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Protein affinity chromatography with purified yeast DNA polymerase α detects proteins that bind to DNA polymerase

(DNA replication/DNA polymerase accessory proteins/protein affinity chromatography)

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ABSTRACT **We have overexpressed the *POL1* gene of the yeast *Saccharomyces cerevisiae* and purified the resulting DNA polymerase α polypeptide in an apparently intact form. We attached the purified DNA polymerase covalently to an agarose matrix and used this matrix to chromatograph extracts prepared from yeast cells. At least six proteins bound to the yeast DNA polymerase α matrix that did not bind to a control matrix. We speculate that these proteins might be DNA polymerase α accessory proteins. Consistent with this interpretation, one of the binding proteins, which we have named POB1 (polymerase one binding), is required for normal chromosome transmission. Mutations in this gene cause increased chromosome loss and an abnormal cell morphology, phenotypes that also occur in the presence of mutations in the yeast α or δ polymerase genes. These results suggest that the interactions detected by polymerase affinity chromatography are biologically relevant and may help to illuminate the architecture of the eukaryotic DNA replication machinery.**

DNA replication in eukaryotic cells appears to require the participation of at least three types of DNA polymerase: α, δ, and ε (reviewed in refs. 1 and 2). While these enzymes clearly play a major role in replication, the observation that a large number of proteins besides the DNA polymerase itself are required to perform the same process in prokaryotes (see refs. 3 and 4) makes it seem likely that the eukaryotic DNA replication machinery is similarly complex. Reconstitution *in vitro* of proteins sufficient to replicate the simian virus 40 genome has revealed some of the cellular DNA synthesis machinery (5, 6), but the properties of this purified system indicate that other accessory factors must also be used *in vivo*.

Identification of eukaryotic replication accessory factors has been slow for several reasons. The replication machinery does not assemble into a complex that is stable enough to allow copurification of the components. [DNA polymerase α does assemble into a stable complex with RNA primase proteins (see ref. 7), but the holoenzyme that makes up the entire active replication fork has not been isolated intact.] Methods for preparing cellular extracts capable of carrying out normal initiation and elongation reactions *in vitro* on cellular genomic DNA have not been devised. Eukaryotes amenable to genetic analysis have thus far not proved suitable for the *in vitro* analysis needed to characterize the products of the small number of genes known to cause defects in DNA replication when mutated. Finally, since many proteins can enhance the activity of DNA polymerases by binding to and removing secondary structure from their single-stranded templates, establishing the biological relevance of such stimulatory factors has been difficult.

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The technique of protein affinity chromatography has been used to detect interactions among replication proteins produced in T4 bacteriophage-infected *Escherichia coli* (8, 9). In the T4 system, polymerase accessory factors are needed for replication *in vivo* and can be shown to interact with the polymerase to form a holoenzyme *in vitro*. However, the T4 replication machinery is not stable enough to allow the components to be isolated as an intact complex. Because of the high concentration of binding sites presented, affinity chromatography allows detection of weak protein–protein interactions among the replication complex components (see ref. 10). Protein affinity chromatography has also been useful for illuminating the nature of other complex protein machines such as those responsible for transcription in bacteriophage λ-infected *E. coli* and in mammalian cells (10–14), for protein sorting (15), as well as for purifying proteins associated with intracellular structures such as microfilaments (16, 17).

We are using DNA polymerase α protein affinity chromatography to identify potential eukaryotic DNA polymerase accessory proteins. We report here the purification of intact yeast DNA polymerase α in quantities large enough to perform affinity chromatography experiments and we demonstrate that this technique can be used to identify DNA polymerase α binding proteins. We will report elsewhere (unpublished data) that elimination of one of these binding proteins, named POB1, produces phenotypes similar to, although less drastic than, mutations of the *POL1* gene itself. These results lend credence to the viability of this approach for identifying polymerase accessory factors.

MATERIALS AND METHODS

Strains and Media. Strains 7311-1-1 (*MATa cdc17-1 leu2 ura3-52 can1 his7 gal1*), 7311-2-4 (*MATα cdc17-1 leu2 ura3 can1 his7 pep4 prb1 gal1*), and 7208-12 (*MATa/MATα his7/his7 ura3/ura3 trp1/trp1 pep4/pep4 prb1/prb1 can1/can1 gal1/gal1*) are isogenic with A364a; the *pep4* and *prb1* mutations reduce the level of proteinase activities in cellular extracts (18). Synthetic medium (SMM) was prepared essentially as described (19) except as noted.

Construction of Plasmids for Overexpressing the *POL1* Gene. All methods were essentially as described (20). A *POL1* gene containing a *Bam*HI linker 7 base pairs (bp) upstream of the open reading frame was constructed by deleting 5′ sequences with BAL-31 (the POL1 clone was kindly provided by B. Garvik, University of Washington). This 5-kbp POL1 fragment was inserted into the *Bam*HI site near the yeast *GAL1* gene promoter in three vectors: pTF56 (derived from pARDB1, contains the *URA3* gene but no yeast replication origin; from A. Reynolds, University of Washington), pTF58 (derived from pBD34, contains the *TRP1* gene and the *ARS1* replication origin; from A. Reynolds), and pTF59 [derived from YEp434, contains the *LEU2* gene and a

Abbreviation: HSA, human serum albumin.

2-μm origin of replication; from D. Botstein (21)]. After linearizing with *Xho* I and transforming yeast cells, pTF56 integrates at the *POL1* locus and is stably maintained at one copy per cell. pTF58 and pTF59 are unstable episomal elements but should be present on average in 10–20 or ≈50 copies per cell, respectively.

Electrophoresis and Blots. SDS/PAGE was performed as described (22). Two-dimensional gel electrophoresis was performed according to the nonequilibrium pH-gradient gel electrophoresis system of O'Farrell *et al.* (23). Protein samples for two-dimensional gel analysis were concentrated by adding sodium deoxycholate to 0.5 mg/ml and cytochrome *c* to 50 μg/ml and then adding trichloroacetic acid to 20% (wt/vol). Samples were incubated on ice for 1 hr and centrifuged at 6000 × *g* for 30 min. The pellets were washed with acetone, collected by centrifugation for 5 min at 6000 × *g*, and washed with diethyl ether, dried, and dissolved in sample buffer (23). Proteins were transferred from polyacrylamide gels to nitrocellulose in a Trans-Blot tank (Bio-Rad) according to the manufacturer's instructions. POL1 protein was detected by using monoclonal antibody 24D9 (a generous gift from G. Brooke and L. Dumas, Northwestern University) and goat anti-mouse antibodies conjugated to horseradish peroxidase (Bio-Rad). Staining was with 4-chloronaphthol according to the supplier's instructions (Bio-Rad).

Purifying POL1. A 500-ml culture of 7311-2-4 cells containing pTF59 was grown to a density of ≈1 × 10^7 cells per ml in SMM lacking leucine and containing 2% glucose. Cells were harvested by centrifugation (5 min at 2800 × *g*), washed with sterile distilled H_2O, and then used to inoculate 5 liters of medium lacking leucine and containing 2% raffinose as the carbon source and 0.3% galactose to induce the *GAL1* promoter. The culture was shaken at 30°C and, after a lag of 12–15 hr, the cells resumed growth. When the cultures reached a density of 1–2 × 10^7 cells per ml they were harvested by centrifugation (4 min at 2800 × *g*), washed with cold distilled H_2O, frozen in liquid nitrogen, and stored at −70°C. Five liters of medium typically yielded ≈15 g (wet weight) of cells.

During all subsequent procedures, protein samples were maintained at 0°C–4°C. Columns were poured in sterile plastic syringes plugged with glass wool, and all solutions were delivered with a Gilson peristaltic pump. Protein concentrations were measured using the Bio-Rad protein assay reagent according to the manufacturer's instructions with immunoglobulin as a standard.

To prepare polymerase α, cells were thawed and transferred to four glass tubes (20 × 180 mm) in an ice water bath; 4 ml of 2× lysis buffer and 10 ml of acid-washed glass beads (0.5 μm diameter; Sigma) were added to each tube, which was then agitated vigorously on a Vortex mixer for a total of 10 min in 1-min bursts with 1-min rests on ice between bursts. The lysate was recovered, the glass beads were washed twice with 2-ml aliquots of 1× lysis buffer, and the liquid fractions were pooled and centrifuged at 17,000 × *g* for 10 min. The supernatant was mixed slowly with an equal vol of saturated ammonium sulfate at 4°C, stirred for 30 min, and the precipitate was collected by centrifugation at 40,000 × *g* for 30 min. The precipitate was resuspended in 7 ml of PC_{100} (see *Buffers* below) and then dialyzed twice against 1 liter of PC_{100}. The dialyzed fraction was centrifuged for 10 min at 40,000 × *g* and the supernatant (extract or F_I) was loaded at 1 column vol/hr to a 20-ml phosphocellulose column (Whatman P11) equilibrated with PC_{100}. The column was washed with 1.5 column vol of PC_{100} and eluted with an 80-ml gradient from 0 to 300 mM NaCl in PC_{100}, followed by a step to 2 M NaCl in PC_{100}. Fractions containing the main peak of polymerase activity were pooled (phosphocellulose or F_{II}) and loaded directly to a 5-ml hydroxyapatite column (Bio-Gel HT, Bio-Rad) equilibrated with PC_{100} at 2–2.5 column vol/hr. Under these conditions, ≈20% of the activity usually failed to bind the matrix, but this activity was associated with a proteolytic fragment of the POL1 protein (T.F., unpublished data). The chromatograph was developed overnight at a flow rate of 1 column vol/hr with a 60-ml gradient from PC_{100} to PC_{400}. The polymerase activity was pooled (hydroxyapatite or F_{III}), dialyzed twice against 1 liter of DC_{100} for 1.5 hr each time, and then loaded at 1 column vol/hr to a 5-ml single-stranded DNA cellulose column (24). The column was washed with 1 column vol of DC_{100} and the polymerase was eluted with a 25-ml gradient from DC_{100} to DC_{300}, followed by a step to DC_{2000}. The polymerase activity often eluted from this column in multiple peaks; SDS/PAGE analysis of these fractions showed each peak to be associated with a different proteolytic fragment of the POL1 protein (T.F., unpublished data), so typically only the main, early peak was retained. The pooled fractions (single-stranded DNA or F_{IV}) were then used to prepare affinity matrices.

Buffers. Lysis buffer contained 0.1 M Tris·HCl (pH 7.5), 1 mM Na_3EDTA, 1 mM 2-mercaptoethanol, 10 mM potassium acetate, 0.1% (vol/vol) Triton X-100, and 10% (wt/vol) glycerol. PC_{100} (or PC_{400}) contained 100 (or 400) mM potassium phosphate (pH 7.6), 1 mM Na_3EDTA, 1 mM 2-mercaptoethanol, and 10% (wt/vol) glycerol. $DC_{100}/DC_{300}/DC_{2000}$ contained 20 mM Tris·HCl (pH 7.5), 2 mM Na_3EDTA, 1 mM 2-mercaptoethanol, 10% (wt/vol) glycerol, and 100/300/2000 mM NaCl. CB_{50}/CB_{800} contained 20 mM Tris·HCl (pH 7.5), 1 mM Na_3EDTA, 1 mM 2-mercaptoethanol, 2 mM $MgCl_2$, 10% (wt/vol) glycerol, 0.5 mM phenylmethylsulfonyl fluoride (Sigma), 0.5 μg of leupeptin per ml, and 0.7 μg of pepstatin per ml (Boehringer Mannheim), and 50/800 mM KCl.

Polymerase Assay. Polymerase activity was assayed in 25 mM Tris·HCl, pH 7.5/5 mM magnesium acetate/5 mM dithiothreitol/50 μM [^{3}H]TTP (Amersham; 200 cpm/pmol)/12.5 μg of poly(dA)$_{250}$ per ml/5 μg of oligo(dT)$_{12-18}$ per ml (Pharmacia)/100 μg of human serum albumin (HSA) per ml (Sigma). Reaction mixtures were incubated at 37°C for 10 min, placed on 2.1-cm Whatman GF/A filters, and dropped into a beaker containing 5% trichloroacetic acid with 1/20th vol of saturated sodium pyrophosphate. The filters were washed three times with 1 M HCl at 4°C, twice with cold 70% ethanol, dried, and placed in scintillation vials with 2 ml of Bio-Safe II scintillation fluid (Research Products International). Radioactivity in the vials was quantitated by using a liquid scintillation counter and the activity in the sample was calculated by the following definition: 1 unit of activity is 1 nmol of TTP incorporated into acid-insoluble material per hr.

Affinity Chromatography: Preparing Columns. Affinity columns were prepared as described (10). Briefly, 0.5–1 mg of polymerase α (F_{IV}) or ≈3-fold more HSA was dialyzed three times for 2 hr each time against at least a 100-fold excess of coupling buffer (20 mM Hepes, pH 7.6/50 mM NaCl/10% glycerol) at 4°C. For each column, a slurry of Affi-Gel 10-activated agarose (Bio-Rad) sufficient to produce 1 ml of packed matrix was washed once in H_2O and twice in coupling buffer. The dialyzed protein was added to the washed matrix and coupling was accomplished overnight at 4°C on a rotator. The matrix was then suspended in ≈5 vol of CB_{50}, poured into a 1-ml sterile syringe plugged with glass wool, and washed with CB_{50}. Coupling (see ref. 10) was typically quantitative for POL1 protein and near 1/3 for HSA.

Extracts for Affinity Chromatography. Strain 7208-12 was grown in 1 liter of SMM containing methionine (1.5 μg/ml) and 5 mCi of [^{35}S]methionine (700 Ci/mmol; 1 Ci = 37 Bq; NEN) to a density of ≈2 × 10^7 cells per ml. The cells were harvested by centrifugation (4 min at 2800 × *g*) and frozen in liquid nitrogen. The cells were thawed, transferred to a tube (20 × 180 mm) on ice, and mixed with 4 ml of LB_{50} (the same as CB_{50} except without glycerol) and 10 ml of glass beads

(Sigma; 0.5 μm diameter). The mixture was agitated for a total of 8 min in 1-min bursts with at least 1-min intervals on ice between bursts. The supernatant was recovered, the glass beads were washed twice with 2-ml aliquots of LB_{50}, and the liquid fractions were pooled and centrifuged 10 min at 13,000 × g. The supernatant was recovered and centrifuged 2 hr at 190,000 × g. The supernatant was recovered carefully, forced through a 0.45-μm cellulose acetate syringe filter, and brought to 10% glycerol. After mixing, the sample was again centrifuged for 2 hr at 190,000 × g and the clear supernatant was recovered.

Under these conditions, ≈80% of the radioactive label was incorporated into cells, and ≈20% of the label was present in the final extract, representing ≈150 mg of protein in a vol of 9 ml with a specific activity of ≈16,000 cpm per μg of protein.

The extract was loaded to the experimental and control affinity columns in parallel at a flow rate of 3 ml/hr. The columns were then washed with 10 column vol of CB_{50} and eluted with CB_{800}. In experiments not shown (T.F., unpublished data), columns were eluted with a gradient to CB_{800} and stripped at room temperature with a mixture of 2% SDS/6 M urea. These experiments showed that specifically bound proteins all eluted by ≈300 mM KCl; no specific interactions were detected by eluting with chaotropic agents.

RESULTS

Overexpression of the Yeast *POL1* Gene. The yeast DNA polymerase α is the product of the *POL1* or *CDC17* gene. It has been difficult to purify sufficient quantities of this protein to attempt affinity chromatography because polymerase α is present at low levels in normal cells and it is sensitive to proteolysis. We have therefore placed the *POL1* gene under control of the strong, inducible yeast *GAL1* promoter (25). In this context, the *POL1* gene is expressed at a high level when cells are grown in medium lacking glucose and containing galactose. We found that the highest levels of expression could be achieved by deleting as much as possible of the 5′ noncoding sequence and by placing cells unable to degrade galactose in medium containing raffinose as a carbon source with low levels of galactose as inducer. Under these conditions, we observe ≈100-fold induction of the *POL1* gene product to ≈5 × 10^4 copies per cell.

We found that recovering the overexpressed POL1 protein intact required the use of yeast strains lacking the major proteinases encoded by the genes *PEP4* and *PRB1* (Fig. 1). We also found that the POL1 protein was sensitive to other aspects of cell physiology: extracts prepared from cells grown to densities higher than ≈2 × 10^7 cells per ml or cells grown on glucose (using a construct in which the *POL1* gene was under control of the constitutive *ADH2* promoter) failed to contain stable, intact POL1 protein. Contrary to our expectations, adding the proteinase inhibitors leupeptin, pepstatin, and phenylmethylsulfonyl fluoride to the cell lysis buffer caused the POL1 protein to be insoluble; the majority of the protein was recovered in the pellet with the cellular debris under these conditions (T.F., unpublished data).

Purification of Intact Yeast DNA Polymerase α. The overexpressed POL1 protein was purified from cells grown under inducing conditions. Polymerase α was purified by successive chromatography on phosphocellulose, hydroxyapatite, and single-stranded DNA cellulose (Table 1). In each case, fractions containing the apparently intact 180-kDa form of the protein (the *POL1* gene sequence predicts a polypeptide of 167 kDa; see ref. 26) as well as the bulk of the polymerase activity were identified, pooled, and prepared for the next chromatographic step as described in *Materials and Methods*. A sample was taken from each pool, the proteins were separated on a polyacrylamide gel in the presence of SDS,

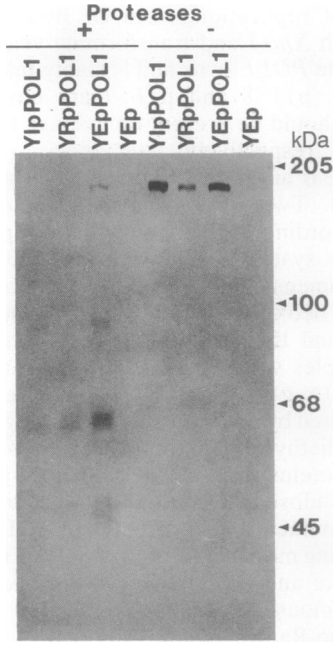

FIG. 1. Overexpressed DNA polymerase α is only stable in protease-deficient strains. Yeast strains were grown in 10 ml of selective medium containing 2% raffinose and 0.3% galactose to an absorbance of ≈0.2 at 600 nm (≈3 × 10^6 cells per ml), cells were collected by centrifugation, suspended in 150 μl of sample buffer (5% glycerol/5% 2-mercaptoethanol/3% SDS/62.5 mM Tris·HCl, pH 6.8/0.05% bromophenol blue/0.05% phenol red), and boiled for 5 min. A 50-μl portion of each sample was loaded on a 7.5% acrylamide gel (22) and electrophoresed for 16 hr at 0.5 mA, the proteins were transferred to nitrocellulose, and POL1 was detected with antibodies. Samples were prepared from 7311-1-1 (protease +) or 7311-2-4 (protease −). The strains contained YIpPOL1 (integrated, single copy of pTF56), YRpPOL1 (intermediate copy number episomal element, pTF58), YEpPOL1 (high copy number episomal element, pTF59), or YEp (high copy vector YEp24, no polymerase gene). Coomassie blue staining of a duplicate gel indicated that all the lanes contained a similar amount of total protein.

and the proteins in the gel were stained with Coomassie blue dye (Fig. 2). According to this analysis, the three-step purification procedure yields nearly homogeneous 180-kDa POL1 protein. While the single-stranded DNA cellulose step permits recovery of most of the POL1 protein, the total activity recovered drops (see Table 1). We have not detected an activator in the column flowthrough.

Affinity Chromatography Reveals Proteins That Bind Specifically to DNA Polymerase α. The purified DNA polymerase α was covalently attached to an agarose matrix with Bio-Rad's Affi-Gel 10-activated agarose. Protease-deficient yeast cells were grown in medium containing [^{35}S]methionine, harvested, and frozen. The cells were lysed and an extract was prepared. We found that it was necessary to perform at least a 2-hr centrifugation at 190,000 × g in order to reduce nonspecific background binding.

Table 1. Purification of overexpressed yeast DNA polymerase α from 13.5 g (wet weight) of yeast cells

Fraction	Protein, mg	Activity, units	Specific activity, units/mg	Elution condition
F_I (extract)	875	2,000	2.3	—
F_{II} (Pcell)	19	17,000	910	140–200 mM NaCl
F_{III} (HAP)	1.8	9,900	5400	130–180 mM KP_i
F_{IV} (ssDNA)	0.3	940	3600	180–210 mM NaCl

Pcell, phosphocellulose; HAP, hydroxyapatite; ssDNA, single-stranded DNA.

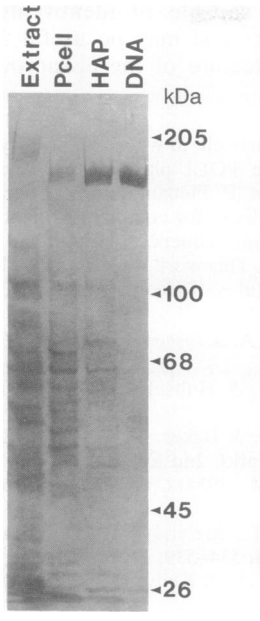

FIG. 2. Purification of yeast DNA polymerase α. A sample was taken from each pool during purification of the POL1 protein and electrophoresed through a 7.5% polyacrylamide gel in the presence of SDS as described in Fig. 1. Proteins were stained with Coomassie blue dye. The lanes contained crude extract (50 μg), the pooled fractions from phosphocellulose (Pcell) (20 μg), hydroxyapatite (HAP) (13 μg), or single-stranded DNA cellulose (5 μg). Molecular mass standards (kDa) migrated as indicated.

The extract was chromatographed on two columns in parallel: one containing agarose to which POL1 protein was attached and one containing agarose to which HSA was attached. The HSA column serves as a control for proteins that bind to the agarose matrix or to proteins in general. After the extract was loaded, the columns were washed extensively with low ionic strength buffer and eluted with buffer containing 800 mM KCl.

Radioactively labeled proteins in each fraction were detected by placing a sample in a liquid scintillation counter. When the fractions containing proteins that eluted from each column (POL1 and HSA) were pooled, concentrated by precipitation with trichloroacetic acid, and subjected to two-dimensional PAGE, the autoradiographs in Fig. 3 were obtained. Comparison of the two gels reveals that the POL1-binding fraction contains at least six and perhaps as many as eight proteins not detected in the eluate from the HSA column, even on longer exposure. The most abundant binding species is a 115-kDa protein, which we have named POB1.

When a POL1 column is used several times in succession, subsequent eluates contain the same proteins found in the initial eluate, demonstrating that the columns are reusable. If an extract is passed over a POL1 column, the column is eluted, and the same extract is passed over the column again; POB1 and other binding proteins are found in the eluates again. They are, however, completely removed from the extract after three or four iterations of this procedure. Since the same proteins can bind to a POL1 column that they previously failed to bind, the column must have been saturated with binding factors during the first run. The total amount of POB1 protein recovered after depleting an extract indicates that it is present in 1000–2000 copies per diploid cell, similar to the number of polymerase α molecules.

We found that a column containing $\approx$700 μg of covalently attached POL1 protein retained $\approx$7.1 μg of protein from an extract containing $\approx$75 mg of protein, while a column containing a similar amount of HSA retained only 3.7 μg. Therefore, the POL1 column specifically retained $\approx$0.5% of its mass in protein. Assuming the average binding protein is about half the size of the POL1 protein, only $\approx$1 molecule of POL1 protein in 100 bound a protein molecule from the extract. For several other proteins, we have found that $\approx$1 in 10 of the immobilized molecules could bind a protein molecule in an extract (see ref. 10).

Yeast DNA polymerase α, like the analogous protein from other eukaryotes, can be found associated in extracts with three proteins: two subunits of an RNA primase and a β

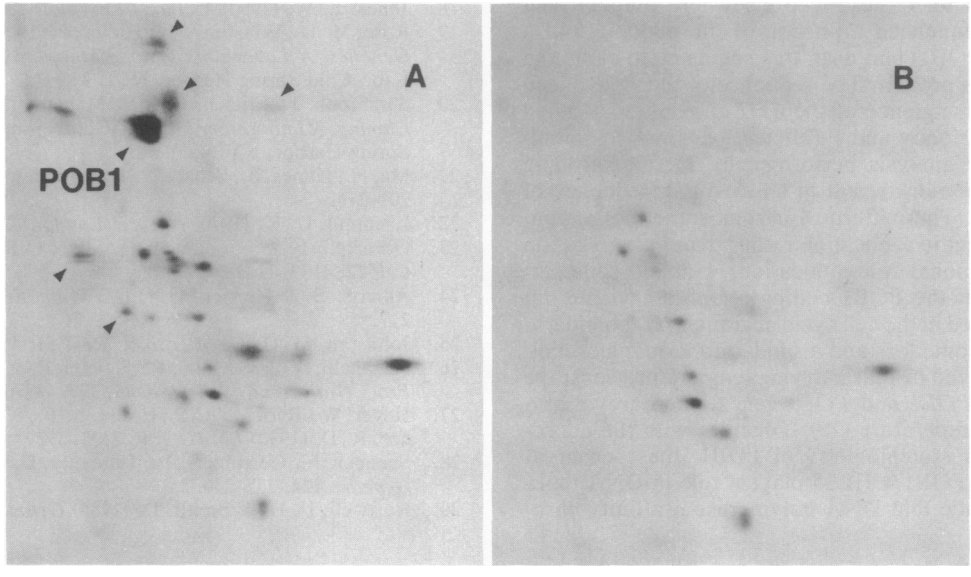

FIG. 3. Two-dimensional gel electrophoresis followed by autoradiography reveals proteins that bind to a DNA polymerase α protein column. Extracts were prepared for chromatography and loaded onto two 1-ml columns in parallel—one containing $\approx$0.7 mg of POL1 protein and one containing a similar amount of HSA. The columns were washed with 10 column vol of CB_{50} and eluted with CB_{800}. Fractions containing eluted proteins were concentrated and subjected to two-dimensional nonequilibrium pH gradient gel electrophoresis/SDS/PAGE (23). The second-dimension gel contained 7.5% polyacrylamide. The gels were dried onto filter paper and exposed to x-ray film at −70°C to produce the autoradiographs shown. (*A*) POL1 column, 1.8 μg of total protein, 28,000 cpm. (*B*) HSA column, 0.9 μg of total protein, 14,000 cpm. Arrowheads indicate proteins that appear only in the POL1 eluate even after overexposing the autoradiographs.

subunit of unknown function (reviewed in ref. 7). Antibodies generated against the yeast primase subunits (the products of PRI1 and PRI2; antibodies were a generous gift from P. Plevani and G. Lucchini, University of Milan) do not detect primase protein in the eluate from the POL1 column (T.F., unpublished data). To detect an interaction, the technique of affinity chromatography requires that two proteins have an appreciable dissociation constant. Otherwise, the interacting protein will remain associated with the test protein present in the cellular extract and will not be presented to the test protein fixed to the affinity matrix. Since primase and polymerase associate tightly, there may be no free primase in the extract. Thus, it is possible that this interaction could be missed by this technique. When tested with antibodies used in a manner similar to that described in Fig. 1, no POL1 protein was detected in the eluted fractions (data not shown).

Other factors such as RF-A and RF-C appear to be components of the eukaryotic replication machinery (see ref. 3). Whether or not these proteins are present in eluates from a POL1 affinity column remains to be determined. POB1 is not a subunit of RF-A by the criteria of size, sequence, and map location (27); the characterization of RF-C in yeast is presently insufficient to draw a similarly firm conclusion.

DISCUSSION

To identify DNA polymerase accessory proteins and obtain information about how these proteins interact to form the DNA replication machinery in a eukaryotic cell, we have used the technique of protein affinity chromatography. After overexpressing the yeast *POL1* gene in yeast cells, we have purified the intact DNA polymerase α, attached it covalently to an agarose matrix, and used this affinity matrix to chromatograph extracts prepared from yeast cells. We have found that at least six proteins remain bound to a POL1 protein column after extensive washing in low ionic strength buffers that are not retained by a similar column containing serum albumin.

These binding proteins are candidates for eukaryotic DNA polymerase α accessory proteins. However, binding alone does not establish biological relevance of the interactions detected. In work to be described elsewhere (unpublished data), we have sequenced a portion of the major 115-kDa binding protein (POB1) and used this sequence to clone the gene encoding this protein. The sequence of the *POB1* gene is identical to the sequence of the *CHL15* gene [also called CTF4 (28); V. Larionov and P. Hieter, personal communications; sequence analysis performed by M. Goebl]. The *chl15* and *ctf4* mutations result in an elevated frequency of chromosome loss. The pattern of chromosome loss is consistent with a defect in replication rather than in segregation (V. Larionov, personal communication). Haploid cells carrying a deletion of the POB1 coding region are viable but appear to be delayed in the cell cycle near the S/G_2 boundary. Elevated chromosome loss and a similar abnormal morphology are also observed in cells carrying temperature-sensitive mutations in the *POL1* and *POL3* genes when grown at a semipermissive temperature (29). Together with the appropriate intracellular stoichiometry of POB1, these observations suggest that POB1/CHL15 plays a role in DNA replication. We conclude that DNA polymerase α affinity chromatography is capable of identifying DNA replication accessory factors and may be useful for revealing details about the architecture of the eukaryotic DNA replication apparatus.

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