

Expression of Herpes Simplex Virus Type 1 DNA Polymerase in *Saccharomyces cerevisiae* and Detection of Virus-Specific Enzyme Activity in Cell-Free Lysates

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The herpes simplex virus type 1 (HSV-1) (strain 17) DNA polymerase gene has been cloned into an *Escherichia coli*-yeast shuttle vector fused to the galactokinase gene (*GAL-1*) promoter. Genes controlled by the *GAL-1* promoter are induced by galactose, uninduced by raffinose, and repressed by glucose. Cell extracts from a strain of *Saccharomyces cerevisiae* harboring this vector (Y-MH202, expresser cells) grown in the presence of galactose and assayed in high salt (100 mM ammonium sulfate) contained a novel DNA polymerase activity. No significant high-salt DNA polymerase activity was detected in extracts from expresser cells grown in the presence of raffinose or in extracts from control cells containing the *E. coli*-yeast shuttle vector without the HSV-1 DNA polymerase gene grown in the presence of raffinose or galactose. Immunoblot analysis of the cell extracts by using a polyclonal rabbit antiserum prepared against a highly purified HSV-1 DNA polymerase preparation revealed the specific induction of the HSV-1 ~140-kilodalton DNA polymerase polypeptide in expresser cells grown in galactose. Extracts from the same cells grown in raffinose or control cells grown in either raffinose or galactose did not contain this immunoreactive polypeptide. The high-salt DNA polymerase activity in the extracts from expresser cells grown in galactose was inhibited >90% by either acyclovir triphosphate or aphidicolin, as expected for HSV-1 DNA polymerase. In addition, the high-salt polymerase enzyme activity could be depleted from extracts by immunoprecipitation by using purified immunoglobulin G from this same polyclonal rabbit antiserum. These results demonstrate the successful expression of functional HSV-1 DNA polymerase enzyme in *S. cerevisiae*.

The herpes simplex virus type 1 (HSV-1) DNA polymerase (pol) has been identified as an approximately 140-kilodalton (kDa) polypeptide which is required for viral DNA replication (5, 6, 26). The viral enzyme shares specific regions of amino acid sequence homology with other herpesvirus DNA pols and with a variety of eucaryotic DNA pols, including the mammalian and yeast alpha pols in regions believed to correspond to substrate and pyrophosphate binding sites (13, 19, 25, 27, 30). Despite these similarities to host cell enzymes, HSV pol activity is readily identified because of the ionic strength optimum of the viral enzyme and its much greater sensitivity to agents such as phosphonoacetate and acycloguanosine (acyclovir) triphosphate (ACVTP) (7, 12, 23). An improved understanding of the structure of the HSV pol could not only aid in designing new antiviral drugs but could also yield information about the regulation of viral replication, DNA synthesis, and cell proliferation.

While mRNA encoding HSV pol appears to be abundant in the infected cell, only low levels of the pol protein are produced (16, 31). Several additional polypeptides appear to remain associated with the 140-kDa pol protein through standard purification procedures (26); one of these polypeptides is the 65-kDa DNA-binding protein (24). Therefore, it is difficult to obtain large quantities of homogeneous 140-kDa protein from infected cells for study. Further mechanistic studies on the HSV DNA pol would be greatly facilitated by

the availability of large quantities of soluble enzyme expressed in a heterologous system such as yeast or bacteria.

Previously, Dorsky et al. (9) successfully expressed a functional form of the HSV pol gene in COS-1 cells from the simian virus 40 early promoter. The low levels of active enzyme expressed in that system were detected by complementation of a temperature-sensitive HSV containing a mutation in *pol*. Dorsky and Crumpacker (11) have also reported the expression of HSV pol protein in an in vitro translation system. In addition, they reported the expression of high levels of HSV 140-kDa pol protein in *Escherichia coli* (10); however, the expressed protein in *E. coli* was insoluble and had no readily detectable enzymatic activity.

In this study, the HSV *pol* gene was expressed in the yeast *Saccharomyces cerevisiae*. This expression system was chosen for several reasons. First, expression of another HSV enzyme (thymidine kinase) in *S. cerevisiae* had led to the production of a soluble, functional product (21). Second, it is possible to construct a high-copy-number, regulatable expression vector to attempt high-level expression of the gene. Finally, the yeast system is easy to manipulate and should readily allow introduction of site-specific mutations into the *pol* gene. Such mutations could then be assessed as to their effects on the enzyme activity, its association with other components of the replication complex, and its intracellular localization.

MATERIALS AND METHODS

Materials. T4 DNA ligase, *E. coli* DNA pol (Klenow fragment), and restriction endonucleases were purchased

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from New England BioLabs or Bethesda Research Laboratories. [³H]TTP (25 Ci/mmol) was obtained from Dupont, NEN Research Products. Unless otherwise noted, chemicals were purchased from Sigma Chemical Co.

Strains and media. The *E. coli* strains used for this study were HB101 (F^- *hsdS20* [$r^- m^-$] *supE44 ara-14 galK2 lacY1 proA2 rpsL20* [Str^r] *xyl-5 leu mtl-1 lambda^- recA13*) obtained from Bethesda Research Laboratories, and XL1-Blue (*endA1 hsdR17* [$r_k^- m_k^+$] *supE44 thi-1 lambda^- recA1 gyrA96 relA1* [Lac⁻] [F' *proAB lacI^q ZΔM15 Tn10* (Tet^r)] supplied by Stratagene Cloning Systems. The yeast strain was Y294 (*MATα leu2-3 leu2-112 ura3-52 trp1 Δhis3 Gal⁺ cir⁺*), obtained from D. Kirsch (Squibb Institute for Medical Research, Princeton, N.J.), and has been previously described (2). Bacteria were grown in Luria broth (22); yeast were grown in minimal media (0.7% yeast nitrogen base [Difco Laboratories]) containing required amino acids (20 to 30 μg/ml) and 2% raffinose or 2% galactose.

Plasmids. The plasmids used in this study were YE_p 352, a high-copy-number *E. coli*-yeast shuttle vector obtained from D. Kirsch (Squibb Institute for Medical Research, Princeton, N.J.) which has been previously described (15). The plasmid YCp125H was obtained from J. Rine (University of California, Berkeley); this plasmid was derived from pBM125 (18) by the addition of a *Hind*III linker at the unique *Bam*HI site. The HSV DNA *pol* gene, derived from HSV-1 strain 17, was obtained from plasmid pD702, a derivative of pD7 in which the sequences for the short open reading frame upstream of the *pol* gene have been deleted (11).

Oligonucleotides. Two 50-base complementary oligonucleotides containing the *CYC-1* termination sequence were synthesized for this study on an Applied Biosystems model 380B DNA synthesizer by using β-cyanoethyl phosphoramidite chemistry. The oligonucleotides were purified by chromatography over a Sepharose G50 medium by previously described procedures (Applied Biosystems User Bulletin, issue 13, p. 17, 1984). Approximately 15 μg of each purified oligonucleotide was annealed by heating to 55°C for 15 min and cooling at room temperature for 30 min before addition to the ligation mixture.

Recombinant DNA methodology. Plasmid DNAs were purified and transformation of *E. coli* was achieved by standard procedures (20). Recombinant colonies were selected by colony hybridization. Radiolabeled probes were prepared by nick translation of the appropriate DNA fragment by using a commercially available kit (Bethesda Research Laboratories) and 5' [α -³²P]dCTP (3,000 Ci/mmol; Dupont, NEN Research Products). Yeast transformation was performed by the spheroplast method (1). DNA fragments used to construct the expression vectors were isolated from low-melting-temperature agarose (Bethesda Research Laboratories) after gel electrophoresis. Where appropriate, DNA fragments containing overhanging 5' ends were filled in by using *E. coli* DNA *pol* (Klenow fragment). All ligations were carried out by using T4 DNA ligase at 13°C overnight.

Protein analysis. The recombinant cultures were screened for the production of the 140-kDa HSV DNA *pol* polypeptide by analysis of the enzyme extracts described below on a 10% polyacrylamide gel by the procedure of Heine et al. (14). After electrophoresis, the gels were stained by using a commercially available silver stain kit (Bio-Rad Laboratories). Protein determinations were performed by using a Coomassie blue protein assay kit (Bio-Rad).

Preparation of antiserum against HSV-1 DNA *pol*. HSV-1 DNA *pol* was purified from HeLa cells infected with strain mP as previously described (26). Sodium dodecyl sulfate-

polyacrylamide gel electrophoresis analysis of the *pol* preparation used for immunization indicated the presence of a 140-kDa band corresponding to the full-size *pol* polypeptide, as well as minor bands at about 65 kDa. The antiserum was raised in a New Zealand White rabbit by a series of five immunizations with 10 μg of purified *pol* per dose; the first injection was with complete Freund adjuvant, and the subsequent injections were with incomplete Freund adjuvant. Purified immunoglobulins were prepared by affinity chromatography on protein A-Sepharose (Beckman Instruments, Inc.).

Immunoblotting. Transfer of proteins to nitrocellulose was done as described by Towbin et al. (28). The processing of filters was performed essentially by manufacturer instructions by using the Immun-Blot assay kit (Bio-Rad), except that bovine serum albumin (BSA) was substituted for gelatin. The dilution of primary antibody was 1:200, and that of alkaline phosphatase-conjugated anti-rabbit secondary antibody was 1:1,000.

Preparation of enzyme extracts. Yeast cells were grown in 100-ml overnight cultures and pelleted at 4°C. Cell lysates were prepared as described by Johnson et al. (17). In brief, cell pellets were suspended in 50 mM Tris hydrochloride (pH 8.0)–50 mM NaCl–1% dimethyl sulfoxide–10% glycerol–5 mM β-mercaptoethanol–10 mM EDTA–2 mM benzamidine–1 mM phenylmethylsulfonyl fluoride (PMSF). Lysis was achieved by vortexing the cells 15 to 20 times for 1 min in the presence of an equal volume of acid-washed glass beads. The extent of lysis was monitored by light microscopy. The lysed extracts were transferred to 1.5-ml Microfuge (Beckman) tubes and centrifuged for 10 min at 12,000 × *g*. Extracts were stored at –80°C in 15% glycerol until use. The final protein concentration of each of the extracts was between 5 and 6 mg/ml.

Infected cell extracts were prepared as follows. HeLa S3 cells were infected with HSV-1 Schooler at a multiplicity of infection of 10 PFU per cell and harvested at 16 h postinfection. Cell pellets (10⁸ cells) were frozen overnight at –80°C, thawed on ice, suspended in 20 ml of hypotonic solution A (10 mM K₂PO₄ [pH 7.0], 10 mM KCl, 1 mM PMSF; 1 mM dithiothreitol [DTT]), and incubated on ice for 30 min with occasional vortexing. An equal volume of solution B (0.7 M K₂PO₄ [pH 7.0], 28% glycerol, 6 mM DTT, 0.4% Nonidet P-40, 1 mM PMSF) was added, and the mixture was incubated on ice for an additional 30 min with occasional vortexing. The mixture was centrifuged for 1 h at 125,000 × *g*, and Nonidet P-40 was added to a final concentration of 0.4%. Aliquots were stored frozen at –80°C. The final protein concentration of the extract was 5 mg/ml.

DNA *pol* assays. The assay for yeast DNA *pol* was performed essentially as described by Celniker and Campbell (3) but without the addition of ribonucleotide triphosphates. Reaction mixtures contained 5 μl of enzyme extract (25 to 30 μg of protein) in 10 mM Tris hydrochloride (pH 7.5)–10 mM MgCl₂–10 mM (NH₄)₂SO₄–0.1 mM DTT–100 μM each of dATP, dCTP, and dGTP–100 μM [³H]TTP (10 cpm/pmol)–40 μg of nicked calf thymus DNA per ml–2 mM spermidine in a final volume of 50 μl. The assay for the HSV-1 DNA *pol* was performed as follows. The reaction mixture contained 5 μl of enzyme extract (25 μg of protein) 50 mM Tris hydrochloride (pH 8.0); 5 mM MgCl₂; 100 mM (NH₄)₂SO₄; 1 mM DTT; 5 μM each of dATP, dCTP, and dGTP; 5 μM [³H]TTP (200 cpm/pmol); 30 μg of nicked calf thymus DNA per ml; and 100 μg of BSA per ml in a final volume of 50 μl. All DNA polymerase assays were performed by incubating the reactants for 15 min at 37°C; the

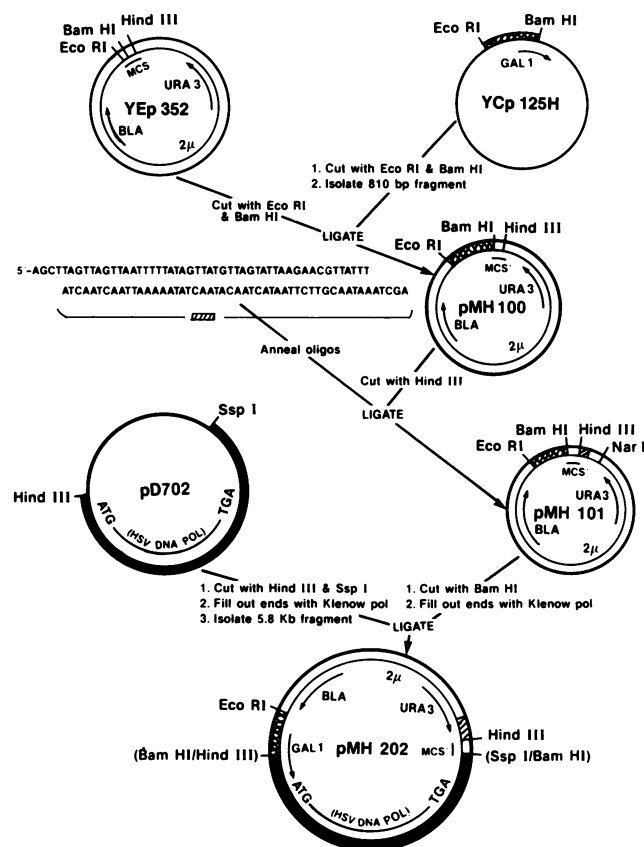


FIG. 1. Scheme for the construction of the yeast expression vector, pMH101 and the HSV DNA pol expression vector, pMH202. kb, Kilobase pairs.

reactions were spotted onto glass fiber filters and quenched by the addition of 10 mM sodium pyrophosphate in 5% trichloroacetic acid. The filters were washed thoroughly (twice with 1 N HCl and once with 95% ethanol), air dried, and quantitated by scintillation counting. All assays were conducted in duplicate.

Immunoprecipitation of DNA pol activity. Protein-A bearing *Staphylococcus aureus* (Pansorbin; Behring Diagnostics) was pretreated with BSA to minimize nonspecific binding by incubating 150 μ l of cell suspension with 0.3 mg of BSA at 4°C overnight. The following day, cells were pelleted, rinsed, and suspended in 125 μ l of 25 mM Tris hydrochloride (pH 8.0) until ready for use. Yeast cell enzyme extract (25 μ l) was incubated with 50 μ l of distilled water containing 0, 1, or 2 μ g of purified immunoglobulin G (IgG) for 3 h on ice. Five microliters of the treated *staphylococcus* suspension was added, and incubation was continued on ice for 1 h. The mixture was centrifuged at 6,500 rpm in a Microfuge, and the supernatant was assayed for DNA pol activity as described above. The results of assays run either without IgG or without both IgG and *Staphylococcus aureus* were averaged and used as a control value (100%).

RESULTS

Construction of the expression vector. The construction of the HSV-1 DNA pol expression vector used in this study is shown in Fig. 1. Plasmid YEp 352 is a high-copy-number, *E.*

coli-yeast shuttle vector used for the expression of genes which contain a functional yeast promoter (15). YEp 352 contains the β -lactamase gene for selective growth in ampicillin-containing media in *E. coli* and the *URA-3* gene for selective growth in uracil-deficient media in *S. cerevisiae*. In addition, the vector contains DNA sequences for autonomous replication in bacteria and in *S. cerevisiae*. We modified this vector to express foreign genes under the control of a regulatable yeast promoter, the galactokinase promoter (*GAL-1*). The *GAL-1* promoter has been described in detail elsewhere (18) and has been used successfully by others to express foreign proteins (18, 29). The use of a regulatable promoter would enable us to grow cells to high densities before inducing foreign protein expression, thus avoiding potential deleterious effects of foreign protein accumulation on cell growth. Expression of genes controlled by the *GAL-1* promoter is induced by galactose, repressed by glucose, and uninduced by raffinose (2, 18).

YEp 352 DNA was digested with restriction endonucleases *EcoRI* and *BamHI* to remove part of the multiple cloning site and to generate overhanging 5' ends. An approximately 810-base-pair (bp) fragment containing the *GAL-1* promoter sequence was isolated from plasmid YCp 125H by digestion with *EcoRI* and *BamHI*. The two DNAs were ligated together to form the plasmid pMH 100.

While some investigators (2, 21) have reported successful expression of foreign proteins in *S. cerevisiae* without the addition of specific transcription termination sequences, others (29) have used these sequences to improve foreign protein expression. To potentially improve the expression of foreign genes, a yeast termination sequence was introduced into the vector. The termination sequence was composed of two synthetic oligonucleotides (Fig. 1) which anneal to form a fragment containing the sequence required for efficient transcription termination from the yeast iso-1-cytochrome *c* (*CYC-1*) gene (32) and translation stop codons in the three possible reading frames. The double-stranded oligonucleotide was designed to be directly inserted into the *HindIII* restriction site in pMH100. When inserted in the correct orientation, the oligonucleotide would retain the *HindIII* site only at the end upstream of the three stop codons. The proper orientation of the insert was established by specific differences in the patterns generated by digestion of recombinant plasmids with appropriate restriction enzymes (data not shown).

pMH101, the new construction containing the correctly oriented oligonucleotide, was digested with *BamHI*, and the overhanging 5' ends were filled in with *E. coli* DNA pol (Klenow fragment) to create blunt ends which could accept a DNA fragment containing the HSV-1 *pol* gene. The HSV *pol* gene was mobilized from pD702, a plasmid in which a *HindIII* linker was inserted 79 bp upstream of the 3,705-bp *pol* open reading frame following BAL 31 mutagenesis. This vector contains a deletion of the short upstream open reading frame, 124 bp proximal of the assigned translation initiator (13, 27).

Plasmid pD702 was digested with restriction enzymes *SspI* and *HindIII*, and the overhanging 5' ends were made blunt as described above. The 5.8-kbp *HindIII*-*SspI* fragment containing the entire HSV-1 *pol* gene was then blunt end ligated into the modified *BamHI* site of pMH101. Recombinant isolates were selected by colony hybridization, and the proper orientation of the inserted gene was determined by digestion with appropriate restriction endonucleases (data not shown).

The final construction (pMH202) is designed to generate a

transcript, under inducing conditions, which contains about 140 bases of 5' noncoding sequence before the natural ATG initiation codon of the HSV-1 *pol* gene and about 2.1 kilobases downstream of the natural TGA termination codon before reaching the oligonucleotide sequence for transcription termination. Therefore, the vector should encode an RNA which can be translated to the complete HSV-1 140-kDa DNA pol polypeptide as predicted from the DNA sequence (13, 27).

Expression of HSV DNA pol in *S. cerevisiae*. To determine whether the 140-kDa HSV pol protein was expressed and whether HSV-specific DNA pol activity could be detected in yeast cells containing pMH202, the following experiments were performed. Yeast cells (Y-294) were transformed with pMH101 (control) or pMH202 (expresser) DNA and selected for uracil prototrophy by growth on minimal media supplemented with leucine, histidine, tryptophan, and glucose but lacking uracil. One colony of each type (designated Y-MH101 or Y-MH202, respectively) was selected at random for further analysis. Overnight cultures were grown from these two strains in the presence of 2% raffinose. On the following day, cultures were divided and cells were pelleted, suspended in fresh media containing either 2% raffinose or 2% galactose, and again grown overnight. On the following day, the four cultures were pelleted and lysates were prepared as described in Materials and Methods. The lysates were run on sodium dodecyl sulfate-polyacrylamide gels, and the separated proteins were transferred to nitrocellulose for immunoblotting with antiserum which we prepared against a highly purified HSV-1 DNA pol preparation. The results are shown in Fig. 2, lanes 3 to 6. For comparison, lane 1 contains HSV-1 DNA pol from the highly purified preparation used as immunogen to prepare our antiserum and lane 2 contains a crude HSV-1 pol preparation from infected HeLa cells prepared as a control for the pol enzyme assays. Analysis of the yeast lysates clearly shows the specific induction of an immunoreactive polypeptide of approximately 140 kDa only in Y-MH202 cells grown under inducing conditions (lane 6). In addition, the yeast-expressed polypeptide exhibits the same electrophoretic mobility as the HSV-1 pol from crude infected-cell extracts. It should be noted that the pol polypeptide in lane 1 (derived from strain HSV-1 mP) has a slightly slower electrophoretic mobility than the pol in lane 2 (derived from HSV-1 Schooler). This difference in electrophoretic mobility may be due to inconsistencies between the two virus strains with respect to DNA sequence or transcriptional or posttranslational modification. In addition, it should be noted that our antiserum also reacts with HSV-specific protein(s) of about 65 kDa in molecular mass. In the yeast cell extracts, this 65-kDa protein most likely represents a degradation product of the 140-kDa HSV DNA pol polypeptide. The generation of such a degradation product during purification of pol from infected cells has been noted previously (W. T. Ruyechan and C. S. Crumpacker, unpublished observations). Alternatively, it is possible that this protein may result from alterations in the transcription or translation of mRNA encoding the HSV *pol* gene. The 65-kDa protein band in lanes 1 and 2, however, may also contain the 65-kDa HSV DNA-binding protein which is often found tightly associated with 140-kDa DNA pol polypeptide through purification procedures (24).

The lysates were also analyzed for DNA pol enzyme activity. Each of the four yeast cell extracts was assayed for DNA pol activity under two sets of conditions. The yeast DNA pol assay conditions were designed to detect the alpha

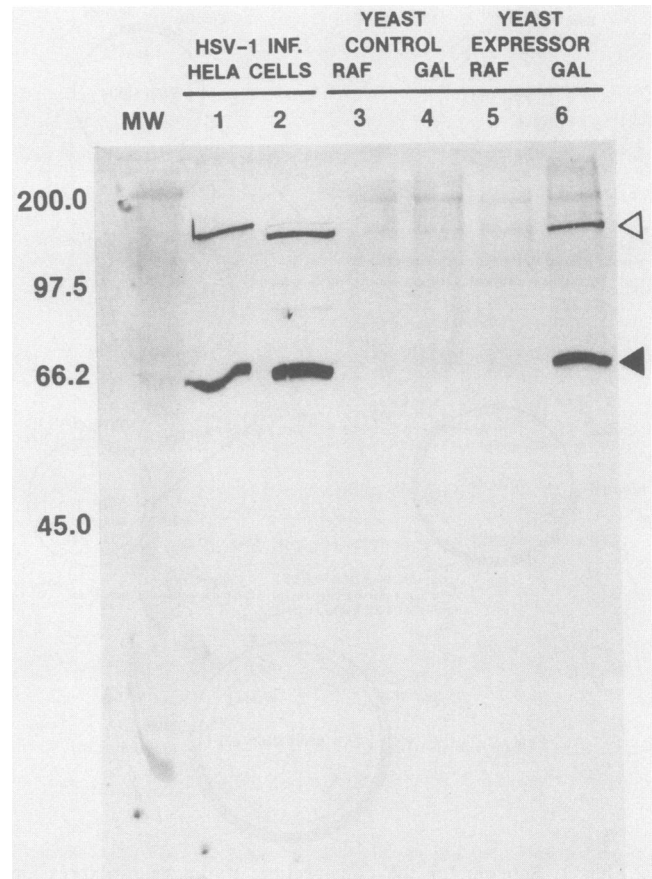


FIG. 2. Immunoblot of the HSV DNA pol expressed in *S. cerevisiae* as described in Materials and Methods. Molecular weights (MW; 10^3) are reported along the left margin. Lanes 1 and 2 contain HSV DNA pol from infected HeLa cells: $\sim 9 \mu\text{g}$ of purified enzyme preparation prepared from HSV-1 (mP)-infected cells and $\sim 60 \mu\text{g}$ of a crude enzyme preparation from HSV-1 (Schooler)-infected cells, respectively. Lanes 3 and 4 contain yeast cell lysates prepared from control cells (Y-MH101) grown in raffinose (RAF) or galactose (GAL), respectively. Lanes 5 and 6 contain extracts prepared from the expresser strain (Y-MH202) grown in RAF or GAL, respectively. Lanes 3 to 6 contain 50 to 60 μg each. \triangle , ~ 140 kDa HSV pol; \blacktriangle , ~ 65 kDa protein(s).

pol which should be present in all lysates. The HSV DNA pol assay conditions were chosen to detect HSV-specific DNA pol activity which is enhanced by high salt (100 mM ammonium sulfate) (23). In contrast, the yeast alpha DNA pol is inhibited under these salt conditions (3). Table 1 reveals that only Y-MH202, HSV DNA pol expresser cells, grown under inducing conditions (galactose) produce significant amounts of high-salt DNA pol activity. In addition, Table 2 reveals that this novel high-salt activity in the Y-MH202 yeast cell extracts is inhibited by ACVTP and aphidicolin, both potent and well-characterized inhibitors of the HSV DNA pol enzyme (8, 16). Table 2 also reveals that the low-salt DNA pol activity in all of the extracts tested is sensitive to aphidicolin, which is characteristic of alpha pols (3, 17) and is not sensitive to ACVTP at the concentration tested.

A more complete comparison of the inhibition of ACVTP of HSV pol produced in *S. cerevisiae* versus HSV pol produced in HeLa cells is shown in Table 3. These results

TABLE 1. DNA pol activity

Extract and growth condition	Result of DNA pol assay ^a			
	HSV		<i>S. cerevisiae</i>	
	I ^b	II ^b	I	II
HSV-1-infected HeLa cells	6.90	5.78	129.1	118.6
Y-MH101 yeast control cells				
Raffinose	0.26	0.18	139.6	113.2
Galactose	0.11	0.87	95.6	125.4
Y-MH202 yeast expresser cells				
Raffinose	0 ^c	0.40	129.1	139.6
Galactose	6.69	5.53	132.4	129.6

^a Pol activity is expressed as picomoles of [³H]TTP incorporated per reaction; assay conditions are as described in Materials and Methods. All values are the average of duplicate assays.

^b I and II are separate experiments.

^c Value below background.

reveal that the HSV pol enzyme from both sources behaves in a similar fashion; the 50% inhibitory dose is at a concentration of between 1.15 and 2.3 μ M ACVTP.

To further demonstrate the HSV-specific nature of this high-salt DNA pol activity, we incubated the cell extracts with purified IgG from our rabbit anti-pol serum or with control IgG from a nonimmunized rabbit. Immune complexes were removed with protein A-*Staphylococcus aureus* by centrifugation, and then residual DNA pol activity from the supernatants was assayed. Two yeast extracts were chosen for comparison; Y-MH101 (galactose-induced) control cell extract was assayed under yeast DNA pol assay conditions to monitor effects of IgG on alpha DNA pol activity, and Y-MH202 (galactose-induced) expresser extract was assayed under HSV DNA pol conditions to monitor effects of the IgG on the high-salt pol activity. Figure 3 reveals that the high-salt activity in galactose-induced Y-MH202 expresser cells can be reduced >50% by immunoprecipitation by using IgG derived from our antiserum to HSV-1 DNA pol, while the alpha pol activity in the galactose-induced Y-MH101 control cell extract is only slightly reduced by reaction with the immune IgG. DNA pol activity in both extracts is only slightly affected by reaction with the control (normal rabbit) IgG.

These results indicate the successful expression of an active form of the HSV-1 DNA pol in *S. cerevisiae*.

TABLE 2. Inhibition of DNA pol activity by ACVTP and aphidicolin

Extract and assay condition ^a	% Inhibition by drug ^b	
	ACVTP	Aphidicolin
Yeast control		
HSV pol	—	—
Yeast pol	0	88
Yeast expresser		
HSV pol	93	94
Yeast pol	0	80
HSV-HeLa		
HSV pol	89	90
Yeast pol	0	89

^a Extracts were prepared as described in Materials and Methods. Yeast control (Y-MH101) and expresser (Y-MH202) cell extracts were prepared from cultures grown in the presence of galactose. Assay conditions were as described in Materials and Methods.

^b ACVTP and aphidicolin were present in reactions at 23 and 5.3 μ M, respectively. 0, Relative activity was ≥ 1.0 ; —, value is not statistically significant because of low pol activity.

TABLE 3. Inhibition of HSV pol by increasing amounts of ACVTP

Concn (μ M) of ACVTP	% HSV pol activity in extract ^a	
	HeLa	Yeast
0	100	100
0.023	78	93
0.115	91	90
0.23	82	81
1.15	60	59
2.3	36	38
11.5	17	6
23.0	6	10

^a HSV assay conditions were as described in Materials and Methods. All values are the average of duplicate assays. Extracts were prepared as described in Materials and Methods. Yeast extracts were prepared from cultures of expresser (Y-MH202) cell extracts grown in the presence of galactose. HeLa extracts were prepared from HSV-1-infected cells.

DISCUSSION

This report details the successful expression of an active eucaryotic DNA pol in a heterologous host. In this study, the HSV pol open reading frame was inserted into a modified yeast expression plasmid such that the HSV DNA pol could be inducibly expressed in yeast cells under the control of the *GAL-1* promoter. The enzyme expressed in *S. cerevisiae* possesses the salt resistance and inhibitor sensitivity profiles of the authentic pol enzyme isolated from HSV-1-infected cells. Furthermore, the expressed product possesses HSV-1 pol antigenic determinants as shown by immunoblotting and by immunoprecipitation of pol enzymatic activity from yeast cell extracts.

Expression of the HSV-1 DNA pol enzyme in quantity from a heterologous system will allow further mechanistic studies as well as characterization of complexes between the HSV-1 DNA pol and the other components of the DNA replication machinery of HSV (4). Even though the amounts of HSV-1 DNA pol obtained from infected cells and the yeast cells appear to be essentially the same when this construct is used, the ability to grow large quantities of yeast

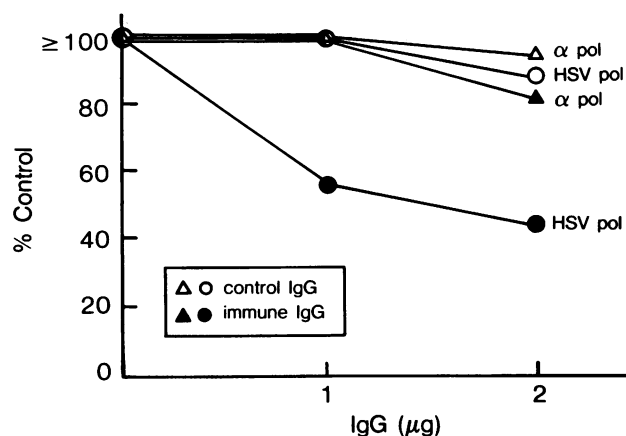


FIG. 3. Depletion of HSV DNA pol activity from yeast lysates by immunoprecipitation with purified immunoglobulins from a rabbit antiserum against HSV-1 DNA pol (immune IgG) or normal rabbit serum (control IgG). Yeast extracts from Y-MH202 grown in galactose were used for the HSV pol enzyme assay. Extracts from Y-MH101 grown in galactose were used for the alpha pol enzyme assay. All values are the averages of duplicate assays.

can potentially result in the availability of large quantities of enzyme. Because the product is expressed from a plasmid, it should be possible to engineer a variety of mutations into the gene to test their effects on such enzyme properties as substrate specificity and sensitivity to inhibitors. Further refinements of this expression system should also allow the bulk purification of a homogeneous product leading to physical-chemical studies designed to elucidate the functional domains and three dimensional structure of the HSV pol enzyme.

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