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Yeast DNA Primase Is Encoded by a 59-Kilodalton Polypeptide: Purification and Immunochemical Characterization†

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ABSTRACT: The DNA primase from the yeast *Saccharomyces cerevisiae* has been purified 9200-fold to homogeneity. The yeast DNA primase is a monomeric protein of molecular weight 59 000, and under conditions described in this report, it is stable at 4 or −80 °C. The primase does not bind to DEAE-cellulose, is not inhibited by a high concentration of α-amanitin (4 mg/mL), and is capable of synthesizing small (up to 15 nucleotides in length) ribo or ribo–deoxy mixed initiator RNA primers. The primer synthesis is stimulated by ATP; however, other ribonucleotides could be replaced by deoxynucleotides without any measurable effect on the overall DNA synthesis. Thus, the purified primase is distinct from the RNA polymerases of *S. cerevisiae*. Immunoblot analysis of the polypeptides in a crude cell extract using a mouse polyclonal antibody prepared against the highly purified primase indicates that the 59-kilodalton polypeptide is the native form and not a degraded form of a larger polypeptide; however, primase is degraded rapidly to smaller polypeptides by yeast proteases especially in the absence of protease inhibitors.

The DNA primase activity has been shown to be essential for the synthesis of primers that enable DNA polymerases to initiate synthesis of Okazaki fragments in the lagging strand of the replication fork (Kornberg, 1980; Oertel & Goulian, 1977). The primase activity was demonstrated in *Escherichia coli* (*E. coli*)[1] to be distinct from the RNA polymerase, and the purified primase has been shown to be a product of the DnaG gene of *E. coli* (Bouche et al., 1975; Zechel et al., 1975; Rowen & Kornberg, 1978). Recent studies in several laboratories (Conaway & Lehman, 1982a,b; Tseng & Ahlem, 1982; Yagura et al., 1983) demonstrated that in eukaryotes, the DNA primase activity is associated at least in part with the DNA polymerase α. Vishwanatha and Baril (1986) have demonstrated recently that HeLa cell DNA primase is a 70-kDa polypeptide and could be separated from primase–polymerase complex by hydrophobic chromatography.

In the yeast *Saccharomyces cerevisiae*, a large fraction of the cellular primase activity remains free (Singh & Dumas, 1984; Wilson & Sugino, 1985; Jazwinski & Edelman, 1985). However, attempts toward purification of this free primase activity have led to ambiguities with respect to its mass, specific activity, subunit structure, and physical properties (Jazwinski & Edelman, 1985; Wilson & Sugino, 1985; Plevani et al., 1985). As primase is a major component of the chromosomal apparatus, purification and characterization of primase are important in deciphering the mechanism of eukaryotic chromosomal DNA replication. The present study has been directed toward developing an appropriate purification protocol for primase, establishing its subunit structure and size, and preparing a polyclonal antibody against primase for immunological characterization and molecular cloning of its gene.

Materials and Methods

Yeast. S. cerevisiae (wild-type bakers' yeast) was obtained as a gift from the American Yeast Corp., Baltimore, MD, and was removed from the fermenter during mid log phase.

Nucleotides, Enzymes, and DNA. All ribo- and deoxynucleotides were obtained from ICN. All radioactive nucleotides were obtained from New England Nuclear. Single-stranded M13 phage DNA was prepared according to the published procedure (Ray, 1969). Calf thymus DNA, yeast tRNA, and α-amanitin were obtained from Sigma Chemical Co. Calf thymus DNA was activated with DNase I according to the procedure of Fansler and Loeb (1974). Poly(dT), de-

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[1] Abbreviations: *E. coli*, *Escherichia coli*; *S. cerevisiae*, *Saccharomyces cerevisiae*; PMSF, phenylmethanesulfonyl fluoride; pepA, pepstatin A; DTT, dithiothreitol; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; ssDNA, single-stranded DNA; Me_2SO, dimethyl sulfoxide; BSA, bovine serum albumin; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography; Bis, *N,N'*-methylenebis(acrylamide); kDa, kilodalton(s); pol I, polymerase I; NEM, *N*-ethylmaleimide.

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phosphorylated $(dA)_8$, $(dA)_{15}$, and polynucleotide kinase were purchased from Pharmacia–P-L Biochemicals. *E. coli* DNA polymerase I was received as a gift from Dr. William E. Brown of Carnegie Mellon University and was further purified by HPLC gel filtration chromatography. Proteinase K was obtained from Boehringer Mannheim.

Chemicals and Chromatographic Supplies. All chemical reagents used to prepare buffers were of analytical reagent grade and were purchased from J. T. Baker. Spectrophotometric-grade dimethyl sulfoxide (Me_2SO) was purchased from Aldrich Chemical Co. Phosphocellulose (P-11) and DEAE-cellulose (DE-52) were purchased from Whatman; Affigel-blue, the HPLC TSK-250 gel filtration column, and all electrophoresis chemicals were from Bio-Rad. Benzamidine hydrochlorides, PMSF, EDTA, EGTA, NP-40, pep A, leupeptin, and the protein standards were from Sigma Chemical Co. DTT was from Calbiochem.

Buffers. Buffer A was 100 mM Tris-HCl (pH 8.0), 10% glycerol, 200 mM KCl, 5 mM $MgCl_2$, and 1 mM EDTA. Buffer B-X consisted of 25% glycerol, 1 mM EDTA, 1 mM EGTA, *X* mM potassium phosphate (pH 7.4) (where "*X*" represents the concentration of potassium phosphate in millimolar), 0.5 mM PMSF, 10 mM benzamidine hydrochloride, 5 mM DTT, and 10 μg/mL each of pep A and leupeptin. Buffer C-X was 25 mM Tris-HCl (pH 7.5), 20% glycerol, 5% Me_2SO, 1 mM EDTA, 0.5 mM PMSF, 0.01% NP-40 (unless otherwise indicated), *X* mM KCl (where "*X*" represents the concentration of KCl in millimolar), 8 mM DTT, and 5 μg/mL each of pepA and leupeptin. Buffer D was composed of 200 mM potassium phosphate, (pH 7.0), 50 mM $(NH_4)_2SO_4$, 20% glycerol, 5% Me_2SO, 0.02% NP-40, and 5 μg/mL each of pepA and leupeptin. Buffer E was 50% glycerol, 125 mM Tris-HCl (pH 7.5), 800 μg/mL BSA, 40 mM DTT, and 40 μg/mL each of pepA and leupeptin.

Primase Assay. The assay was carried out essentially as described by Conaway and Lehman (1982a). Briefly, the reaction mixture contained 5 μL of buffer E, 10 mM $MgCl_2$, 1000 pmol (as nucleotide) of M13 and mp18 ssDNA, 4 mM ATP, 125 μM each of GTP, CTP, and UTP, 100 μM each of [^{3}H]dTTP (200–400 cpm/pmol), dATP, dGTP, and dCTP, 1 unit of *E. coli* DNA polymerase I, and an appropriate amount of primase sample in a total volume of 25 μL and was incubated for 30 min at 30 °C. The reactions were terminated by adding 1 mL of 10% trichloroacetic acid/0.1 M sodium pyrophosphate followed by chilling in ice. Samples were allowed to stand in ice for 15 min so that the precipitation of the products could occur. The precipitates were collected on Whatman GF/C filters and washed with 20 mL of 1 N HCl/0.1 M sodium pyrophosphate followed by 5 mL of ethanol. The filters were dried under a heat lamp and counted in a toluene-based scintillator using a Packard Tri-Carb liquid scintillation spectrometer. One unit of DNA primase activity is defined as that amount of activity which incorporates 1 nmol of nucleotide in 30 min at 30 °C.

Polymerase Assay. The assay was carried out essentially as described for the primase assay except M13 ssDNA, rNTPs, and pol I were omitted and 3 μg of activated calf thymus DNA was added to the reaction mixture. The remainder of the assay was identical.

Other Assays. The ATPase assay was carried out as described (Biswas et al., 1986; Biswas & Biswas, 1987). The exodeoxyribonuclease assay was carried out in a manner similar to that described by Skarnes et al. (1986).

Protein Assay and Gel Electrophoresis. Protein concentrations were determined by the method of Bradford (1976) with bovine serum albumin as a standard. Polyacrylamide slab gel electrophoresis was carried out according to the procedure of Laemmli (1970).

Primer Analysis. The reaction conditions were essentially the same as described under Primase assay except that DNA polymerase I and all deoxynucleotides were omitted. Twenty nanograms of purified primase per reaction was examined for primer synthesis on φX174, M13mp18 ssDNA, and poly(dT) templates. In addition, the reaction mixture contained 20 μCi of [α-^{32}P]ATP and 50 μM each of the four rNTPs [for poly(dT), only 50 μM ATP was present]. The reactions were terminated by the addition of 5 μL of 5% SDS/375 mM EDTA. Yeast tRNA (10 μg) and proteinase K (10 μg) were added to each sample, and the samples were incubated at 37 °C for 60 min. The primers were extracted successively with phenol/chloroform, chloroform, and ether. The rpimers were precipitated with 0.3 M sodium chloride and 2.5 volumes of ethanol at −80 °C for 30 min. The precipitates were collected by centrifugation at 4 °C in a microfuge. The pellets were dried and resuspended in 50% Me_2SO, 100 mM Tris–borate (pH 8.3), 1 mM EDTA, 0.1% Xylene cyanol, and 0.1% Bromophenol blue and then placed in a 100 °C bath for 3 min, followed immediately by chilling in an ice/water bath. The samples were loaded on a preelectrophoresed 20% polyacrylamide (acrylamide:Bis ratio = 19:1) gel containing 8 M urea and electrophoresed in 100 mM Tris–borate, pH 8.3, and 1 mM EDTA at a constant power of 18 W for 1–2 h. The gels were autoradiographed for 12 h at −80 °C, using Kodak XAR-5 film.

Production of Primase Antiserum. Three female Balb/C mice, 8 weeks old, were primed by injecting subcutaneously with 10 μg of the highly purified primase (fraction VII) in complete Freund's adjuvant. Booster injections of 10 μg of primase protein in incomplete Freund's adjuvant were given after 3 weeks and repeated at 2-week intervals. Samples of blood were obtained by tail bleeding. The sera were tested for primase antibody by their ability to neutralize primase activity in a standard primase assay.

RESULTS

Primase Purification. (A) Preparation of Yeast Extract. Fresh yeast cells were taken directly from the fermenter at mid log phase (6–7 h after infection) and chilled in ice. The subsequent steps were carried out at 0–4 °C. The cells were sedimented at 5000 rpm for 5 min in a GS3 rotor. The cells were washed several times with water and once with buffer A and resuspended in buffer A containing 5 mM DTT, 5 mM sodium bisulfite, 1 mM PMSF, and 5 μg/mL each of pepA and leupeptin to OD_{600} = 1000. The resuspended cells were homogenized by grinding in a Biospecs bead beater. Following cell rupture, the pH of the suspension was adjusted to 8.0 with 2 M Tris. Ammonium sulfate was added to 0.056 g/mL, and the mixture was stirred for 30 min. The mixture was centrifuged at 100000*g* for 60 min; the supernatant was filtered through a VWR 617 filter, and the primase and polymerase activities were precipitated by the addition of 0.19 g/mL $(NH_4)_2SO_4$ followed by stirring for 60 min at 0 °C. The precipitate was centrifuged at 100000*g* for 60 min. The pellets (fraction II) were stored at −80 °C.

(B) Phosphocellulose Chromatography. A 200-mL phosphocellulose column was equilibrated with buffer B-20. The primase fraction II pellets were resuspended in buffer B-20. The homogenate was dialyzed for 4 h in buffer B-20. The dialyzate was clarified by centrifugation at 19000 rpm for 30 min in an SS 34 rotor. The supernatant solution was filtered through a Whatman No. 1 filter, diluted with 20% glycerol,

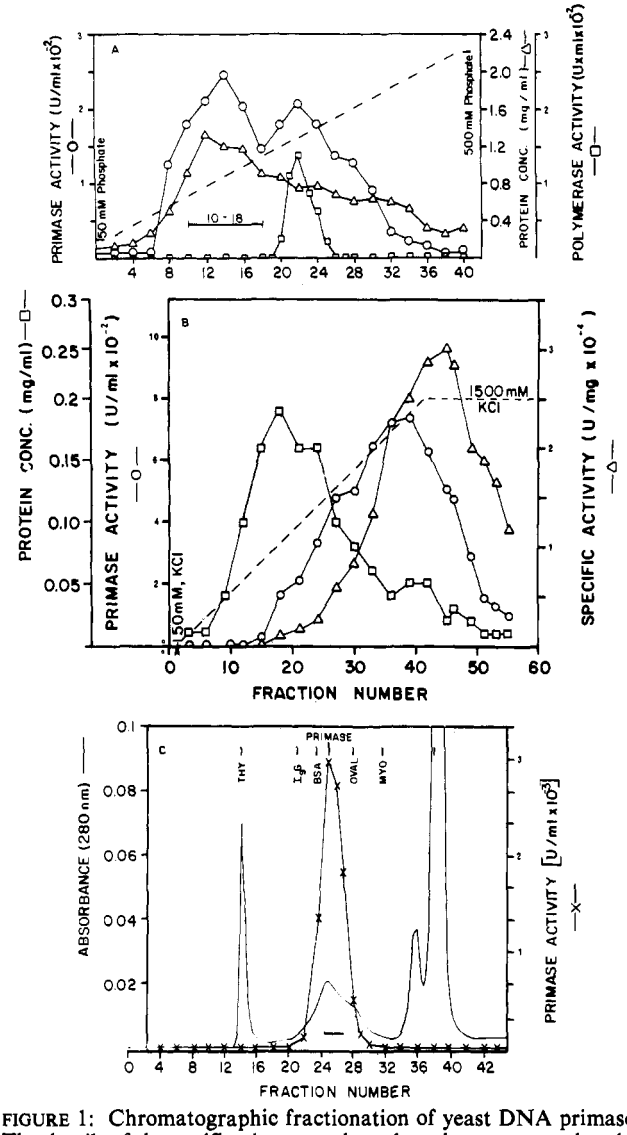

FIGURE 1: Chromatographic fractionation of yeast DNA primase. The details of the purification procedures have been presented under Results. (A) Phosphocellulose chromatography; (B) Affigel-blue chromatography; (C) size-exclusion high-performance liquid chromatography (size markers were as follows: THY, thyroglobulin; IgG, immunoglobulin G; BSA, bovine serum albumin; OVAL, ovalbumin; MYO, myoglobin).

8 mM DTT, and 1 mM EDTA (pH 7.5) to the conductivity of buffer B-40, and loaded on the phosphocellulose column. The column was washed with 100 mL of buffer B-20 and 200 mL of buffer B-50. A 1000-mL gradient of buffer B-50 to B-500 was used to fractionate the primase and polymerase activities. Twenty-milliliter fractions were collected in tubes containing 20 μL of 10% NP-40. Following assays for protein, primase, and polymerase activities, the DNA polymerase free primase activity (fractions 10–18) was pooled as shown in Figure 1A (fraction III).

(*C*) *Affigel-blue Chromatography.* The primase pool (fraction III), devoid of DNA polymerase activity, obtained from the phosphocellulose chromatography was dialyzed extensively in buffer C-20. The dialyzate was adjusted to the conductivity of buffer C-50, made 8 mM in $MgCl_2$, and loaded on a 20-mL Affigel-blue column equilibrated with buffer C-20 containing 8 mM $MgCl_2$. The flow through was recycled through the column once, and the column was washed with 200 mL of buffer C-50. A 250-mL gradient of buffer C-50 to C-1500 was used to fractionate the primase activity. Following the gradient, the column was washed with 100 mL of buffer C-1500. The fractions were assayed for protein, primase, and polymerase activities. The results of the fractionation are shown in Figure 1B. Fractions containing high primase activity (fractions 36–52) were pooled (Figure 1B).

Table I: Purification Table[a]

fraction	act. (units)	protein (mg)	sp act. (units/mg)	purification (*x*-fold)
fraction II[b] (ammonium sulfate)	1.6×10^4	890	18	1
fraction III (phosphocellulose)	2.5×10^4	100	250	14
fraction IV (Affigel-blue)	2.0×10^4	1.2	17000	930
fraction V (DEAE-cellulose)	1.6×10^4	0.5	32000	1800
fraction VI[c] (HPLC)	1.4×10^4	0.085	165000	9200

[a] Purification from 300 g of cells. [b] The activities of fractions I and II are highly variable due to the presence of inhibitors, nucleases, etc. [c] The overall yield varies between 20% and 40%, perhaps due to slight variations in the purification conditions.

(*D*) *High-Performance Liquid Chromatography.* Primase (fraction IV pool) was concentrated to approximately 10 mL in an Amicon ultrafiltration cell using an Amicon YM30 membrane. The concentrated fraction IV was dialyzed against 500 mL of buffer C-20 and then passed slowly through a 10-mL DEAE-cellulose column equilibrated with buffer C-30. The column was washed with an additional 30 mL of buffer C-30, and 3-mL fractions were collected. The fractions were assayed for protein and primase activity. The active fractions were concentrated to a volume of approximately 1 mL by ultrafiltration as described earlier (fraction V). A 200-mL aliquot of primase fraction V was injected in a Varian liquid chromatograph apparatus equipped with a Bio-Rad TSK 250SW gel filtration column equilibrated with buffer D. Fractions of 400 μL were collected at 0 °C. The chromatography was repeated in order to fractionate the entire pool, and fractions in each run were collected repeatedly in the same set of tubes. Protein concentration and primase activity were determined (Figure 1C), and the fractions with high specific activity (fractions 24–26) were pooled and stored at −80 °C. The primase (fraction VI) is essentially pure (>90%) at this stage as determined by SDS–polyacrylamide gel electrophoresis (Figure 2A). To achieve further purification, the HPLC pool (fractions 24–26) was concentrated and rechromatographed (fraction VII) as described. SDS–polyacrylamide gel electrophoresis followed by silver staining demonstrated a single band of 59 kDa (Figure 2B, lane 3).

Comments on Purification. Due to the presence of proteases in yeast and the instability of the DNA primase activity, the purification of yeast DNA primase has been very difficult. We have used a combination of rapid purification schemes and a series of protease inhibitors in order to suppress the protease activities in the yeast extract. In the absence of protease inhibitors, the purified primase, although active, becomes a mixture of shorter polypeptides (Figure 2B). We have also been able to stabilize the primase activity by the use of a set of stabilizers. Addition of Me_2SO (5%) and NP-40 (0.01–0.025%) was required in order to stabilize the primase activity following phosphocellulose chromatography where the primase activity is most unstable. The primase activity remained stable following the Affigel-blue chromatography. We have taken advantage of the fact that primase bound strongly to the cibracron blue dye in Affigel-blue in the presence of Mg^{2+} and can be eluted with a high ionic strength buffer

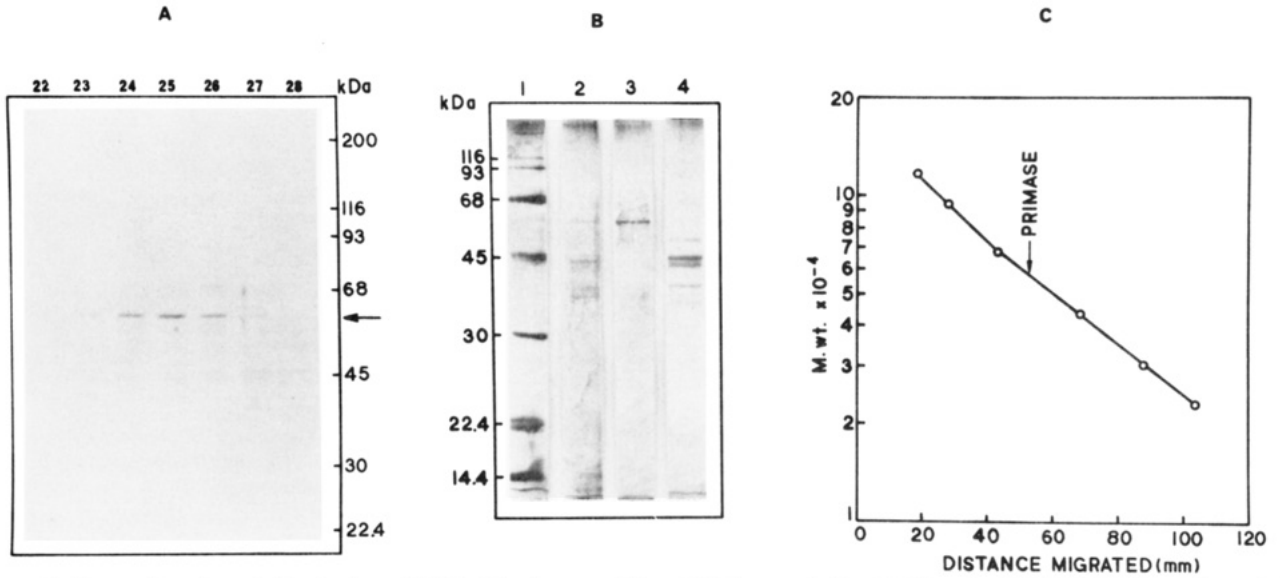

FIGURE 2: Electrophoretic analysis of polypeptide(s) with primase activity. (A) Coomassie blue R-250 stained SDS–PAGE (5–15%) analysis of HPLC fractions (Figure 1C, the arrow indicates the position of the 59-kDa band; (B) silver-stained SDS–PAGE (12.5%) analysis of primase fraction V (lane 2), primase fraction VII (lane 3), primase fraction VI purified in the absence of protease inhibitors (lane 4), and protein standards (lane 1: β-galactosidase, 116 kDa; phosphorylase B, 93 kDa; BSA, 68 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 22.4 kDa; lysozyme, 14.4 kDa); (C) determination of molecular weight of primase from a plot of log molecular weight vs. relative mobility as determined from panel B.

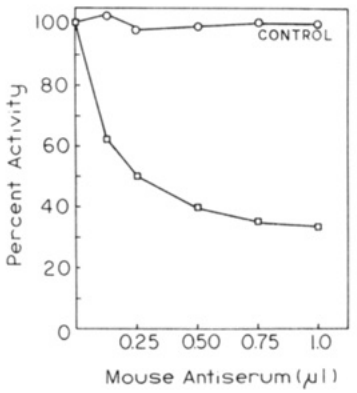

FIGURE 3: Inhibition of primase activity by mouse polyclonal anti-primase antiserum. The details of the production of primase antisera and assay are described under Materials and Methods. Control refers to serum obtained from an unimmunized mouse.

lacking Mg^{2+}. This step provided a 20–50-fold purification (Table I). Primase also did not bind to DEAE-cellulose at low ionic strength whereas 70% of the total protein bound to DEAE-cellulose, providing a rapid 2–3-fold purification (Table I). This step also eliminated any contaminating yeast RNA polymerases (data not shown). It is interesting to note that Tseng and Ahlem (1983) reported that the mouse DNA primase also does not bind to DEAE-cellulose under similar chromatographic conditions. HPLC size-exclusion chromatography separates proteins on the basis of their size and provided an excellent final prufication step for yeast primase.

Structure of the Yeast DNA Primase. The HPLC gel filtration of yeast DNA primase indicated that (i) the enzyme eluted with a native molecular weight of 59 000 (Figure 1C) and (ii) the primase activity corresponded to a 59-kDa polypeptide band in SDS–polyacrylamide gel electrophoresis (Figure 2). The primase is therefore a monomeric protein of molecular weight 59 000. Although the highly purified primase displayed a single 59-kDa polypeptide in coomassie or silver-stained gels (Figure 2) and had a high specific activity, it was still questionable whether the 59-kDa polypeptide was a protease-degraded form of a larger polypeptide. Therefore,

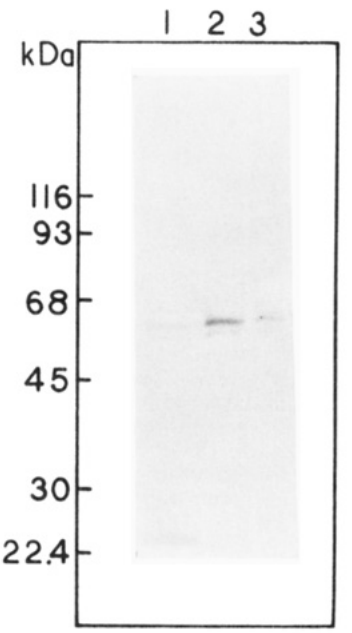

FIGURE 4: Immunoblot analysis of primase at various stages of purification using mouse polyclonal antiserum. Primase fraction II (lane 1); primase fraction IV (lane 2); primase fraction VI (lane 3). The polypeptides were separated by 5–15% SDS–PAGE and transferred to nitrocellulose filters (BA85, Schleicher & Schuell) in a Bio-Rad "Trans-Blot apparatus" according to the manufacturer's suggestions. The antibody detection of polypeptide(s) was carried out by using a "ProtoBlot" immunoblotting system (Promega Biotech) employing a goat anti-mouse IgG–alkaline phosphatase conjugate as the second antibody and nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates of alkaline phosphatase for detection. The immunodetection was carried out following manufacturer's instructions.

we prepared polyclonal antibody in mice against the highly purified primase (as shown in Figure 2B). The mouse antisera inhibited primase activity in an in vitro assay to an extent of 70%, indicating the presence of primase-specific immunoglobulin in the antisera (Figure 3).

We analyzed different primase fractions from various stages of purification by immunoblot analysis using the mouse po-

Table II: Characterization of Primase Activity: DNA Synthesis (Picomoles of Nucleotide) in the Primase Assay

conditions	DNA synthesis	% activity
complete	139	100
−GTP, CTP, UTP	125	90
−ATP	75	54
+α-amanitin (4 mg/mL)	124	89
+aphidicolin	130	94
−*E. coli* pol I	2	1
−dNTP	0	0
$-MgCl_2$	2	1
−DNA	2	1
−primase	5	4

lyclonal antibody. In the crude primase preparations (fractions II and IV), the antibody specifically detected 59- and 56-kDa polypeptides and a few smaller polypeptides and only a 59-kDa polypeptide in the highly purified fraction (Figure 4). Consequently, the 59-kDa polypeptide that corresponded to the primase activity is the likely native form of primase.

Characterization of the Primase Activity. Purified yeast DNA primase did not exhibit any detectable polymerase, ATPase (DNA-dependent or -independent), or exonuclease (5′ → 3′ or 3′ → 5′) activities (data not shown). The primase activity was not inhibited by aphidicolin or α-amanitin (Table II), and thus different from DNA and RNA polymerases of *S. cerevisiae*. The yeast DNA primase requires either ATP or GTP for primer synthesis in the presence of a full complement of deoxynucleotide triphosphates. The pyrimidine nucleotides are dispensable under these conditions.

The purified primase synthesized primers up to 15 nucleotides long as determined by the analysis of the primers synthesized on M13, φX174, and poly(dT) DNA templates (Figure 5). Yeast DNA primase absolutely requires $MgCl_2$, and the stimulation remained unaltered between 5 and 30 mM $MgCl_2$. The activity was inhibited approximately 70% by 50 mM $MgCl_2$. The primase activity was not inhibited by KCl, potassium phosphate (pH 7.4), and $(NH_4)_2SO_4$ (pH 7.4) up to 20 mM concentration (Table II). However, the activity decreased at higher concentrations of these salts (~100 mM). The primase activity was extremely sensitive to heat denaturation, and the half-life ($t_{1/2}$) of primase was less than 4 min at 45 °C (data not shown). The activity was also extremely sensitive to treatment with *N*-ethylmaleimide. The activity was inhibited >80% by 2-fold molar excess of NEM over DTT.

FIGURE 5: Analysis of primers synthesized by primase in vitro. The details of the primer synthesis and analysis are described under Materials and Methods. Primers synthesized by primase (fraction VII) on φX174 ssDNA (1000 pmol of nucleotide, lane 1), on M13mp18 ssDNA (1000 pmol of nucleotide, lane 2), and on poly(dT) (100 pmol of nucleotide, lane 3; 1000 pmol of nucleotide, lane 4). $(dA)_8$ and $(dA)_{15}$ were phosphorylated with [γ-^{32}P]ATP and T4 polynucleotide kinase and were used as standards for octanucleotide (8 nt) and pentadecanucleotide (15 nt).

Discussion

We have purified a stable yeast DNA primase with high specific activity in its native form from wild-type *S. cerevisiae*. We have demonstrated that the yeast DNA primase is encoded by a 59-kDa polypeptide. HPLC gel filtration chromatographic and SDS–polyacrylamide gel electrophoretic analyses indicated that the yeast DNA primase is a monomeric protein with a molecular weight of 59 000. The purified yeast DNA primase had a specific activity of 165 000 units/mg. Previous attempts to purify yeast DNA primase resulted in the purification of various polypeptides with molecular weights of 65 000 with a specific activity of 1000 units/mg (Jazwinski & Edelman, 1985), 60 000 with a specific activity of 3000 units/mg (Wilson & Sugino, 1985), and an unstable dimer of 58 000 and 48 000 of unknown specific activity (Plevani et al., 1985). The previous reports lack any immunochemical characterizations of primase, making unequivocal characterization difficult. We have encountered a great difficulty in the stabilization of yeast DNA primase under the previously reported purification conditions ($t_{1/2}$ ~ 24 h at 4 °C; data not shown), an observation similar to that reported by Plevani et al. (1985). The stability is perhaps the contributing factor in the ambiguity of the reported results. We have developed conditions at which primase is stable indefinitely at 0 or −80 °C, allowing us to purify it to homogeneity and with high specific activity. The addition of Me_2SO and NP-40 increases both the recovery and stability of primase activity during the purification procedures. Tseng and Ahlem (1983) and Vishwanatha and Baril (1986) made similar observations with the stability of polymerase-free mouse DNA primase and HeLa cell DNA primase, respectively.

Primase synthesizes up to 15 nucleotide long initiator RNAs or RNA–DNA hybrids that can function as primers for DNA synthesis by DNA polymerases (Figure 5). Primase requires ATP or GTP to synthesize primers; however, other ribonucleotides could be replaced by deoxynucleotides without any detrimental effect (Table II) in primase function. This lack of requirement of GTP, CTP, and UTP in the presence of ATP remains relatively unchanged during the purification. Primase is distinctly different from known eukaryotic cellular RNA polymerases by its resistance to α-amanitin and its inability to bind to DEAE-cellulose at very low ionic strength.

Proteolysis of enzymes during purification presents an obvious problem in the yeast *S. cerevisiae*. Consequently, it is essential to consider this phenomenon in confirming the true molecular size of the protein. Immunoblot analysis indicated that the largest polypeptide that cross-reacts with anti-primase antibody was 59 kDa. However, shorter polypeptides, likely produced by proteolytic degradation, were observed in the

crude extract. These degraded polypeptides were removed during purification. Although the DNA primase activity has been assigned to an assembly of two polypeptides of approximate molecular weights 58K and 48K (Tseng & Ahlem, 1983; Plevani et al., 1985), recent studies (Vishwanatha & Baril, 1986; this report) indicate that the eukaryotic primase activity resides in a single polypeptide. However, the studies of Vishwanatha et al. (1986) and our laboratory (E. E. Biswas, unpublished results) indicate that other subunits in the primase–polymerase complex have significant influence on the primase function. Further analyses are required in order to delineate the involvement of this 59-kDa yeast DNA primase in the primase–polymerase complex, with regard to both the structure and the functions of yeast DNA polymerase–primase complex. However, the purification and especially the stabilization conditions reported here should greatly facilitate such studies.

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