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

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

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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.

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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 MudApR, 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 imply that this enzyme in the processes of DNA repair and mutagenesis.

EXPERIMENTAL PROCEDURES

Strains. The E. coli strains used were GW1002 [lacΔ (U169), recA441 (tif-1), sfa11/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, ΔpolA, Km'p/CJ102 (F' 5'ExoCm')], kindly provided by C. Joyce (Yale University) (12); and NM522 (husd, Δ(lac-pro), [F', pro'], lacI2ΔM15]), 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 Sequase 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 (~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).

Abbreviation: pol II, DNA polymerase II.
Degenerate Oligonucleotide Probe Synthesis. The degenerate oligonucleotide 5'-CA(A/G)TGGCGIGA(T/C)ACIC- CICA(A/G)GGICIGA(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-32P]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 μg 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 μg of each) from pGW511 and Kohara λ phage clone 7H9 was digested with Bgl II and HinfI, 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) BamHI/HindIII 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 M13K07 (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-32P]thiotriphosphate.

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 Gin-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-32P]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 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 32P-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. 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 μg 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.

![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 μg) digested with HinfI; 2, 7H9 (1 μg) digested with HinfI; 3, pGW511 (0.2 μg) digested with HinfI; 4, λ/HindIII molecular size markers; 5, pGW511 (0.2 μg) digested with Bgl II; 6, 7H9 (1 μg) digested with Bgl II; 7, pGW511 (1 μg) digested with Bgl II. (A) Ethidium-stained gel. (B) Autoradiograph of gel shown in A.](https://example.com/fig1.png)
and amino-terminal coding region of the dinA gene on a 540-base-pair (bp) Hinfl fragment in plasmid pGW511. This Hinfl 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 Hinfl 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 Hinfl 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

![Diagram](image-url)

**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 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*, 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 polynucleases (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 −900 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 araD operon. The map order in this interval is then 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 linkage map 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 only to two of these clones, Kohara phase 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 HindIII fragment present in both clones.

DNA sequence determination of the amino-terminal codon 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)-araDABC-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>
<thead>
<tr>
<th>Polymerase</th>
<th>Domain II</th>
<th>Domain IV</th>
</tr>
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<tbody>
<tr>
<td>E. coli (pol II)</td>
<td>V V L D Y K S L Y P S I I</td>
<td>D P D V I G W V Y V Q P D L R M L</td>
</tr>
<tr>
<td>Human (pol α)</td>
<td>I L L D D F N S L Y P S I I</td>
<td>D P D I I V G H I Y G P E L E V L</td>
</tr>
<tr>
<td>Yeast (pol I)</td>
<td>V V V W M D F N S L Y P S I I</td>
<td>D P D V I I G H R L Q N V Y L D V L</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>V V V F D F A S L Y P S I I</td>
<td>G P E F V T G Y M I N P D W P F L</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>V V V F D F A S L Y P S I I</td>
<td>A P A F V T G Y M I N S D P L K Y I</td>
</tr>
<tr>
<td>Epstein–Barr virus</td>
<td>V V V W M D F N S L Y P S I I</td>
<td>S V E V I T G Y M V A N P D W P Y I</td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td>I H S P D L T S L Y P S I I</td>
<td>Y V V T E N G H N -- -- -- P D L R Y Y</td>
</tr>
<tr>
<td>Adenovirus 2</td>
<td>L Y V V D I C G M Y S A S A L</td>
<td>L E L Y I V G H M I N G P D -- E I V</td>
</tr>
<tr>
<td>PRD1</td>
<td>L Y V V D I C G M Y S A S A L</td>
<td>L E L Y I V G H M I N G P D -- E I V</td>
</tr>
</tbody>
</table>

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 deoxyribonucleotide 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 polypeptide 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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