Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 3772–3776, June 1988
Biochemistry

DNA polymerase I gene of *Saccharomyces cerevisiae*: Nucleotide sequence, mapping of a temperature-sensitive mutation, and protein homology with other DNA polymerases

ANTONELLA PIZZAGALLI, PAOLA VALSASNINI, PAOLO PLEVANI*, AND GIOVANNA LUCCHINI*

Dipartimento di Genetica e Biologia dei Microrganismi, Università di Milano, Via Celoria 26, Milan 20133, Italy

Communicated by I. Robert Lehman, February 16, 1988

ABSTRACT **A 5600-base-pair segment spanning the coding region of the *Saccharomyces cerevisiae* DNA polymerase I gene was sequenced and found to contain an open reading frame of 1468 codons, corresponding to a polypeptide of M_r 166,794. A *pol1* temperature-sensitive mutation, encoding a DNA polymerase–primase complex with altered stability, has a single base-pair substitution that changes the glycine at position 493 to a positively charged arginine. Protein sequence comparison with other prokaryotic and eukaryotic DNA polymerases reveals three major regions of homology. This observation suggests that certain DNA polymerases might require the conservation of critical amino acid residues for activity.**

Progress in defining the structure and function of eukaryotic DNA replication components is the result of improved purification procedures and of the observation that DNA polymerase α can be purified in a multisubunit protein complex with DNA primase (for review, see ref. 1). Several laboratories, working with organisms as phylogenetically distant as yeast, *Drosophila*, and mammals, have isolated DNA polymerase–primase complexes with similar composition. A 170- to 180-kDa polypeptide has been identified as the core catalytic DNA polymerase subunit, and two polypeptides of ≈50 kDa and ≈60 kDa have been associated with DNA primase activity.

The DNA polymerase–primase complex from *Saccharomyces cerevisiae* has been purified to near homogeneity (2), and specific antibodies have been used to isolate the genes encoding DNA polymerase I (3–5) and the 48-kDa subunit of DNA primase (p48) (6). Both genes are unique in the haploid yeast genome and perform essential functions (4–6). The availability of the cloned DNA polymerase I gene has allowed the production, by *in vitro* mutagenesis, of temperature-sensitive mutants that will be extremely useful to establish the role of this enzyme in DNA metabolism (7, 8). Moreover, direct analysis of POL1 mRNA showed that the amount of DNA polymerase I mRNA fluctuates during the cell cycle and meiosis and that DNA polymerase I mRNA is induced after DNA damage (9). The finding that the expression of the *POL1* gene is under cell-cycle control leads to questions about the nature of the cis- and trans-acting genetic elements that regulate the expression of this essential gene.

To meaningfully address such questions, the nucleotide sequence of the DNA polymerase I gene and of its putative upstream regulatory region is required. Moreover, the nucleotide sequence will permit calculation of the molecular weight of the *POL1* encoded protein and identification of possible functional domains as homologies with other DNA polymerase sequences are found and as *pol1* mutants are characterized. We have determined the nucleotide sequence of the *POL1* gene† and have identified regions of homology between the yeast DNA polymerase polypeptide and DNA polymerases from animal viruses and bacteriophages. We also report the sequence of one point mutation, *pol1-1* (8), that confers a temperature-sensitive phenotype by altering the stability of the DNA polymerase–primase complex and that possibly defines a functional domain involved in protein–protein interactions.

MATERIALS AND METHODS

Plasmids and Strains. Plasmids, pAP70, pAP33, and pAP1, were constructed by cloning, respectively, the 3.4-kilobase (kb) *Sal* I segment, in both orientations, and the 2.2-kb *Sal* I–*Bam*HI segment (Fig. 1) into pGEM4 (Promega Biotec, Madison, WI). The two restriction fragments, derived from plasmid pGL7-4 (4), span the entire *POL1* coding sequence plus 700 base pairs (bp) of the 5′-noncoding region that are sufficient for cell viability (8). Plasmid DNA was digested with *Kpn* I and *Bam*HI (pAP1, pAP33, and pAP70) or with *Sph* I and *Sal* I (pAP1) and serial deletions, ≈150 bp apart, were made with exonuclease III (10). Plasmid pCM54, carrying the *pol1-1* temperature-sensitive allele, was obtained from the DNA of the original yeast mutant TD28-ts1 (8), in which the mutation had been introduced by integration at *POL1* of one mutagenized copy of plasmid pCM1 (a YIp5 derivative containing the 3.4-kb *Sal* I fragment). Digestion of this DNA with *Bam*HI, ligation, and *Escherichia coli* transformation gave rise to a plasmid containing the *POL1* segment depicted in Fig. 1, inserted into the *Sal* I–*Bam*HI region of YIp5. The same procedure was used to construct plasmid pCM57, containing the wild-type *POL1* allele derived from the yeast strain TD28[pCM1] that carries one copy of the nonmutagenized pCM1 integrated at *POL1*. Plasmid pCM54 contains the *pol1-1* allele, since its integration at the *POL1* locus of a *ura3-52 pol1-1* yeast strain did not give rise to Ura$^+$ transformants with a temperature-resistant phenotype. Integration of plasmid pCM57 at the *POL1* locus of a *ura3-52 pol1-1* yeast strain gave rise to Ura$^+$ transformants. The *ura3-52 pol1-1* strain used to map the *pol1-1* mutation was a TD28-ts1 derivative from which the plasmid had been segregated, leaving the mutant allele at the *POL1* locus.

DNA Sequencing. DNA sequencing was performed by the dideoxynucleotide chain-termination method (11) by using deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate, denatured plasmid DNA template (12), and *E. coli* Klenow fragment of DNA polymerase I. Either SP6 or T7 phage promoter primers were used to sequence the *POL1* region in both directions in the pAP1, pAP33, and pAP70 derivative deletion plasmids. The region including the *pol1-1* mutation was sequenced in pCM54 and pCM57 by using synthetic oligonucleotides as primers (13). Protein data-base searches and DNA sequence

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

†This sequence is being deposited in the EMBL/GenBank data base (Intelligenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03268).

*To whom reprint requests should be addressed.

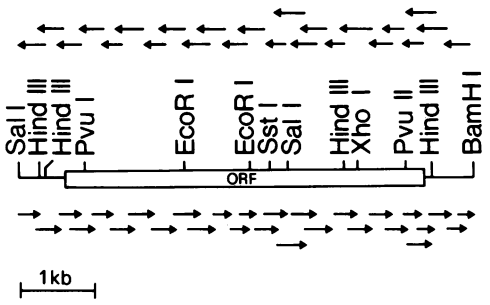

FIG. 1. Restriction map and DNA sequence strategy for the *POL1* region. The figure depicts the 5635-bp DNA segment containing the entire *POL1* coding sequence (ORF, open reading frame). The direction of transcription is from left to right (3). To generate plasmids for direct sequencing, the 3.4-kb *Sal* I fragment and the 2.2-kb *Sal* I–*Bam*HI fragment were cloned in the pGEM vector, and a set of targeted deletion mutants was created. The arrows indicate the extent and direction of the sequenced fragments. Sequences around the internal *Sal* I site and the termination codon were confirmed in pCM57 by using appropriate synthetic oligonucleotides.

analysis were performed with an IBM microcomputer with the MicroGenie program (Beckman).

RESULTS

Nucleotide Sequence of the *POL1* Gene. Fig. 2 shows the sequence of 5600 bp in the *POL1* region of the yeast *S. cerevisiae* that we have mapped (4, 8) to the left arm of chromosome XIV at a position between *MET4* and *TOP2*. A 1468-codon open reading frame starting with an ATG (position +1) and ending with a TAG stop signal is found within this sequence. The 4404-bp open reading frame encodes a polypeptide with a calculated molecular weight of 166,794. The size of the larger polypeptide associated with DNA polymerase activity in our purified enzyme preparations was estimated to be ≈180 kDa by $NaDodSO_4$/polyacrylamide gel electrophoresis (2). Given the uncertainties in the electrophoresis results in the high-molecular-weight range and the possibility that the DNA polymerase polypeptide might be subjected to some posttranslational modification (16), the agreement between the measured and the calculated molecular sizes is reasonable.

The consensus sequence for the initial AUG context in highly expressed yeast genes is AAAAAAAUGUCU, where the adenosine residues at positions −1 and −3 occur at a frequency of 89% and 100%, respectively, and serine is preferentially present as the second amino acid, possibly because of its protein-stabilizing role (17). The nucleotide sequences around the first AUG codon of the *POL1* gene only partially match the above consensus sequence, in that it lacks the adenosine residues at positions −6 to −4. Since the AUG consensus has been found for highly expressed yeast genes, this discrepancy can be related to the observation that the levels of DNA polymerase and of its mRNA are very low (2, 3). Also the codon usage in *POL1* is typical of poorly expressed yeast genes; in fact, all 61 possible codons are used with little, if any, codon bias (data not shown), which correlates with the observation that there is a direct correspondence between the extent of codon bias and the level of mRNA and protein expression (18).

Translation of the 4404-bp open reading frame shows that about one-third of the residues are basic (219 lysines, arginines, and histidines) or acidic (214 glutamic and aspartic acids). The predicted polypeptide does not have strongly hydrophobic domains, and the amino terminus is predominantly hydrophilic. In a number of DNA-binding proteins, DNA contacts are made by two α-helices that are linked by a tight turn (19). A computer search for secondary structure of the *POL1* gene product revealed at least three helix-turn-helix motifs (residues 88–130, 405–427, and 1099–1133), whose role is unknown. It has also been suggested that a cysteine- and/or histidine-rich region forms a metal-binding domain that interacts with nucleic acids through hydrophilic residues (20). Interestingly, a potential metal-binding domain,

HSIRAHISLYYAGWLQCDDSTC

(presented in the single-letter code), can be found at the carboxyl terminus (residues 1332–1353) of the POL1 protein.

Amino Acid Homologies with Other DNA Polymerases. Several groups have noted a limited amino acid sequence homology among DNA polymerases from herpesviruses, vaccinia virus, adenovirus, and phage φ29 (21–24). We have extended this analysis and found that there is homology in regions I, II, and III (Fig. 3) among DNA polymerases from the yeast *S. cerevisiae*, several animal viruses (21–27), phage φ29 (24), the *Kluyveromyces lactis* killer plasmid pGKL1 (28), and the S-1 mitochondrial particle from maize (29). Region I is characterized by the presence of 5–7 contiguous conserved amino acids. Region II is larger, and, besides a central conserved sequence of 5–10 residues, there is up to 57% sequence identity on both sides of the conserved sequence. Region III does not have a stretch of identical amino acids but rather has identical residues at regular intervals. Moreover, these three conserved regions in the DNA polymerase polypeptides are in the same linear arrangement (II–III–I), and the relative distances between the regions are also similar. Although the role of region I is unknown, the sites of mutations that alter drug sensitivity to acyclovir and phosphonoacetic acid fall in regions II and III (21, 30). Wong *et al.* (31) have isolated cDNA clones encoding the human DNA polymerase α catalytic polypeptide. By sequence comparison, they also found the three conserved sequences described above and identified three additional regions, IV, V, and VI, also present in the yeast DNA polymerase I polypeptide (residues 659–669, 904–922, and 1069–1079, respectively).

Sequencing of a *pol1* Temperature-Sensitive Mutant. We have constructed *pol1* temperature-sensitive mutants by *in vitro* mutagenesis of the cloned gene and *in vivo* replacement of the wild-type allele with the mutated copy (8). Preliminary physiological and biochemical characterization of the *pol1-1* temperature-sensitive mutant indicates that it is a conformational mutant defective in the DNA polymerase–primase complex stability (8). To map the position of the mutation, we transformed a *ura3-52 pol1-1* strain (8) to a Ura^+ phenotype by integrating YIp5 derivative plasmids carrying the 3.4-kb *Sal* I fragment, the 5′ *Sal* I–*Eco*RI fragment, or the 5′ *Eco*RI–*Bam*HI wild-type fragment at the *POL1* locus. Fifty Ura^+ clones from each transformation were tested for growth at 37°C on rich medium. Only transformation with the plasmid containing the entire 3.4-kb *Sal* I fragment gave rise to temperature-resistant clones. Therefore, a mutation crucial for the pol1-1 phenotype must be located within the internal *Eco*RI fragment which is not overlapped by the sequences present in the other plasmids used (see Fig. 1).

The *pol1-1* allele was deleted from the original mutant, and synthetic oligonucleotides spanning the region between positions +1000 and +2800 were used as primers to localize, at the nucleotide level, the position of the *pol1-1* mutation. As it is shown in Fig. 4, the temperature-sensitive mutation is a single G·C → A·T transition at position +1477, which results in the replacement of the glycine with a positively charged arginine at codon 493 that is far upstream from the conserved regions found by sequence comparison. Since this mutation seems primarily to affect the stability of the DNA polymerase–primase complex, it might define a structural domain important for specific protein–protein interactions.

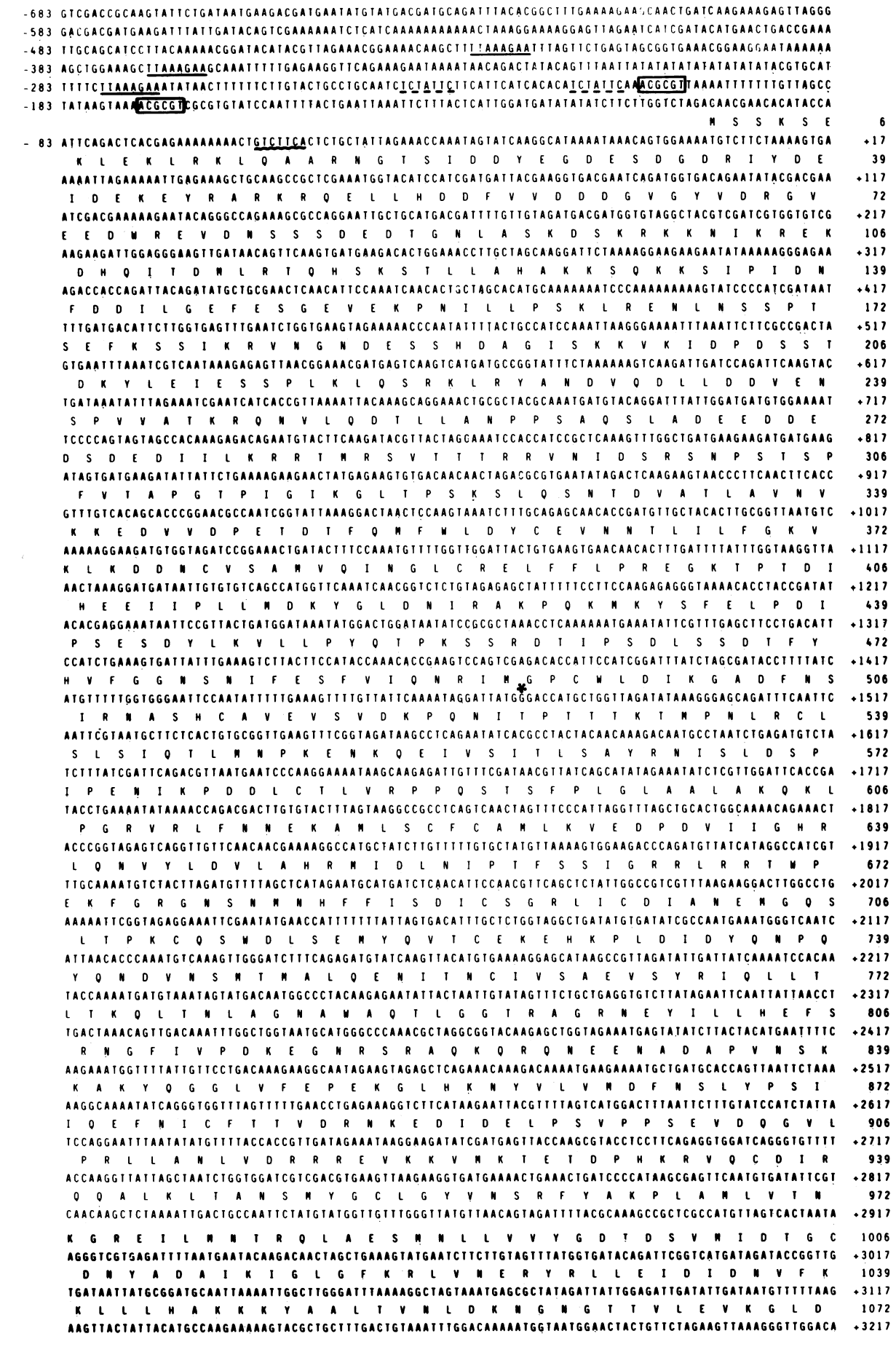

FIG. 2. *(Figure continues on the opposite page.)*

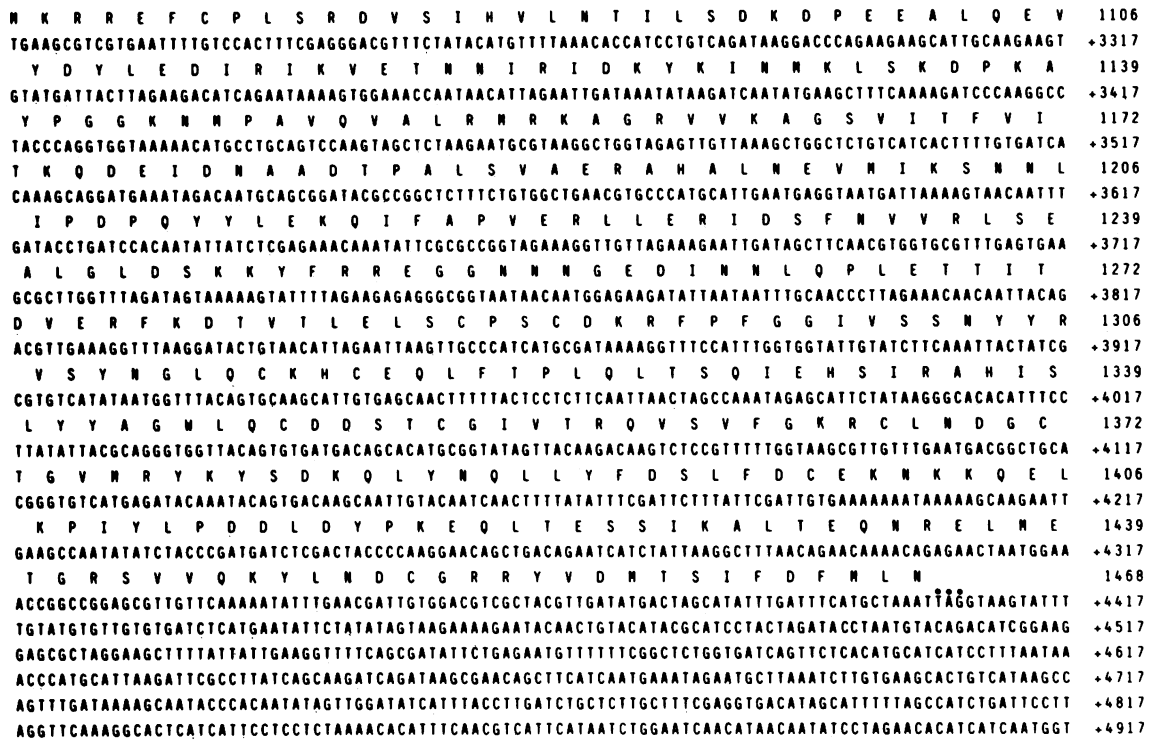

FIG. 2. Nucleotide sequence and deduced amino acid sequence of *POL1*. The nucleotide sequence is numbered from the ATG initiation codon of the *POL1* open reading frame (position +1). The amino acid sequence has been translated from the DNA sequence and amino acids (in the single-letter code) are numbered from the first methionine. Repeated DNA sequences in the 5′-noncoding region are boxed or underlined with continuous or dashed lines. A wavy underline indicates the position (−55 ± 3 bases) of the major 5′ terminus of the POL1 transcript detected by RNase mapping (14) and primer extension analysis (15). The asterisk at position +1477 indicates the location of the nucleotide changed in the *pol1-1* allele.

Sequences Upstream of the *POL1* Coding Region. In many of the yeast genes characterized to date, the consensus TATAAA has been found at distances of 50–150 nucleotides from the transcription start site, and many of the poorly expressed yeast genes do not have canonical TATA homology (15, 32–34). The 5′-noncoding region of the *POL1* gene has a high A+T content (>70%) with several "TATA-like" sequences. We have shown that the *POL1* gene is periodically transcribed around the G_1/S phase boundary of the cell cycle (9). It is, therefore, plausible that transcription regulatory elements are present in the 5′-noncoding region of *POL1*. Short repeated DNA sequences (indicated in Fig. 2) are present in the region from position −500 to +1. The sequence 5′ ACGCGT 3′ (boxed in Fig. 2) is repeated twice in the region from positions −208 to −168, and the second hexanucleotide is immediately followed by the direct repetition CGCGT. The same sequence is present, at least once, in the 5′-noncoding regions of other genes involved in DNA metabolism, *CDC8* (35), *CDC9* (33), *CDC21* (36), and *TOP2* (15), and the related sequence 5′ ACGTGT 3′ is found upstream of the coding region of the DNA primase gene (34).

DISCUSSION

In yeast, the isolated preparation of core DNA polymerase with catalytic activity has a heterogeneous composition, consisting of antigenically related polypeptides ranging from 140 kDa to 180 kDa in size (2, 5, 6). DNA sequence determination of the *POL1* gene indicates that native DNA polymerase I is a 1468-amino acid polypeptide with a calculated molecular weight of 166,794.

DNA polymerase is a complex enzyme that binds deoxynucleoside 5′-triphosphates, interacts with the template–primer molecule and with other protein of the DNA replication machinery, and probably has an associated proofreading exonuclease activity (37). The analysis of the POL1 protein sequence revealed helix-turn-helix motifs and a potential metal-binding domain that can be involved in the interactions with nucleic acids. Moreover, several DNA polymerases, including that of yeast, possess clusters of highly conserved amino acids, suggesting that their mode of action might require the conservation of critical residues. It has been shown that herpes simplex virus mutants in region II and III have altered sensitivity to antiviral drugs and aphidicolin, drugs that mimic or compete for binding with the natural deoxynucleoside 5′-triphosphates and pyrophosphate analogs (21, 30). Therefore, regions II and III, located in the carboxyl-terminal half of the protein, may contain the pyrophosphate- and nucleotide-binding sites. The amino-terminal half of the gene product might be involved in other physiological functions, such as interactions with other components of the DNA replication apparatus. We have isolated a DNA polymerase I mutant that appears to be defective in the assembly of a stable DNA polymerase–primase complex (8). This mutation has been mapped to the amino-terminal half of the protein, and it may define a functional domain necessary for productive protein–protein interactions. We have shown that POL1 transcription is under cell-cycle control (9). Detailed functional studies will be necessary to define the boundaries of the regulatory region and the DNA sequences controlling the fluctuation of POL1 mRNA during the cell cycle. The 700-bp sequence upstream of the *POL1* coding region contains the repeated hexanucleotide 5′ ACGCGT 3′ that is also present in the 5′-noncoding regions of other DNA synthesis genes (*CDC8*, *CDC9*, and *CDC21*) whose mRNAs fluctuate in the same pattern during the cell cycle as does POL1 mRNA (9, 38). It is tempting to speculate that the above sequence might be involved as a cis-acting element in the regulation of the expression of these genes during the cell cycle. However, the regulatory circuits governing the expression of these genes are likely to be very complex, because their transcripts are also induced during meiosis, but with

REGION I

Yeast	983	QLAESMNLLVVYGDTDSVMIDTGCDNYADA
HSV	873	----------YGDTDS----------A--
CMV	896	----------V-YGDTDSV----------
VZV	839	----------V-YGDTDSV-I----------
EBV	742	-L-----L-V-YGDTDS--I----------
Vaccinia	713	----------YGDTDSV----------
Ad 2	838	---E---L---YGDTDS----------
pGKL1	861	----------Y-DTDS----------A--
S-1	704	----------Y-DTDSV----------
ø29	440	--A--------Y-DTDS----------A--

REGION II

Yeast	843	YQGGLVFEPEKGLHKNYVLVMDFNSLYPSIIQEFNICFTT
HSV	696	YQG--V--P--G-H-N-V-V-DF-SLYPSIIQ--N-----
CMV	696	YQG--VFEPE-G-----V-V-DF-SLYPSII---N-C--T
VZV	662	Y-G---F-P--G-----V-V-DF-SLYPSIIQ--N-CFTT
EBV	563	YQG--V-EP--G-----VLV-DF-SLYPSIIQ--N-C--T
Vaccinia	503	Y-GG-VF-P------N-VL--D-NSLYP------N----T
Ad 2	522	--GG-------G-------V-D----Y-S----------
pGKL1	628	----------------VL--D--SLYP----------
S-1	464	----------D-NSLYPS----------T
ø29	225	----------K--------V-D-NSLYP----------

REGION III

Yeast	940	QQALKLTANSMYGCLGYVNSRFYAKPLAMLVTNKGREIL
HSV	807	Q-A-K---NS-YG--G----------A--VT--GRE-L
CMV	807	Q-ALK-T-N--YG--G-VN------P-A---T--GR--L
VZV	771	Q-A-K---NS-YG--G----------A--VT--GR--L
EBV	677	Q-A-K-T-N--YG--G--N--F-----A--VT--GR--L
Vaccinia	633	Q---K--ANS-YG--G--NS--Y----A---T--GR---
Ad 2	681	--A-KL--N--YG----------I-
pGKL1	780	----K---NS--G---------F----------E--
S-1	613	----K-T-NS-YG--G----------
ø29	375	----KL--NS-YG----------P--------G----

FIG. 3. Regions of homology in yeast DNA polymerase I and other DNA polymerases. The figure shows the three major homologous regions found in the DNA polymerases listed, and the amino acids in common with DNA polymerase I are indicated. A dash indicates no absolute conservation, but no attempt was made to distinguish between conservative and nonconservative changes. The numbers indicate the position of the first residue in each sequence. HSV, herpes simplex virus; CMV, human cytomegalovirus; VZV, varicella–zoster virus; EBV, Epstein–Barr virus; Vaccinia, vaccinia virus; Ad 2, adenovirus type 2; pGKL1, killer plasmid from *K. lactis*; S-1, mitochondrial particles from maize; ϕ29, bacteriophage ϕ29.

different kinetics, and some of them are induced by DNA damage (9, 38). It is, therefore, possible that different cis- and trans-acting elements regulate their expression.

We thank Dr. D. Martini and J. Malyszko (Farmitalia Carlo Erba, Milan) for assistance with the computer programs and for providing the synthetic oligonucleotides. We also thank M. Muzi for performing some experiments. P.V. was supported by a fellowship from Fondazione Anna Villa Rusconi. This work was partially supported by a grant from Progetto Finalizzato Ingegneria Genetica e Basi Molecolari delle Malattie Ereditarie, Consiglio Nazionale delle Ricerce, Rome, Italy.

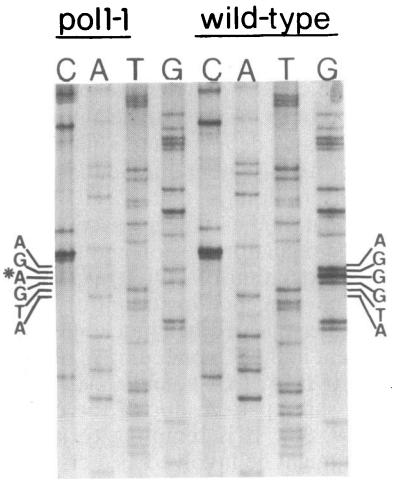

FIG. 4. DNA sequence of the *pol1-1* mutant gene. The DNA sequence of *pol1-1* shows a G·C → A·T transition (position +1477) compared to the wild-type allele.

1. Campbell, J. L. (1986) *Annu. Rev. Biochem.* **55**, 733–771.
2. Plevani, P., Foiani, M., Valsasnini, P., Badaracco, G., Cheriathundam, E. & Chang, L. M. S. (1985) *J. Biol. Chem.* **260**, 7102–7106.
3. Lucchini, G., Brandazza, A., Badaracco, G., Bianchi, M. & Plevani, P. (1985) *Curr. Genet.* **10**, 245–252.
4. Plevani, P., Lucchini, G., Foiani, M., Valsasnini, P., Brandazza, A., Bianchi, M., Magni, G. & Badaracco, G. (1987) *Life Sci. Adv.* **6**, 53–60.
5. Johnson, L. M., Snyder, M., Chang, L. M. S., Davis, R. W. & Campbell, J. L. (1985) *Cell* **43**, 369–377.
6. Lucchini, G., Francesconi, S., Foiani, M., Badaracco, G. & Plevani, P. (1987) *EMBO J.* **6**, 737–742.
7. Budd, M. & Campbell, J. L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2838–2842.
8. Lucchini, G., Mazza, C., Scacheri, E. & Plevani, P. (1988) *Mol. Gen. Genet.*, in press.
9. Johnston, L. H., White, J. H., Johnson, A. L., Lucchini, G. & Plevani, P. (1987) *Nucleic Acids Res.* **15**, 5017–5029.
10. Henikoff, S. (1984) *Gene* **28**, 351–359.
11. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5413–5417.
12. Hattori, M. & Sakaki, Y. (1986) *Anal. Biochem.* **152**, 232–238.
13. Strauss, E. C., Kobori, J. A., Siu, G. & Hood, L. E. (1986) *Anal. Biochem.* **154**, 353–360.
14. Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12**, 7035–7056.
15. Giaver, G., Lynn, R., Goto, T. & Wang, J. C. (1986) *J. Biol. Chem.* **261**, 12448–12454.
16. Wong, S. W., Paborsky, L. R., Fisher, P. A., Wang, T. S. F. & Korn, D. (1986) *J. Biol. Chem.* **261**, 7958–7968.
17. Hamilton, R., Watanabe, C. K. & deBoer, H. A. (1987) *Nucleic Acids Res.* **15**, 3581–3593.
18. Sharp, P. M., Tuohy, T. M. F. & Mosurski, K. R. (1986) *Nucleic Acids Res.* **14**, 5125–5143.
19. Pabo, C. O. & Sauer, R. T. (1984) *Annu. Rev. Biochem.* **53**, 293–321.
20. Berg, J. M. (1986) *Science* **232**, 485–487.
21. Larder, B. A., Kemp, S. D. & Darby, G. (1987) *EMBO J.* **6**, 169–175.
22. Gingeras, T. R., Sciaky, D., Gelinas, R. E., Bing-Dong, J., Yeu, L. E., Kelley, M. M., Bullock, P. A., Parsons, B. L., O'Neill, K. E. & Roberts, R. J. (1982) *J. Biol. Chem.* **257**, 13475–13491.
23. Earl, P. L., Jones, E. V. & Moss, B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3659–3663.
24. Yoshikawa, H. & Ito, J. (1982) *Gene* **17**, 323–335.
25. Baer, R., Bankier, A. T., Biggin, M. D., Deininger, P. L., Farrel, P. J., Gibson, T. J., Hutfull, G., Hudson, G. S., Satchwell, S. C., Seguin, C., Tuffnell, P. S. & Barrell, B. G. (1984) *Nature (London)* **310**, 207–211.
26. Davison, A. & Scott, J. E. (1986) *J. Gen. Virol.* **67**, 1759–1816.
27. Kouzarides, A., Bankier, A. T., Satchwell, S. C., Weston, K., Tomlinson, P. & Barrell, B. G. (1987) *J. Virol.* **61**, 125–133.
28. Stark, M. J. R., Mileham, A. J., Romanos, M. A. & Boyd, A. (1984) *Nucleic Acids Res.* **12**, 6011–6030.
29. Paillard, M., Sederoff, R. R. & Levings, C. S., III (1985) *EMBO J.* **4**, 1125–1128.
30. Gibbs, J. S., Chiou, H. C., Hall, J. D., Mount, D. W., Retondo, M. J., Weller, S. K. & Coen, D. M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7969–7973.
31. Wong, S. W., Wahl, A. F., Yuan, P. M., Arai, N., Pearson, B. E., Arai, K., Korn, D., Hunkapiller, M. W. & Wang, T. S. F. (1988) *EMBO J.* **7**, 37–47.
32. Sentenac, A. & Hall, B. D. (1982) in *The Molecular Biology of the Yeast S. cerevisiae*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), Vol. 2, pp. 561–606.
33. Barker, D. G., White, J. H. M. & Johnston, L. H. (1985) *Nucleic Acids Res.* **13**, 8323–8337.
34. Plevani, P., Francesconi, S. & Lucchini, G. (1987) *Nucleic Acids Res.* **15**, 7975–7989.
35. Birkenmeyer, L. G., Hill, J. C. & Dumas, L. B. (1984) *Mol. Cell. Biol.* **4**, 583–590.
36. Taylor, G. R., Lagosky, P. A., Storms, R. K. & Haynes, R. H. (1987) *J. Biol. Chem.* **262**, 5298–5307.
37. Cotteril, S. M., Reyland, M. E., Loeb, L. A. & Lehman, I. R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5635–5639.
38. Johnston, L. H., Johnson, A. L. & Barker, D. G. (1986) *Exp. Cell Res.* **165**, 541–549.