The nucleotide sequence of the PRII gene related to DNA primase in Saccharomyces cerevisiae

Paolo Plevani*, Stefania Francesconi and Giovanna Lucchini

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

Received July 30, 1987; Revised and Accepted September 11, 1987 Accession no. Y00458

ABSTRACT
The PRII gene of Saccharomyces cerevisiae encodes for the p48 polypeptide of DNA primase. We have determined the nucleotide sequence of a 1,965 bp DNA fragment containing the PRII locus. The entire coding sequence of the gene lies within an open reading frame, and there are 409 amino acids in the single polypeptide protein if translation is assumed to start at the first ATG in this frame. The 5' and 3' end-points of PRII mRNA have been determined by S1 mapping and primer extension analysis. The primary structure and the codon usage of PRII suggest that this essential gene is poorly expressed in yeast cells.

INTRODUCTION
Purified DNA polymerases from both prokaryotic and eukaryotic organisms are unable to initiate DNA synthesis de novo (1). Several evidences indicate that the nascent DNA chains (Okazaki fragments), found on the lagging strand of the replication fork, are synthesized by elongation of RNA primers (1). A specialized enzyme, called DNA primase, devoted to the synthesis of such oligoribonucleotides, is involved in the initiation of DNA replication (1). Recently, a DNA primase activity has been found to be associated with the replicative DNA polymerase purified from a variety of eukaryotic organisms (2). The polypeptide structure and the catalytic activity of these DNA polymerase-DNA primase complexes appear to be highly conserved, thus suggesting that they play some critical role in DNA replication. Because the mechanism of priming on the leading strand at an origin of replication may be similar to the initiation of synthesis on the lagging strand, it has been proposed that eukaryotic DNA polymerase-primase complexes might be involved in the forma-
tion of the initiation complex, rather than simply have a function in the elongation reaction (for a review, see ref.2).

We have purified the yeast DNA polymerase-primase complex by rapid immuno-affinity chromatography by using a mouse monoclonal antibody (3). A catalytic subunit exhibiting a heterogeneous composition of antigenically related polypeptides, ranging from 140 kDa to 180 kDa, has been identified as the DNA polymerase core enzyme (3,4). The corresponding gene has been cloned by using specific antibodies, and Southern analysis and gene disruption experiments showed that yeast DNA polymerase I is encoded by a single, essential gene (5-7). The mechanistic properties of yeast DNA primase have been extensively characterized, because this enzymatic activity can be easily dissociated from DNA polymerase and, eventually, the bifunctional complex can be reconstituted in vitro (3,8). DNA primase, in yeast as well as in other eukaryotic organisms, correlates with two polypeptides of approximately 48 kDa and 58 kDa (2). Even if it is not yet known whether the yeast p48 polypeptide itself is sufficient for DNA primase activity, an antiserum raised against this isolated polypeptide inhibits the enzymatic activity, thus providing the first direct evidence that this protein is related to DNA primase (4). We isolated the gene encoding the p48 polypeptide by using this specific antiserum and we demonstrated that it is unique in the yeast haploid genome and that its function is essential (4). We called this gene PRII, because it is the first cloned gene related to an eukaryotic DNA primase.

In order to facilitate further studies on the mechanistic and functional properties of DNA primase, we undertook the determination of the nucleotide sequence of the PRII gene. The knowledge of this sequence will be essential to overproduce the p48 polypeptide both in yeast and in E.coli, to construct thermosensitive mutants by site directed mutagenesis and to analyze the regulation of the expression of this essential enzyme involved in DNA replication. Moreover, the knowledge of the sequence of yeast DNA primase might be helpful in identifying genes performing similar functions in other eukaryotic cells.

The analysis of the sequence of a 1,965 bp segment containing the entire PRII gene revealed the presence of an open reading frame of 1,227 nucleotides which encodes for a protein of 47,623 daltons. The mapping of the 5' and 3'
end-points of PRII mRNA provides necessary information to study the mechanisms controlling PRII transcription.

MATERIALS AND METHODS

Subcloning and construction of deletions spanning the PRII gene.

The cloning of the PRII gene of Saccharomyces cerevisiae has been reported previously (4). The pGEM series of plasmids (Promega Biotec., Cambridge, MA) containing a polylinker cloning segment flanked by T7 and SP6 promoters was used in the sequencing work reported here. The PRII coding sequence is contained within a 1.92 kb NruI-SstI fragment (4), which was ligated into the SmaI and SstI sites of plasmid pGEM-2, to create the derivative plasmid pPP1. Exonuclease III was used to construct a set of unidirectional deletions according to the procedure described by Henikoff (9). Because exonuclease III fails to initiate digestion at DNA ends with four-base 3'-protrusions and digestion progresses at an uniform rate, an ordered set of deletion clones in the 5' to 3' direction of the PRII gene can be made by cutting pPP1 with BamHI and PstI before the exonuclease treatment. In fact, both these enzymes cut the plasmid only once in the polylinker region, being BamHI the most proximal to the PRII insert, while PstI leaves a 3'-four-base protrusion that protects the remainder of the vector from nuclease attack. Aliquots were removed from the exonuclease III digestion mixture at uniform intervals, treated with S1 nuclease, Klenow DNA polymerase, T4 DNA ligase and then used to transform E.coli competent cells (9). Plasmid DNA was prepared from several transformants at each time point and the extent of the deletions was determined by restriction analysis. Sequencing of the PstI-NruI region shown in Figure 1 and sequencing of the opposite DNA strand were achieved by subcloning appropriate restriction fragments into the pGEM-2 vector and by using either the T7 or SP6 promoter primers. In one case, the sequence in the 3' to 5' direction of a portion of the PRII gene was obtained by using as primer in the sequencing reaction a 20-mer synthetic oligonucleotide complementary to the sequence of the opposite strand previously determined.

DNA sequencing.

The subclones were sequenced by the dideoxy-chain termination method (10),
using denatured plasmid templates (11). Preparation of plasmid DNA and alkaline denaturation were performed essentially as described (11). The sequencing reactions were carried out with the Promega GemSeq™ K/RT system according to the instructions of the manufacturer, using $^{35}$S-dATP as the labeled nucleotide. The Klenow fragment of E.coli DNA polymerase was generally used, while AMV reverse transcriptase was utilized to resolve the ambiguities eventually found in the sequence ladder obtained with the first enzyme.

**Preparation of RNA and transcript mapping of the 5' and 3' end-points.**

Poly(A)$^+$ yeast RNA was prepared from strain TD28 (MATa, ura3.52, inos1, can1) as previously described (4). The S1 nuclease protection experiments were carried out essentially as described by Natsoulis et al. (12). Our probes to map the 5' and 3' end-points of the PRI1 transcript were, respectively, the NruI-ScaI and NdeI-SstI fragments of plasmid pPP1 labeled with T4 polynucleotide kinase at the ScaI site and with Klenow enzyme at the NdeI site. The fragments were hybridized to 20 and 40 µg of poly(A)$^+$ RNA and the quantities of S1 required for complete digestion of the non-protected fragments were determined empirically. Protected DNA was electrophoresed on a 6% polyacrylamide denaturing gel in parallel with the Maxam and Gilbert sequence (13) of the fragments used in the S1 mapping experiments.

The primer extension experiment was done with a synthetic primer 5' GTCTTTAC TGAATTGGTCAT 3' complementary to the PRI1 mRNA (+1 to +20) synthesized with an Applied Biosystems DNA synthesizer. The primer was hybridized to 20 and 40 µg of poly(A)$^+$ RNA and extended with AMV reverse transcriptase in the presence of $^{32}$P-TTP as the labeled nucleotide essentially as described by Giaver et al. (14). The products of the reaction were analyzed on a 8% sequencing gel together with a Sanger sequencing reaction of plasmid pPP1 performed using the same synthetic oligonucleotide as primer.

**Computer assisted analysis of the DNA sequence.**

DNA sequence data to deduce the amino acids composition of the PRI1 protein, the codon usage and the hydrophilicity profile were analyzed with an IBM microcomputer using the MicroGenie program (Beckman).
Figure 1. Restriction map and DNA sequencing strategy for the PRII region. The horizontal lines with arrowheads indicate the extent and direction of the sequenced fragments. The extent of the PRII open reading frame is indicated at the top of the figure.

RESULTS AND DISCUSSION

Sequencing strategy.

The cleavage sites of a number of 6 bp restriction enzymes in a previously cloned 2,450 bp region containing the entire yeast PRII gene is shown in Figure 1. From previous experiments it was known that the coding sequence was located to the right of the NruI site and it terminated before the SstI site. In fact, the 1.45 kb mRNA identified as the PRII mRNA was detected on Northern blots of yeast poly(A)^+ RNA only by using DNA probes spanning the NruI-BalI region. The upstream PstI-NruI fragment and the SstI-NcoI fragment, spanning a 1 kb region further downstream from the SstI site, identified on Northern blots two mRNA species of 0.92 kb and 2.25 kb respectively (data not shown), which correspond to transcripts of two unidentified yeast genes which lie on each side of the PRII locus. The direction of transcription of the PRII gene was known to be from left to right as demonstrated by in vitro transcription and translation of a pGEM-2 derivative plasmid in which the NruI-SstI fragment was inserted under the control of the phage T7 promoter (4).

To generate plasmids for direct sequencing with the dideoxy-chain termination method (10), plasmid pPP1 containing the NruI-SstI fragment inserted into the polylinker region of pGEM-2, was cut with PstI and BamHI and a set of targeted 5' deletions was created by unidirectional digestion with exonuclease III as described in Materials and Methods. A number of overlapping deletions was se-
sequenced by using the denatured plasmid template and the phage T7 promoter primer to directly obtain the sequence of the mRNA (non-coding) strand of the PRI1 gene. The opposite DNA strand was sequenced after subcloning suitable restriction fragments into pGEM plasmids and using T7 or SP6 phage promoter primers or synthetic oligonucleotides complementary to proper regions of the previously determined non-coding strand of the PRI1 gene (15). The large majority of the clones yielded high quality sequencing ladders, but the purity of the plasmid DNA is crucial for this DS sequencing method (11).

Nucleotide sequence of the yeast PRI1 gene.

Figure 2 depicts a sequence of 1,965 bp in the PRI1 region of the yeast Saccharomyces cerevisiae, that we previously located on chromosome IX (4). The indicated restriction sites deduced from the nucleotide sequence are in complete agreement with the independently found restriction map shown in Figure 1, within the limits of agarose gel resolution. Furthermore, the entire coding sequence of the yeast PRI1 gene is contained in the NruI-SstI region of the restriction map, as it was deduced from previous data (4).

An open reading frame (ORF) encoding 409 amino acids and ending with a TGA stop signal is identified within this sequence. The 1,227 bp ORF encodes a polypeptide with a calculated molecular weight of 47,623 daltons, provided that the first ATG downstream from the transcription start is recognized by the translation machinery. PRI1 encodes for one of the two polypeptides of yeast DNA primase and the polypeptide mass of this enzyme subunit has been estimated to be around 48,000 daltons from its electrophoretic mobility in polyacrylamide gel in the presence of sodium dodecyl sulfate (3). Therefore, it appears that the molecular weight of the PRI1 protein deduced by DNA sequence analysis and that of the p48 polypeptide of yeast DNA primase are in complete agreement.

There is a second in frame ATG codon at nucleotides +45 to +47 that could act as the initiator codon and it will generate a protein with a slightly reduced molecular weight of 46,210 daltons which could still be in reasonable accordance with the peptide mass of DNA primase determined by electrophoretic analysis. The gene encoding the yeast histidine-tRNA synthetase (HTS1) has two
Figure 2. Nucleotide sequence and deduced amino acids sequence of PRII. The nucleotide sequence is numbered from the ATG initiation codon of the PRII protein (+1). The amino acids sequence has been translated from the DNA sequence. The two major 5' end-points of the mature PRII transcripts are designated by horizontal arrows at position -47 and -61. The three closed circles at position +1373 (+1) indicate the 3' end-point of the PRII mRNA. The 6 bp restriction endonuclease recognition sites are also indicated in the sequence.

in frame translation start sites located 60 bp apart, and it has been shown that one set of HTS1 transcripts initiates upstream of both ATG codons, while the other set initiates between the two ATGs, and these messages encode the
mitochondrial and cytoplasmic hystidine-tRNA synthetase, respectively (12). Because transcription mapping of the PRI1 mRNA (see below) places the 5' end-points of the transcripts upstream to the first ATG codon, it will be necessary to destroy the ATGs by in vitro mutagenesis and/or to construct PRI1-lacZ fusions which include only the first or both the in frame ATGs to assess their role in the initiation of translation. Recent examination of the ribosome scanning hypothesis (18) indicates that, when the first AUG lies in an unfavorable nucleotide context and in the same reading frame there is a second AUG codon, ribosomes may initiate at both sites, producing long and short versions of the encoded polypeptide, and this could be the case for the PRI1 gene. The optimal context for the initiator AUG codon in higher eukaryotes is CCACCAUGG (16), while the consensus sequence for the AUG context in highly expressed yeast genes is AAAAAAUGUC (17). Neither one of these sequences is found at the two possible translation initiation codons of PRI1. A 72 bp long ORF is also present upstream to the 5' transcription start at position -201 to -126. However, the function, if any, of this short ORF is, at the present, unknown.

**Codon usage in PRI1 and the predicted structure of its gene product.**

As it is shown in Table 1, translation of the 1,227 bp ORF of the PRI1 gene indicates that the PRI1 protein has 40.3% non polar, 27.9% polar, 17.1% positively charged and 14.7% negatively charged amino acids. This represents a random distribution of the four types of amino acids. Table 1 also shows the frequency of codon usage. It has been shown that there is a correlation between the extent of codon bias within a gene and the level of both mRNA and protein expression (19,20). An examination of the codon usage of the PRI1 gene reveals that there is little if any codon bias. In fact, except for the CGC arginine codon, all the other 60 possible codons are used in PRI1. This pattern of codon usage is common for poorly expressed yeast genes and it is in agreement with our observation that PRI1 mRNA is low in abundance. The codon usage of the highly expressed ADH1 gene is also shown in Table 1 to facilitate a direct comparison (27).
### Table 1.

#### A. Codon usage in the PRII protein.

| Amino Acid | PRII | ADNI |
|------------|------|------|
| Phe(f) | TTT 10(2.4) 48 0 | Tyr(Y) TAC 11(2.7) 73 0 |
| Phe | TIC 11(2.7) 57 0 | Tyr TAC 4(1.0) 27 100 |
| Leu(1) | TTA 11(2.7) 28 0 | Ter TAA - - |
| Leu | TIG 13(3.2) 33 79 | Ter TAG - - |
| Leu | CIT 4(1.0) 10 0 | His(H) CAI 6(1.5) 67 9 |
| Leu | CTC 4(1.0) 10 0 | His CAC 3(0.7) 33 91 |
| Leu | CTA 3(0.7) 8 13 | Gln(Q) CAA 13(3.7) 87 100 |
| Leu | CTC 4(1.0) 10 0 | Gln CAG 2(0.5) 13 0 |
| Ile(1) | ATT 12(2.9) 53 42 | Asn(N) AA1 13(3.7) 67 0 |
| Ile | ATC 4(1.0) 18 57 | Asn AAC 8(2.0) 38 100 |
| Ile | ATA 6(1.5) 27 0 | Lys(K) AAA 19(4.8) 53 17 |
| Met | ATG 9(2.7) 100 100 | Lys AAG 17(4.2) 47 83 |
| Val(Y) | GTT 4(1.0) 21 53 | Asp(D) GAT 25(6.1) 71 13 |
| Val | GTC 3(0.7) 16 47 | Asp GAC 10(2.4) 29 87 |
| Val | GTA 5(1.7) 26 0 | Glu(E) GAA 18(4.4) 72 100 |
| Val | GTG 7(1.7) 37 0 | Glu GAG 7(1.7) 28 0 |
| Ser(S) | TCT 9(2.2) 31 0 | Cys(C) TGT 5(1.2) 63 100 |
| Ser | TCC 3(0.7) 10 33 | Cys TGC 3(0.7) 37 0 |
| Ser | TCA 8(2.0) 26 0 | Ter TGA - - |
| Ser | TCG 3(0.7) 10 0 | Trp(W) TGG 7(1.7) 100 100 |
| Pro(P) | CCA 12(2.9) 55 15 | Arg(R) CGT 5(1.2) 20 0 |
| Pro | GAC 1(0.5) 9 0 | Arg CCG 0(0.0) 0 0 |
| Pro | CAA 5(1.7) 23 77 | Arg CGA 2(0.5) 8 0 |
| Pro | GGG 3(0.7) 14 0 | Arg CGG 7(1.5) 8 0 |
| Thr(T) | ACT 5(1.2) 31 36 | Ser AG1 10(2.4) 3 0 |
| Thr | ACC 1(0.2) 6 64 | Ser AGC 5(1.2) 17 0 |
| Thr | ACA 5(1.2) 31 0 | Arg AGA 10(2.4) 40 100 |
| Thr | ACC 5(1.2) 31 0 | Arg AGG 6(1.5) 24 0 |
| Ala(A) | GCC 12(2.9) 46 54 | Gly(G) GGT 6(1.5) 60 93 |
| Ala | GCC 6(1.5) 23 46 | Gly GGC 1(0.2) 10 7 |
| Ala | GCA 7(1.7) 27 0 | Gly GGA 1(0.2) 10 0 |
| Ala | GCC 1(0.2) 4 0 | Gly GGG 2(0.5) 20 0 |

#### B. Predicted amino acid composition of the PRII protein

| Amino Acid | % | Amino Acid | % | Amino Acid | % | Amino Acid | % |
|------------|---|------------|---|------------|---|------------|---|
| Ala | 28 6.4 | Glu | 15 3.7 | Leu | 39 9.5 | Ser | 29 7.1 |
| Arg | 25 6.1 | Glu | 25 6.1 | Lys | 36 8.8 | Thr | 16 3.9 |
| Asn | 21 5.1 | Gly | 10 2.4 | Met | 9 2.7 | Trp | 7 1.7 |
| Asp | 35 8.6 | His | 9 2.2 | Phe | 21 5.1 | Tyr | 15 3.7 |
| Cys | 8 2.0 | Ile | 22 5.4 | Pro | 27 5.4 | Val | 10 4.6 |

7983
Figure 3. Hydrophilicity profile and secondary structure of the mature PRI1 amino acids sequence predicted from DNA sequence analysis. Positive and negative numbers correspond to hydrophilic and hydrophobic regions, respectively (22). \( \alpha = \alpha \)-helix; \( \beta = \beta \)-sheet; \( T = \beta \)-turn.

The local secondary structure and the hydrophilicity value of the PRI1 gene product were predicted by computer assisted analysis (21,22). As it is shown in Figure 3, several regions of \( \alpha \)-helical structure of 10 or more contiguous amino acids were located at residues 43-52, 94-114, 136-151, 214-233, 294-313, 320-329, 354-363, 387-403. The longest contiguous \( \beta \)-turns and \( \beta \)-sheets regions were located at residues 124-135 and 179-195, respectively. The hydrophilicity index of the PRI1 protein (Figure 3), might be of some interest, because hydrophilicity values have been shown to be useful to predict protein antigenic determinants from amino acids sequences (22). In fact, the point of highest local average hydrophilicity is invariably located in, or adjacent to, an antigenic determinant. Our attempts to produce specific monoclonal antibodies against the DNA primase moiety of the yeast DNA polymerase-DNA primase complex have been until now unsuccessful (7). The use of proper synthetic peptides derived from the PRI1 sequence will possibly help us to reach this goal. Such specific immunological reagents can be very useful in purifying yeast proteins interacting in vivo with DNA primase. In fact, the yeast DNA polymerase-primase complex itself was purified by using a monoclonal antibody raised against isolated DNA polymerase I (3).

Transcription mapping of the PRI1 gene.

Poly(A)' RNA prepared from Saccharomyces cerevisiae strain TD28 was used in
Figure 4. Determination of the 5' and 3' end-points of the mature PRII transcript. The 5' termini of the PRII transcripts were mapped by S1 protection (Panel A) and primer extension (Panel B), according to the procedures described in Materials and Methods. Panel C, shows the S1 mapping of the 3' end-point of PRII transcription. Lanes 1 and 2 show the results obtained when using 20 and 40 μg, respectively, of poly(A)+ RNA in the S1 mapping and primer extension experiments. After treatment with S1, the protected fragments were run on a 6% sequencing gel (Panel A and C). The products of the primer extension shown in Panel B were analyzed on a 8% sequencing gel.

S1 mapping experiments (23) and for primer extension with AMV reverse transcriptase (24) to identify the 5' end-points of the mature PRII message. Both methods allow to measure the distance between the 5' mRNA terminus and a site whose exact position in the sequence is known.

To map the 5' terminus by S1 digestion, plasmid pPPl was cut with ScaI, 5' end labeled with T4 polynucleotide kinase and [γ-32P]-ATP and the NruI-ScaI fragment was used as probe. As shown in Figure 4, S1 mapping of the 5' terminus
identifies two major bands corresponding to two mRNA start sites at position -61 and -47 and the strength of the signal was dependent upon the concentration of poly(A)$^+$ mRNA. The existence of these two transcription initiation points was confirmed by primer extension experiments, using a synthetic oligonucleotide complementary to nucleotide +1 to +20 of the mRNA sequence which was incubated with poly(A)$^+$ RNA in the presence of reverse transcriptase and triphosphates (Figure 4). Again, the intensity of the signals increased by doubling the RNA concentration.

A comparison of a number of yeast leader sequences shows that transcription initiation occurs at the nucleotide sequence PyAAPu (25), but this does not seem to be a strict rule, since other yeast genes initiate transcription into a different nucleotide context (14). The sequence GAAAAG is found at the PRI1 upstream transcription start and the related sequence GAAG is found at the 5' end of the CDC9 (DNA ligase) mRNA (27). A similar motif is present in PRI1 at position -126 to -121 and the sequences GAAG, GAAAG, GAAAAG are also found in the 5' non-coding region of other yeast genes such as CDC8 (thymidylate kinase) and CDC9 (26,27). The PRI1 gene is periodically transcribed around the G1/S phase boundary in the same interval as the yeast DNA synthesis genes CDC8, CDC9, CDC21 (thymidilate synthetase) and POL1 (DNA polymerase I) (28).

Once complete sequence and deletion analysis data will be available, a comparison of the upstream regions of these genes will eventually identify sequence similarities suggesting possible common regulatory mechanisms. The consensus sequence TATAAA (29) for the yeast TATA box is not found in the PRI1 gene 5' to the transcription start, but an identical sequence is located at position -10 to -5. In many of the yeast genes characterized to date, TATA like sequences are quite heterogeneous and may be found at distances varying from 50-150 nucleotides from the transcription start (30). Furthermore, many of the poorly expressed yeast genes do not have a recognisable TATA homology (31), and this indeed appears to be the case not only for the PRI1 gene, but also for the CDC9 and TOP2 genes (14,27). Perhaps for the same reason, a sequence complementary to the yeast 18S rRNA (32) cannot be found in these poorly expressed yeast genes. However, the PRI1 5' non-coding region has a high
A+T content, which is typical of all the yeast promoter regions (17). From position -200 to -1 the A+T composition is 67.5% as compared to 56.5% in the PRI1 coding region.

The determination of the 3' end-point of the PRI1 transcript was performed by labeling plasmid pPPl at the NdeI site and by using the NdeI-SstI fragment as probe in S1 nuclease protection experiments. As it is shown in Figure 4 panel C, the 3' end-point of the PRI1 transcript maps at position +1373(±1). This site is located 142 bp beyond the TGA termination codon. The sequence between the termination codon and the 3' end-point is 64% A+T, a characteristic commonly found in other yeast genes. The specific signals responsible for polyadenylation and transcription termination in Saccharomyces cerevisiae are not well defined. Sequences similar to the consensus elements proposed by Bennetzen and Hall (33), Zaret and Sherman (34) or Henikoff et al. (35) are not readily apparent in the 3' non-coding region of the PRI1 gene, except for the presence of short runs of T residues in the region between the termination codon and the 3' end-point. The sequence AATAA, which is found necessary for the polyadenylation of many genes from higher eukaryotes (34), is found within a larger sequence located at position +1251 to +1258. A deletion analysis in the 3' region of the PRI1 gene or nuclear runoff experiments will be necessary to determine the effect of specific sequences on the PRI1 transcript stability and on its translation efficiency and to distinguish between polyadenylation and termination of transcription.

The PRI1 gene of the yeast Saccharomyces cerevisiae is the first sequenced gene related to an eukaryotic DNA primase. A search for possible homology with prokaryotic DNA primases such as the E.coli dnaG protein (36), the T7 gene 4 protein (37) and the T4 genes 41 and 61, did not reveal any significant homology. The polypeptide structure of eukaryotic DNA primases seems to be highly conserved. In fact, DNA primase is associated with 48 kDa and 58 kDa polypeptides in the DNA polymerase-DNA primase complexes purified from several eukaryotic organisms (2). Once other eukaryotic DNA primase genes will be identified and characterized, it will be very interesting to evaluate whether the above conserved structure is also maintained at the amino acids level,
Nucleic Acids Research

thus defining possible functional domains in an essential enzyme involved in the initiation of DNA replication.

ACKNOWLEDGMENTS

This work was partially supported by a grant from Progetto Finalizzato Ingegneria Genetica e Basi Molecolari delle Malattie Ereditarie, CNR, Roma, Italy.

*To whom correspondence should be addressed

REFERENCES

1. Kornberg, A. (1980) DNA replication, W.H.Freeman and Co.,San Francisco.
2. Campbell, J.L. (1986) Ann.Rev.Biochem. 55, 733-771
3. Plevani, P., Foiani, M., Valsasnini, P., Badaracco, G., Cherithundam, E. and Chang, L.M.S. (1985) J.Biol.Chem. 260, 7102-7107.
4. Lucchini, G., Francesconi, S., Foiani, M., Badaracco, G. and Plevani, P. (1987) EMBO J. 6, 737-742.
5. Lucchini, G., Brandazza, A., Badaracco, G., Bianchi, M. and Plevani, P. (1985) Curr.Genet. 10, 245-252.
6. Johnson, L.M., Snyder, M., Chang, L.M.S., Davis, R.W. and Campbell, J.L. (1985) Cell. 43, 369-377.
7. Plevani, P., Lucchini, G., Foiani, M., Valsasnini, P., Brandazza, A., Bianchi, M., Magni, G. and Badaracco, G. (1987) Life Sci. Adv. 6, 53-60.
8. Badaracco, G., Valsasnini, P., Foiani, M., Benfante, R., Lucchini, G. and Plevani, P. (1986) Eur.J.Biochem. 161, 435-440.
9. Henikoff, S. (1984) Gene. 28, 351-359.
10. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc.Natl.Acad.Sci.USA. 74, 5413-5417.
11. Hattori, M. and Sakaki, Y. (1986) Anal.Biochem. 152, 232-238.
12. Natsoulis, G., Hilger, F. and Fink, G.R. (1986) Cell. 46, 235-243.
13. Maxam, A. and Gilbert, W. (1980) Methods Enzymol. 65, 499-560.
14. Giaver, G., Lynn, R., Goto, T. and Wang, J.C. (1986) J.Biol.Chem. 261, 12448-12454.
15. Strauss, E.C., Kobori, J.A., Siu, G. and Hood, L.E. (1986) Anal.Biochem. 154, 353-360.
16. Kozak, M. (1986) Cell. 44, 283-292.
17. Hamilton, R., Watanabe, C.K. and deBoer, H.A. (1987) Nucleic Acids Res. 15, 3581-3593.
18. Kozak, M. (1986) Cell. 47, 481-483.
19. Bennetzen, J.L. and Hall, B.D. (1982) J.Biol.Chem. 257, 3026-3031.
20. Sharp, P.M., Tuohy, T.M.F. and Mosurski, K.R. (1986) Nucleic Acids Res. 14, 5125-5143.
21. Chou, P.Y. and Fasman, G.D. (1978) Ann.Rev.Biochem. 47, 251-276.
22. Hopp, T.P. and Woods, K.R. (1981) Proc.Natl.Acad.Sci.USA. 78, 3824-3828.
23. Berk, A.J. and Sharp, P.A. (1978) Proc.Natl.Acad.Sci.USA. 75, 1274-1278.
24. Sollner-Webb, B. and Reeder, R.H. (1979) Cell. 18, 485-499.
25. Burke, R.L., Tekamp-Olsen, P. and Najarian, R. (1983) J. Biol. Chem. 258, 2193-2201.
26. Birkenmeyer, L.G., Hill, J.C. and Dumas, L.B. (1984) Mol. Cell. Biol. 4, 583-590.
27. Barker, D.G., White, J.H.M. and Johnston, L.H. (1985) Nucleic Acids Res. 13, 8323-8337.
28. Johnston, L.H., White, J.H.M., Johnson, A.L., Lucchini, G. and Plevani, P. Nucleic Acids Res. (in press).
29. Sentenac, A. and Hall, B.D. (1982) in The Molecular Biology of the Yeast S. cerevisiae (Strathern, J.N., Jones, E.W., Broach, J.R., Eds.) vol.2, pp.561-606, Cold Spring Harbor Laboratory, Cold Spring Harbor N.Y.
30. Reynolds, P., Higgins, D.R., Prakash, L. and Prakash, S. (1985) Nucleic Acids Res. 13, 2357-2372.
31. Laughon, A. and Gesteland, R.F. (1984) Mol. Cell. Biol. 4, 260-267.
32. Shine, J. and Dalgarno, L. (1974) Biochem. J. 141, 609-615.
33. Bennetzen, J.L. and Hall, B.D. (1982) J. Biol. Chem. 257, 3018-3025.
34. Zaret, K.S. and Sherman, F. (1982) Cell. 28, 563-573.
35. Henikoff, S., Kelly, J.D. and Cohen, E.H. (1983) Cell. 33, 607-614.
36. Smiley, B.L., Lupski, J.R., Svec, P.S., McMacken, R. and Godson, G.N. (1982) Proc. Natl. Acad. Sci. USA. 79, 4550-4554.
37. Dunn, J.J. and Studier, F.W. (1983) J. Mol. Biol. 166, 477-535.