located 8 bp upstream of the likely initiation GTG codon. Nucleotide sequences homologous to the conserved –35 and –10 boxes common to several prokaryotic promoters were found. A putative LexA operator site overlaps the *dinA* promoter as has been seen with other SOS-regulated genes (8). Although the –10 region is a relatively good match to the consensus TATAAT (five of six), there is considerably less homology to other bacterial promoters in the –35 region (three of six), which contains an overlapping CAG conserved triplet from the LexA operator. A similar location of the LexA operator overlapping the –35 interval has been reported for the *uvrA* gene (21).

The *dinA* (pol II) Gene Is Adjacent to *araBAD*. We sequenced ≈200 nucleotides upstream of the *dinA* (pol II) promoter. When this sequence was compared to sequences in the *E. coli* data base, we discovered that this region was identical to the sequence of the end of the *araD* gene determined by Lee *et al.* (22). As shown in Fig. 3B, the *araD* coding region terminates 2 bp upstream of the LexA operator of *dinA* (pol II). This result was surprising, not only because of the relatively short intergenic spacing between *araD* and *dinA* (pol II), but because earlier linkage data had placed the *polB* gene more than 1 minute away on the *E. coli* genetic map (4, 19). The *polB* mutant was shown to be defective in pol II activity. Our DNA sequencing results locate the structural gene for pol II immediately counterclockwise of the *araBAD* operon. The map order in this interval is *thr-dinA* (pol II)-*araDABC-leuDABC-ilvIH*. Moreover, based upon the sequence information and the orientation of the *ara* operon, we conclude that the *dinA* (pol II) gene is transcribed in the same direction as the arabinose (*araBAD*) operon.

pol II Contains at Least One Conserved Sequence Element Common to Eukaryotic and Prokaryotic DNA Polymerases. Conserved sequence motifs have been found in several DNA polymerases, and these amino acid sequences as well as their positions in the polypeptide chains are conserved among a diverse set of polymerases. Examination of the predicted protein sequence of pol II identified a seven-residue region, Ser-Leu-Tyr-Pro-Ser-Ile-Ile, which was identical to sequences found in polymerases from human, yeast, herpes simplex virus, cytomegalovirus, and Epstein-Barr virus as well as phage T4 (see Table 1 and Fig. 3). The location of this motif within the polypeptide chain is also conserved between pol II and these other group B (“ α -like”) polymerases (23, 24).

Also in Fig. 3 and Table 1 is a stretch of 18 amino acids that is 50% identical to sequences found in human polymerase α and yeast polymerase I (which are also 50% identical to each other). These 18 amino acids are part of another domain (IV) found in several group B polymerases (24). Domain IV is less well conserved than domain II; within the entire 42-amino acid stretch, *E. coli* pol II is 33% identical to human polymerase α , whereas human polymerase α and yeast polymerase I are 36% identical. Additional DNA sequence determination will be needed to determine whether the other conserved regions found in group B (α -like) polymerases are contained within the pol II enzyme.

DISCUSSION

We have purified the 84-kDa pol II to near homogeneity and determined the sequence of 27 residues at the amino terminus. Guided by this sequence, we designed an oligonucleotide probe that was used to clone the polymerase structural gene. The labeled degenerate oligonucleotide was used to probe a group of eight clones that originated from the interval between 1 and 3 minutes on the *E. coli* linkage map.

Six clones from the Kohara collection, 7H9, 8D2, 8H11, 15B8, 6C1 and 6F3, were probed as well as plasmid pLC26-6, which contained DNA from the interval between *leuA* and *murEF* (11, 20). Furthermore, the plasmid pGW511, which contained a portion of the *dinA* gene, was also included. Hybridization was observed to only two of these clones, Kohara phage 7H9 and the pGW511 plasmid. Restriction digestion analysis of these two clones demonstrated that they contained DNA fragments in common, and the oligonucleotide probe hybridized to a 1.78-kb *Bgl* II fragment and a 540-bp *Hinf*I fragment present in both clones.

DNA sequence determination of the amino-terminal coding portion of *dinA* demonstrated convincingly that this gene encoded pol II. Furthermore, consistent with our earlier biochemical demonstration that pol II activity increased in cells after exposure to DNA-damaging agents in *lexA*⁺ cells (5), we identified a LexA operator site within the *dinA* promoter. Kenyon *et al.* (10) demonstrated that transcription from the *dinA* promoter *in vitro* was blocked by added LexA protein. These results clearly show that expression of pol II is regulated by the SOS response in *E. coli*.

Additional DNA sequence analysis of the *dinA* region has unambiguously localized this gene immediately adjacent to *araD*. Indeed the proposed LexA operator of pol II is just two nucleotides away from the end of the *araD* coding region. This result establishes the gene order in this interval to be *thr-dinA* (pol II)-*araDAB-araC-leu*, a result that differs significantly from the earlier studies (4, 19) using the original mutation that abolished pol II activity, *polB100*. Transductional mapping experiments (4, 19) had localized this mutation clockwise of *leu* on the genetic map, more than a minute

Table 1. Sequence homology between *E. coli* pol II and other prokaryotic and eukaryotic DNA polymerases

Polymerase	Domain II	Domain IV
<i>E. coli</i> (pol II)	V L V L D Y K S L Y P S I I	D P D V I I G W N V V Q F D L R M L
Human (pol α)	I L L L D F N S L Y P S I I	D P D I I V G H N I Y G F E L E V L
Yeast (pol I)	V L V M D F N S L Y P S I I	D P D V I I G H R L Q N V Y L D V L
Herpes simplex virus	V V V F D F A S L Y P S I I	G P E F V T G Y N I I N F D W P F L
Cytomegalovirus	V A V F D F A S L Y P S I I	A P A F V T G Y N I N S F D L K Y I
Epstein-Barr virus	V L V V D F A S L Y P S I I	S V E I V T G Y N V A N F D W P Y I
T4	I M S F D L T S L Y P S I I	R P A I F T G W N I E G F D V P I I
Vaccinia virus	V L I F D Y N S L Y P N V C	Y V V T F N G H N - - - F D L R Y I
Adenovirus 2	L Y V Y D I C G M Y A S A L	L E L Y I V G H N I N G F D - E I V
PRD1	I K V Y D V N S M Y P H A N	

Amino acids found in *E. coli* pol II that are identical in four or more of the other polymerases are indicated by boldface letters. Amino acid sequences other than the *E. coli* pol II are from ref. 24. pol, Polymerase.

from the location of the pol II structural gene identified in this study.

The predicted primary sequence of pol II reveals considerable similarity with both prokaryotic and eukaryotic polymerases. One particular example of striking similarity is found between human DNA polymerase α and pol II in a region (region II) that is highly conserved in several other DNA polymerases. It is interesting that within this region pol II shows greater similarity to human polymerase α and yeast DNA polymerase I than to phage T4 DNA polymerase. That this region is found in the primary sequences of DNA polymerases of bacteriophages, DNA viruses, yeast, and vertebrates has suggested that this region functions in deoxynucleotide binding or phosphodiester bond cleavage (24).

A less well conserved region, designated region IV, was also identified in the pol II sequence. This result is especially intriguing because region IV has been implicated in the interaction between yeast polymerase I and primase (24). There is no evidence at the present time that would indicate an interaction between pol II and the *E. coli* primase, and a role for pol II in replication has not been determined.

Recently, Chen *et al.* (25) reported the cloning of the gene for *E. coli* pol II. The restriction map of their clone (figure 3 in ref. 25) is compatible with the restriction map shown in Fig. 3A, suggesting that the same gene has been cloned by different methods. They have also presented evidence that pol II is degraded to several different sized polypeptides retaining polymerizing activity. Based on their data, they concluded that breakdown products of 82 kDa and 55 kDa were derived from a 99-kDa precursor by proteolysis. We originally reported a large molecular mass protein (102 kDa) in highly purified preparations of pol II that was active in an *in situ* DNA polymerization assay (5). This activity was also present in corresponding fractions from *polB* mutant HMS83, which lacked the 84-kDa protein.

Although we can only speculate as to the relationship between pol II and this 102-kDa polypeptide, our sequence analyses demonstrate that the 84-kDa enzyme corresponds to the amino-terminal portion of the *dinA* coding region. It is possible that pol II is initially synthesized as a large (102-kDa) polypeptide, which is processed to a mature 84-kDa polymerase in wild-type cells but not in *polB* mutants. Such a model raises interesting questions regarding the nature of the *polB100* mutation and the control of pol II activity by the SOS response.

Note Added in Proof. We have completed the sequence of the gene for pol II and have identified three additional domains (III, I, and V) found

in group B (α -like) polymerases (24). The pol II gene has an open reading frame of 2349 nucleotides and predicts a protein of 89.9 kDa.

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