JOURNAL OF VIROLOGY, Aug. 1992, p. 4884–4892
0022-538X/92/084884-09$02.00/0

Vol. 66, No. 8

The UL8 Subunit of the Herpes Simplex Virus Helicase-Primase Complex Is Required for Efficient Primer Utilization

G. SHERMAN, J. GOTTLIEB, AND M. D. CHALLBERG*

Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, 9000 Rockville Pike, Bethesda, Maryland 20892

Received 24 March 1992/Accepted 11 May 1992

The herpes simplex virus (HSV) type 1 helicase-primase is a three-protein complex, consisting of a 1:1:1 association of UL5, UL8, and UL52 gene products (J. J. Crute, T. Tsurumi, L. Zhu, S. K. Weller, P. D. Olivo, M. D. Challberg, E. S. Mocarski, and I. R. Lehman, Proc. Natl. Acad. Sci. USA 86:2186–2189, 1989). We have purified this complex, as well as a subcomplex consisting of UL5 and UL52 proteins, from insect cells infected with baculovirus recombinants expressing the appropriate gene products. In confirmation of previous reports, we find that whereas UL5 alone has greatly reduced DNA-dependent ATPase activity, the UL5/UL52 subcomplex retains the activities characteristic of the heterotrimer: DNA-dependent ATPase activity, DNA helicase activity, and the ability to prime DNA synthesis on a poly(dT) template. We also found that the primers made by the subcomplex are equal in length to those synthesized by the UL5/UL8/UL52 complex. In an effort to uncover a role for UL8 in HSV DNA replication, we have developed a model system for lagging-strand synthesis in which the primase activity of the helicase-primase complex is coupled to the activity of the HSV DNA polymerase on ICP8-coated single-stranded M13 DNA. Using this assay, we found that the UL8 subunit of the helicase-primase is critical for the efficient utilization of primers; in the absence of UL8, we detected essentially no elongation of primers despite the fact that the rate of primer synthesis on the same template is undiminished. Reconstitution of lagging-strand synthesis in the presence of UL5/UL52 was achieved by the addition of partially purified UL8. Essentially identical results were obtained when *Escherichia coli* DNA polymerase I was substituted for the HSV polymerase/UL42 complex. On the basis of these findings, we propose that UL8 acts to increase the efficiency of primer utilization by stabilizing the association between nascent oligoribonucleotide primers and template DNA.

The herpes simplex virus (HSV) genome is a 153-kb linear DNA duplex that contains three origins of DNA replication and codes for at least 72 proteins (25). Seven viral genes code for proteins that are essential for HSV origin-dependent DNA synthesis (2–6, 24, 34, 35, 40–43). The products of these genes include an origin-binding protein (UL9) (30), a DNA polymerase (UL30) (5, 6, 34) and its associated processivity subunit (UL42) (14–16, 32), a single-stranded DNA-binding protein (UL29 or ICP8) (8, 33, 41), and the three members of a primase-helicase complex (UL5, UL8, and UL52) (10).

The UL5/UL8/UL52 complex has been isolated both from HSV-infected cells and from insect cells triply infected with baculovirus recombinants expressing each of the proteins (10, 12). The recombinant enzyme is indistinguishable from that isolated from HSV-infected cells with respect to both structure and enzymatic activity. It comprises a heterotrimer containing one each of the subunits (9). As a helicase, it can utilize ATP or GTP to unwind DNA in a 5′-to-3′ direction (9a) and is stimulated by the HSV single-stranded DNA-binding protein, ICP8 (18). Kinetic analyses have shown that the helicase has two nucleoside triphosphate (NTP) sites; one site hydrolyzes ATP and GTP, and the other hydrolyzes only ATP (8a). The primase activity of the complex synthesizes short oligoribonucleotides 8 to 12 bases in length and can utilize both poly(dT) and natural DNAs as templates (9–11).

There is very little information available regarding the functions of the individual subunits of the helicase-primase complex. Although the UL5 gene contains regions bearing considerable homology to several conserved motifs in other known helicases, including a likely ATP binding site (26), neither DNA-dependent ATPase nor helicase activity has been associated with the protein during its purification from cells infected with a UL5-expressing baculovirus recombinant (1). A biochemical characterization of the UL52 protein has not been possible because of the insolubility of this protein when overexpressed in the absence of UL5 protein (unpublished observations). A subcomplex consisting of UL5 and UL52 proteins has recently been shown to possess the enzymatic activities that have been associated with the heterotrimer: DNA-dependent ATPase and GTPase activities, ability to displace a 3′-tailed oligonucleotide annealed to M13 single-stranded DNA, and ability to prime DNA synthesis on poly(dT), albeit at a rate somewhat lower than that of the heterotrimer (1, 12). It therefore appears likely that UL5 and UL52 proteins constitute a functional unit in which both helicase and primase activities are dependent on the presence of both subunits. The UL8 protein has no detectable ATPase, primase, or helicase activities (1, 12).

To better understand the role that the UL8 protein plays in viral replication, we compared purified preparations of UL5/UL8/UL52 and UL5/UL52 complexes in a number of assays. We found that UL5/UL52 is very similar to UL5/UL8/UL52 in DNA-dependent ATPase activity, helicase activity, and ability to prime DNA synthesis on a poly(dT) template, confirming previous findings (1, 12). In addition, we demonstrated that UL5/UL52 makes primers of the same size and at the same rate as does UL5/UL8/UL52. We detected striking differences, however, when we compared the heterotrimer and subcomplex in primase-primed replication

* Corresponding author.

assays on single-stranded M13 circles. We showed that M13 single-stranded DNA was converted to nicked or gapped double-stranded circles by either the HSV DNA polymerase (Pol/UL42) or *Escherichia coli* DNA polymerase I (Pol I) when the reaction was primed by using the UL5/UL8/UL52 complex. In contrast, little or no DNA synthesis was observed with either DNA polymerase in the presence of UL5/UL52; activity was restored in this system, however, by the addition of partially purified UL8. Our data indicate, therefore, that UL8 is required for efficient primer utilization on natural DNA templates. Our results are consistent with a model in which UL8 acts during lagging-strand synthesis to stabilize the association of primers with the template before they are elongated by DNA polymerase.

MATERIALS AND METHODS

Buffers. Buffer A contained 20 mM Tris hydrochloride (pH 8.0), 0.5 mM dithiothreitol (DTT), 0.5 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 10% glycerol. Buffer B contained 20 mM HEPES (*N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; pH 7.6), 0.5 mM DTT, 0.5 mM $MgCl_2$, 10 mM $NaHSO_3$, 0.5 mM PMSF, and 2.0 μg each of leupeptin and pepstatin A per ml. Buffer C contained 20 mM HEPES (pH 7.6), 0.5 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, and 10% glycerol. Buffer D contained 20 mM Tris hydrochloride (pH 7.5), 4 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 50 mM NaCl, and 10% glycerol.

Cells and viruses. *Spodoptera frugiperda* (Sf9) cells were maintained in TMNFH medium (GIBCO) containing 10% fetal bovine serum. Recombinant baculoviruses containing the UL5, UL8, and UL52 genes (*Autographa californica* nuclear polyhedrosis virus [AcNPV]/UL5, AcNPV/UL8, and AcNPV/UL52) (31) were propagated as described previously (37).

Enzyme assays. (i) DNA-dependent ATPase assays were performed as described previously (10) except that incubations were for 1 h at 37°C. (ii) Indirect (coupled) primase assays (40 μl) contained 50 mM Tris hydrochloride (pH 7.5), 8 mM $MgCl_2$, 1 mM DTT, 0.5 mM ATP, 5.0 μM poly(dT)$_{3500}$, 2.35 μM [^{3}H]dATP (1×10^4 to 2×10^4 cpm/pmol), and 3 to 5 U of *E. coli* DNA Pol I. Reaction mixtures were incubated at 37°C for 1 h, at which time they were terminated by the addition of 10 μl of 0.5 M EDTA. Incorporation of [^{3}H]dATP into acid-insoluble material was measured in a liquid scintillation counter. One unit of enzyme activity is defined as the incorporation of 1.0 pmol of [^{3}H]dATP per h (10). (iii) Assays for lagging-strand synthesis (50 μl) contained 60 μM each GTP, CTP, and UTP, 1 mM ATP, 60 μM each dGTP, dATP, and dTTP, 2.5 μg of bovine serum albumin (BSA), 20 μM [^{32}P]dCTP (0.8×10^4 to 1.6×10^4 cpm/pmol), 20 fmol of single-stranded M13mp19(+) DNA, 400 fmol of HSV Pol/UL42 complex, 7.0 pmol of ICP8, and 200 to 1,600 fmol of UL5/UL8/UL52 or UL5/UL52 complex in buffer D. Reaction mixtures were incubated for 30 min at 37°C in the presence of HSV Pol/UL42 complex and then chilled on ice for the sequential addition of [^{32}P]dCTP, UL5/UL8/UL52 or UL5/52, and ICP8; they were then incubated for 2 h at 25°C. Reactions were terminated by adding an equal volume of 1% sodium dodecyl sulfate (SDS)–40 mM EDTA–0.6 M sodium acetate–100 μg of calf thymus DNA per ml. After phenol-chloroform extraction of protein and precipitation in ethanol, the reaction products were analyzed by gel electrophoresis in 1% neutral agarose gels and autoradiography. Singly primed M13 DNA was prepared as described previously (16). The synthetic oligonucleotide used was 5'-CAGTCACGACGTTGTAAAACG ACGGCCAGT-3', which is complementary to nucleotides 6300 to 6271 of M13mp19(+) DNA.

Determination of the length of the RNA primers. Standard reaction mixtures (50 μl) contained 20 mM Tris acetate (pH 7.2), 3 mM Mg^+ acetate, 1 mM DTT, 50 μM each GTP, CTP, and UTP, 8.3 μM [^{32}P]ATP (1.6×10^5 to 3.2×10^5 cpm/pmol), and either 50 μM (nucleotide concentration) M13mp19(+) or poly(dT)$_{3500}$ (17). After addition of HSV primase-helicase, reaction mixtures were incubated for 2 h at 37°C, and reactions were terminated with the addition of an equal volume of 1% SDS–40 μM EDTA–50 μg of tRNA per ml. After phenol-chloroform extraction of protein and ethanol precipitation, reaction products were resuspended in deionized formamide and analyzed by electrophoresis on denaturing gels containing 20% polyacrylamide, 0.7% methylene bisacrylamide, 7 M urea, and 50 mM Tris borate (pH 8.3). Gels were dried and exposed to Kodak XAR-5 X-ray film at −80°C. Size standards, generated either by 5' ^{32}P labeling of *Cla*I linkers or by partial alkaline hydrolysis of 5'-^{32}P-labeled poly(rA) (13), were electrophoresed in parallel lanes on the same gels. The lengths of RNA primers, relative to that of p(Ap)$_n$ size standards, were corrected for the presence of an expected 5' triphosphate.

Protein purification. (i) UL5/UL52 complex. Forty 225-cm^2 flasks of Sf9 cells were coinfected with baculovirus recombinants AcNPV/UL5 and AcNPV/UL52 at a multiplicity of infection of 10 PFU of each virus per cell. At 60 h after infection, cells were dislodged from the flasks and harvested from the cell culture medium by centrifugation for 10 min at 3,000 rpm in a Sorvall HA-6 rotor. Pelleted cells were gently resuspended in 500 ml of ice-cold serum-free Grace's medium and collected by centrifugation at 4°C as described above. The cells were resuspended in 40 ml of buffer B, allowed to swell on ice for 15 min, and then lysed by 15 strokes in a Kontes all-glass Dounce homogenizer (B-type pestle). The homogenate was centrifuged for 10 min at 3,000 rpm in a Sorvall SS34 rotor to pellet nuclei. The supernatant was decanted and clarified by centrifugation at 12,000 × *g* for 10 to 20 min at 4°C. Nuclei were resuspended in 20 ml of buffer B, to which an equal volume of buffer B containing 3.4 M NaCl was added. The lysate was incubated on ice for 1 h, clarified by centrifugation at 100,000 × *g* for 1 h, and dialyzed to equilibrium against buffer C containing 50 mM NaCl.

The cytosolic extract (fraction I) was made 25 mM in NaCl and applied to a DEAE-Sephacel column (35 ml) previously equilibrated with buffer A containing 25 mM NaCl. Proteins were eluted from the column in a linear salt gradient (350 ml) from 25 to 600 mM NaCl in buffer A. Fractions (7 ml) containing the peak of DNA-dependent ATPase activity were pooled and dialyzed to equilibrium against buffer C containing 50 mM NaCl. The clarified dialysate (fraction II) was applied to a column of heparin-agarose (25 ml) that had been equilibrated with buffer C containing 50 mM NaCl. Proteins were eluted from the column in a linear gradient (250 ml) from 50 to 600 mM NaCl in buffer C. Fractions (5 ml) containing the peak of DNA-dependent ATPase activity were pooled and dialyzed to equilibrium against buffer C containing 50 mM NaCl. The clarified dialysate (fraction III) was applied to a single-stranded DNA agarose column (5 ml) previously equilibrated in the same buffer. Proteins were eluted from the column in a linear gradient (50 ml) from 50 to 600 mM NaCl in buffer C. Fractions (1 ml) were assayed for DNA-dependent ATPase and primase activities. Peak fractions were pooled and concentrated 20-fold in Centricon 30

concentrators (Amicon). The concentrate (0.2 ml) (fraction IV) was applied to a prepacked Superose 12 gel filtration column equilibrated in buffer C containing 0.2 M NaCl and 0.01% Nonidet P-40. The column was eluted with the same buffer at a flow rate of 0.25 ml/min, and fractions, collected every 2.0 min, were assayed for DNA-dependent ATPase activity and primase activity. Peak fractions (fraction V) were pooled and stored at −80°C. The polypeptide composition of this fraction was examined by SDS-polyacrylamide gel electrophoresis (PAGE) and staining with Coomassie blue and with silver. By this analysis, we estimated that the UL5/UL52 complex comprised approximately 60% of the total protein. A more accurate determination of the concentration of UL5/UL52 was obtained by performing SDS-PAGE and Western immunoblot analysis of this fraction, using rabbit antisera to the UL5 and UL52 gene products. Proteins were quantitated by comparing the intensities of these bands, in densitometric scans, with that of a reference standard of pure UL5/UL8/UL52 (see below).

(ii) UL5/UL8/UL52. Forty 225-cm^2 flasks were triply infected with baculovirus recombinants AcNPV/UL5, AcNPV/UL8, and AcNPV/UL52 at a multiplicity of 10 PFU per cell for each virus. Preparation of infected cell extracts and the procedure for purification of the UL5/UL8/UL52 primase-helicase complex from the cytosolic lysate were exactly as described above for the UL5/UL52 complex. An examination of the final fraction (fraction V) by staining of SDS-protein gels with Coomassie blue and with silver indicated that UL5, UL8, and UL52 proteins together constituted greater than 95% of the total protein. An early preparation of UL5/UL8/UL52 and UL5/UL52 used in this study was initially applied to a phosphocellulose column. Since the majority of UL5/UL8/UL52 and UL5/UL52 proteins failed to bind to the column, this step was omitted from subsequent preparations.

(iii) UL8. Partially purified UL8 was obtained during the purification of the UL5/UL8/UL52 complex from a cytosolic lysate prepared from triply infected Sf9 cells. The lysate, prepared as described above from 40-cm^2 flasks, was made 25 mM in NaCl and applied to a 25-ml DEAE-Sephacel column previously equilibrated with buffer A containing 25 mM NaCl. Proteins were eluted in a linear salt gradient from 25 to 500 mM NaCl in buffer A. UL5/UL8/UL52-containing fractions, as determined by DNA-dependent ATPase activity and Western blotting, were pooled, dialyzed against 50 mM NaCl in buffer C, and applied to a 25-ml heparin-agarose column. Proteins were eluted from the column in a linear salt gradient from 50 to 500 mM NaCl in buffer C. Western analysis and DNA-dependent ATPase assays indicated that the flowthrough material contained UL8, free of UL5 and UL52 proteins, whereas the column fractions contained UL8 complexed with UL5 and UL52. Uncomplexed UL8 constituted approximately 30% (0.5 mg/ml) of the total protein in the flowthrough material.

(iv) HSV DNA polymerase and ICP8. The HSV Pol/UL42 complex and ICP8 were purified as described previously (16).

RESULTS

Enzymatic activities of purified proteins. UL5/UL52 and UL5/UL8/UL52 complexes were purified from insect cells that were dually or triply infected with the appropriate recombinant baculoviruses. During the purification, the peaks of both DNA-dependent ATPase and primase activities coincided with the peak of UL5/UL52 or UL5/UL8/UL52 proteins. In agreement with previous reports (11, 12), the elution profile for each complex from a Superose 12 column was consistent with an equimolar (1:1 or 1:1:1) association of subunits. In addition, we found that both the UL5/UL52 complex and the UL5/UL8/UL52 complex displayed helicase activity on a substrate consisting of single-stranded M13 annealed to an oligonucleotide (30-mer) having a 15-base free 3′ tail. Thus, our results confirm previous studies demonstrating that the UL5/UL52 subcomplex has the helicase, DNA-dependent ATPase (1, 12), and primase (12) activities that have been associated with UL5/UL8/UL52. Our data are also in close agreement with previously published values for the specific activities associated with these related enzyme complexes (Table 1) (12).

TABLE 1. Comparison of the specific activities of the UL5/UL8/UL52 complex, UL5/UL52 subcomplex, and UL5 protein

Enzyme(s)	DNA-dependent ATPase (nmol of ATP hydrolyzed/pmol of enzyme)	DNA primase (pmol of dATP incorporated/pmol of enzyme)	
		Poly(dT) primer	M13 primer
UL5/UL8/UL52	5.3	4.4	0.63
UL5/UL52	5.6	3.4	0.008
UL5	0.03	ND[a]	ND

[a] ND, not done.

During the purification of UL5/UL52 on a heparin-agarose column, immunoblot assays of column fractions revealed that the UL5 polypeptide eluted from the column both as an uncomplexed polypeptide and in association with UL52

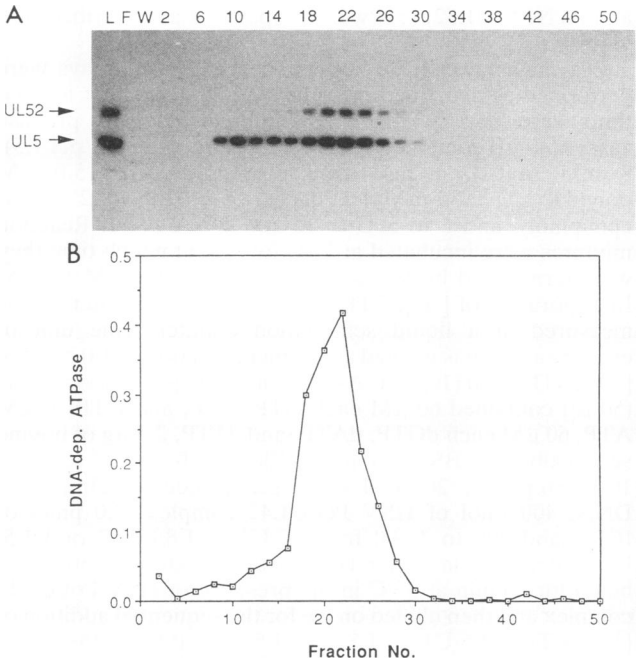

FIG. 1. Evidence that UL5 alone has negligible DNA-dependent ATPase activity. (A) Western blot of fractions eluting from heparin-agarose, probed with UL5- and UL52-specific antisera. L, column load material; F, column flowthrough fraction; W, column wash fraction. (B) Plot of DNA-dependent ATPase activity versus column fractions from the heparin-agarose column shown in panel A. DNA-dependent ATPase activity was assayed as described in Materials and Methods.

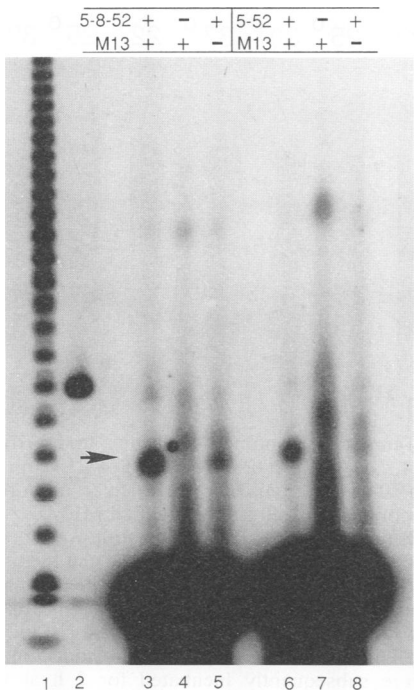

FIG. 2. Sizes of RNA primers made by HSV UL5/UL8/UL52 (lane 3) or UL5/UL52 (lane 6). Primase reactions products were analyzed as described in Materials and Methods. Lanes 4 and 7 represent control reactions lacking primase-helicase; reaction mixtures in lanes 5 and 8 lacked single-stranded M13mp19(+) DNA. Lane 1, 5′-^{32}P-labeled p(Ap)$_n$ size standards generated by partial alkaline hydrolysis of poly(rA) (13); lane 2, 5′-^{32}P-labeled *Cla*I linker (5′-CATCGATG-3′). The arrow indicates the location of primers.

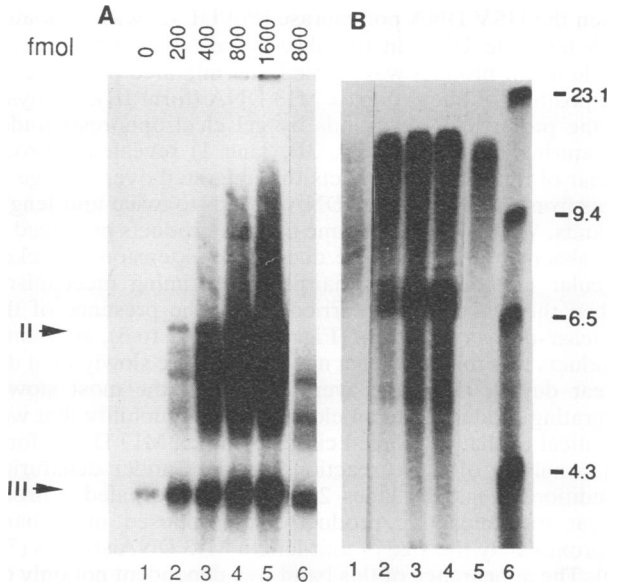

FIG. 3. Primase-primed DNA synthesis on a single-stranded M13 DNA template. Reaction mixtures (50 μl) contained 20 fmol of single-stranded M13mp(+) circular DNA, 1.0 mM ATP, 2.5 μg of BSA, 60 μM each dATP, dGTP, and dTTP, 20 μM [^{32}P]dCTP (1.6×10^4 cpm/pmol); 60 μM each GTP, CTP, and UTP, 7.0 pmol of ICP8 (where indicated), 400 fmol of HSV Pol/UL42 complex, and 0 to 1,600 fmol of UL5/UL8/UL52 in buffer D. (A) Each reaction mixture contained the indicated amount of UL5/UL8/UL52. Lanes 1 to 5 contained 7.0 pmol of ICP8. Reaction products were analyzed by neutral agarose gel electrophoresis as described in Materials and Methods. Arrows indicate the locations of nicked double-stranded M13 circles (form II) and linear M13 duplexes (form III). (B) Reaction conditions were as described for panel A. Reaction products were dissolved in 15 μl of 0.1 N NaOH–5% glycerol–1 mM EDTA–0.025% bromocresol green and analyzed on a 1% alkaline agarose gel. All lanes contained 7.0 pmol of ICP8 and 0 fmol (lane 1), 200 fmol (lane 2), 400 fmol (lanes 3 and 5), and 800 fmol (lane 4) of UL5/UL8/UL52. In lane 5, ATP, GTP, CTP, and UTP were omitted from the reaction mixture. Lane 6, phage lambda DNA digested with *Hin*dIII and end labeled with ^{32}P. The sizes of the *Hin*dIII restriction fragments (in kilobases) are indicated by the numbers at the right.

(Fig. 1); UL5 was evidently expressed at a higher level than was UL52 in the doubly infected cells. This finding provided an opportunity to assay uncomplexed UL5 polypeptide for DNA-dependent ATPase under conditions in which the complexed form of the polypeptide isolated from the same infected cell preparation was known to be active. The results clearly showed that the peak of DNA-dependent ATPase activity corresponds to the peak of UL5/UL52 antigen. We estimate that specific ATPase activity of free UL5 (Fig. 1, fractions 8 to 14) is less than 5% of that of the UL5/UL52 complex. As others have reported (1, 12), we were not able to find conditions in which uncomplexed UL52 could be isolated in soluble form.

Determination of the length of primers made by UL5/UL52 and UL5/UL8/UL52 complexes. To determine whether UL8 plays a role in regulating the size of primers made by the helicase-primase complex, we analyzed reaction products made by purified UL5/UL8/UL52 and UL5/UL52. The two enzyme preparations were incubated with single-stranded M13 DNA template and ^{32}P-labeled rNTPs. The reaction products were then analyzed by electrophoresis on a 20% polyacrylamide sequencing gel. As shown in Fig. 2, both proteins synthesized short oligoribonucleotides approximately 6 to 8 nucleotides in length (lanes 3 and 6). The primers made by both enzymes appeared to be quite homogeneous, with greater than 90% of the reaction products in a single band on the gel. The nucleotide composition of this band has not yet been determined. Some preparations of UL5/UL8/UL52 and UL5/UL52 synthesized primers in the absence of template (lane 5). Control experiments showed that this template-independent primase activity could be selectively removed by passing the enzyme preparation over a DEAE-Sephacel column (data not known). Quantitation of the radioactive oligoribonucleotide products on the gel demonstrated that, as in the case when primase activity was tested by using the indirect assay on a poly(dT) template, the specific primase activities of the two enzymes were similar (Table 1). We conclude that UL8 has no effect either on the rate of synthesis or on the length of the RNA oligonucleotide synthesized by the helicase-primase complex.

Assay system for lagging-strand DNA synthesis. Since UL8 has no apparent role in either of the known catalytic activities of the UL5/UL8/UL52 complex, we considered the possibility that UL8 provides a link between the helicase-primase and the HSV DNA polymerase at a replication fork. To test such a possibility, we developed a model assay system for lagging-strand DNA synthesis. This assay system utilized ICP8-coated single-stranded M13 circles as a template and measured the incorporation of ^{32}P-labeled dNTPs into partially or fully duplex circular DNA molecules by agarose gel electrophoresis. As shown in Fig. 3A, lane 1,

when the HSV DNA polymerase (Pol/UL42) was incubated with template DNA in the absence of helicase-primase, a single major product was formed that migrated on the gel at the position of linear duplex M13 DNA (form III). Analysis of the product DNA strands by gel electrophoresis under denaturing conditions (Fig. 3B, lane 1) revealed a broad smear of radioactive products that migrated over a range of sizes from unit-length M13 DNA strands to twice-unit-length strands. We therefore presume that the products produced in the absence of primase are due to the extension of nicked circular molecules by a hairpin self-priming mechanism. When the reaction was carried out in the presence of the helicase-primase complex (Fig. 3A, lanes 2 to 5), additional products that migrated on a neutral gel more slowly than did linear duplex molecules were observed; the most slowly migrating products had an electrophoretic mobility that was identical to that of relaxed circular duplex M13 DNA (form II). Analysis of these reaction products under denaturing conditions (Fig. 3B, lanes 2 to 4) again revealed a broad smear of radioactive products superimposed on a band approximately the size of unit-length M13 DNA strands (7.6 kb). The appearance of this band was dependent not only on the presence of the helicase-primase but also on the presence of rNTPs (Fig. 3B, lane 5). We conclude that the product DNA migrating more slowly than linear duplex DNA under nondenaturing conditions represents partially or fully duplex M13 DNA circular molecules formed by the extension by DNA polymerase of primers synthesized by the helicase-primase. We consider that the synthesis of such products represents a model system for studying the synthesis of lagging strands at an HSV DNA replication fork.

Reaction conditions were systematically varied to optimize lagging-strand synthesis. Reaction mixtures containing the HSV DNA polymerase alone on a template of singly primed, ICP8-coated M13 DNA were used as controls. Although in most respects the optimal conditions for the two reactions were similar, the effects of incubation temperature on the two reactions were significantly different (Fig. 4). With the DNA polymerase alone (Fig. 4, lane 4), the optimal temperature was ~37°C, and at that temperature, the product was predominately fully duplex M13 circular molecules, as observed previously. At lower temperatures, fewer products were formed and there was an increasing proportion of product that had an electrophoretic mobility between those of form II and form III. It is likely that such products represent partially duplex circular molecules formed as a consequence of the failure of the HSV DNA polymerase to synthesize through regions of template secondary structure. In contrast, although the distributions of product sizes at the various temperatures were similar in the two reactions, the temperature optimum of the primase-primed reaction was considerably lower, between 25 and 30°C (Fig. 4, lanes 6 and 7); at 37°C, product formation was less than 10% of that observed at 25°C (Fig. 4; compare lanes 6 and 8). Control experiments (not shown) demonstrated that the primase activity per se of the UL5/UL8/UL52 complex [as measured by the indirect assay on a poly(dT) template] had a temperature optimum of 37°C. We therefore think it likely that the low temperature optimum of the lagging-strand synthesis reaction reflects the relative instability of the interaction between template DNA and the short oligonucleotide primers synthesized by the helicase-primase. Accordingly, all subsequent assays for lagging-strand DNA synthesis were performed at between 25 and 30°C.

We also investigated the effect on ICP8 on the lagging-strand synthesis reaction. As shown in Fig. 3A, the reaction

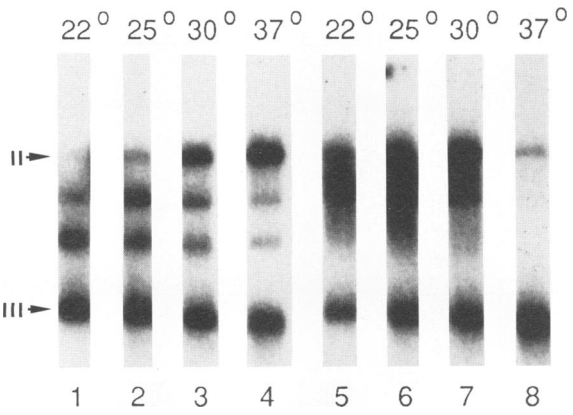

FIG. 4. Temperature optimum of lagging-strand DNA synthesis. Lanes 1 to 4, DNA replication by HSV Pol/UL42 on M13 circles, primed by a 30-base oligonucleotide. Reaction mixtures containing HSV DNA polymerase and single-stranded M13 DNA primed with a 30-base synthetic oligodeoxyribonucleotide were preincubated at 37°C as described in Materials and Methods and then, following the addition of [^{32}P]dCTP, incubated for 2 h at the indicated temperatures. In lanes 5 to 8, reaction mixtures were preincubated at 37°C as described in Materials and Methods, and then [^{32}P]dCTP, 800 fmol of UL5/UL8/UL52, and 7 fmol of ICP8 were added on ice. Reaction mixtures were subsequently incubated for 2 h at the indicated temperatures. Reaction products were analyzed by agarose gel electrophoresis. Arrows indicate the location of nicked, double-stranded M13 circles (form II) and linear M13 duplexes (form III).

is almost completely dependent on ICP8 (compare lanes 4 and 6). Analysis of the small amount of product produced in the absence of ICP8 by denaturing gel electrophoresis revealed that the product strands were very short, with an average length of less than 500 nucleotides. It seems likely that the requirement for ICP8 in the lagging-strand reaction reflects the ability of ICP8 to alter the secondary structure of the template DNA, thereby increasing the lengths of the strands synthesized by the HSV DNA polymerase. Such an effect of ICP8 has been reported previously (18).

The UL5/52 complex does not support lagging-strand synthesis. The purified UL5/UL52 subcomplex was tested for its ability to support the lagging-strand synthesis reaction. As is evident from the experiment shown in Fig. 5, lagging-strand synthesis was not observed when UL5/UL52 was substituted for UL5/UL8/UL52 (lanes 4 and 5). The highest concentration of UL5/UL52 tested was at least eightfold higher than a level of UL5/UL8/UL52 at which synthesis was readily detectable. The activity of the UL5/UL52 complex was restored by the addition of partially purified UL8 (lanes 6 and 7), ruling out the possibility that the preparation of UL5/UL52 had simply become inactivated. We therefore conclude that although UL8 has no effect on the two enzymatic activities of helicase-primase complex, UL8 does have an essential function in lagging-strand DNA synthesis.

The failure of the UL5/UL52 complex to prime lagging-strand synthesis by the HSV DNA polymerase suggested that UL8 may play a role in the elongation of nascent primers by interacting directly with the Pol/UL42 complex. As a preliminary test of this hypothesis, we substituted a heterologous DNA polymerase, *E. coli* DNA Pol I, for HSV Pol/UL42 in the lagging-strand assay. Since primers synthesized by both forms of the enzyme are elongated by *E. coli* Pol I when poly(dT) is used as a template, we expected that such would also be the case with M13 DNA as a template.

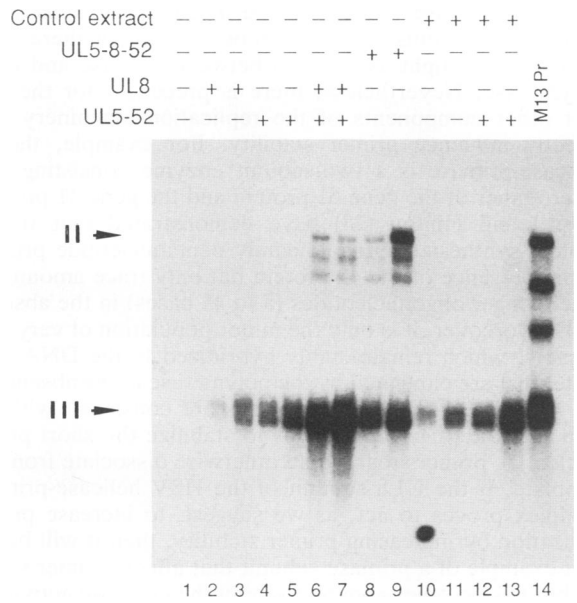

FIG. 5. Reconstitution of lagging-strand DNA synthesis. Reaction mixtures (50 μl) contained the basic components as described in the legend to Fig. 3 and were preincubated at 37°C prior to addition of [^{32}P]dCTP, ICP8, and the components indicated above each lane. Control extract refers to an extract on insect cells infected with a recombinant baculovirus expressing the HSV UL42 polypeptide (16) that was subjected to the same chromatographic purification procedures as was the extract containing UL8. Additions were as follows: lane 2, 10 pmol of UL8 (3 μg of total protein); lane 3, 50 pmol of UL8 (15 μg of total protein); lane 4, 400 fmol of UL5/UL52; lane 5, 1,600 fmol of UL5/UL52; lane 6, 10 pmol of UL8 and 1,600 fmol of UL5/UL52; lane 7, 50 pmol of UL8 and 1,600 fmol of UL5/UL52; lane 8, 400 fmol of UL5/UL8/UL52; lane 9, 1,600 fmol of UL5/UL8/UL52; lane 10, control extract, 3 μg of total protein; lane 11, control extract, 15 μg of total protein; lane 12, control extract, 15 μg of total protein and 400 fmol of UL5/UL52; lane 13, control extract, 15 μg of total protein and 1,600 fmol of UL5/UL52. The reaction mixture in lane 14 contained M13 DNA primed with a 30-base complementary oligodeoxynucleotide (see Materials and Methods) and no primase. Arrows indicate the locations of nicked, double-stranded M13 circles (form II) and linear M13 duplexes (form III).

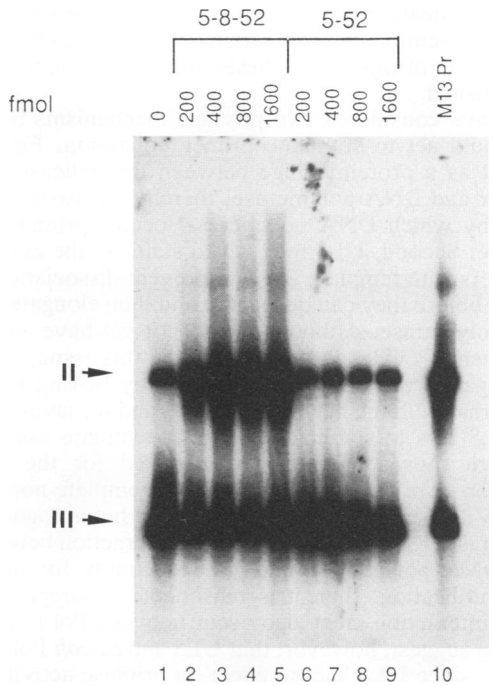

FIG. 6. Primase-primed DNA synthesis by *E. coli* Pol I. Reaction mixtures contained the indicated amounts of UL5/UL8/UL52 (lanes 2 to 5) or UL5/UL52 (lanes 6 to 9). Reaction conditions were identical to those described in the legend to Fig. 3 except that 2 U of DNA Pol I was substituted for 400 fmol of HSV Pol/UL42. Although we are not certain of the cause, we suspect that the background of form II product synthesized in the absence of primase (lane 1) is due the presence of contaminating polynucleotides in the commercial preparation of *E. coli* Pol I. The reaction mixture in lane 10 contained M13 primed with a 30-base complementary oligodeoxynucleotide (see Materials and Methods) and no primase. Arrows indicate the locations of nicked, double-stranded M13 circles (form II) and linear M13 duplexes (form III).

As shown in Fig. 6, such was not the case; UL5/UL8/UL52 efficiently primed DNA synthesis by Pol I (lanes 2 to 5), but UL5/UL52 did not (lanes 6 to 9). While this experiment does not completely exclude a specific interaction between UL8 and DNA polymerases in general, we think it more likely that UL8 affects the efficiency of primer utilization by some other mechanism.

DISCUSSION

The HSV-encoded helicase-primase complex is composed of a 1:1:1 association of three polypeptides, UL5, UL8, and UL52. Although it is clear from genetic analyses that each of these three subunits is absolutely essential for viral DNA replication in infected cells, the contributions of each subunit to the two enzymatic activities of the complex and the precise roles of each subunit in DNA synthesis have not yet been elucidated. It has been shown previously that a stable subcomplex of two of the subunits, UL5 and UL52, can be isolated from insect cells after coinfection with the appropriate recombinant baculoviruses (1, 12). The UL5/UL52 subcomplex was shown to display both helicase and primase activities in standard assays (1, 12); UL8, therefore, is not required for either of these two enzymatic activities. In this report, we have confirmed published results regarding the enzymatic activities of the UL5/UL52 subcomplex. We purified both the three-subunit helicase-primase complex and a UL5/UL52 subcomplex from recombinant baculovirus-infected insect cells and showed that the specific activities of the two proteins in standard assays for DNA-dependent ATPase, DNA-dependent GTPase, DNA helicase, and primase were essentially identical. In addition, we found that the lengths of the oligoribonucleotide primers synthesized by the enzyme were not affected by the presence of UL8. None of these data, therefore, suggest a role for UL8 in viral DNA replication.

As one approach to understanding the function of the virus-encoded replication proteins in greater detail, we have begun to develop assays involving these proteins that more closely resemble the process of DNA replication than do the standard assays for the component activities such as DNA polymerase, helicase, and primase. In this study, we used a model system for lagging-strand synthesis in which the primase activity of the helicase-primase complex is coupled to the activity of the HSV DNA polymerase on ICP8-coated single-stranded M13 DNA. Using this assay, we found that the UL8 subunit of the helicase-primase is critical for the

efficient utilization of primers; in the absence of UL8, we detected essentially no elongation of primers despite the fact that the rate of primer synthesis on the same template was undiminished.

We have considered two possible mechanisms by which UL8 could act to stimulate primer utilization. First, UL8 may act as a protein bridge between the helicase-primase complex and DNA polymerase, thereby increasing the efficiency by which DNA polymerase locates primers on the template. Second, UL8 may act to stabilize the association of primers with template DNA to prevent dissociation of the primers before they can be located and then elongated by the DNA polymerase. Although we do not yet have any direct experimental evidence that addresses this issue, our data argue against the idea that UL8 acts by linking the DNA polymerase with the helicase-primase, and we favor the view that UL8 acts to stabilize the primer-template association. We have shown that UL8 is required for the efficient utilization of primers on an M13 DNA template not only by the HSV DNA polymerase but also by a heterologous DNA polymerase, *E. coli* Pol I. Thus, if an interaction between the HSV DNA polymerase and UL8 accounts for increased primer utilization, then it is reasonable to suppose that a similar interaction must also occur between Pol I and UL8. Our data suggest, however, that UL8 and *E. coli* Pol I do not interact, since UL8 had no effect on primase activity in the standard indirect primase assay, in which primers synthesized on a poly(dT) template are elongated by *E. coli* Pol I. The fact that UL8 is required for the efficient elongation of primers by *E. coli* Pol I when M13, but not poly(dT), is used as a template argues against the idea that UL8 increases primer utilization by mediating an interaction between DNA polymerase and the helicase-primase. There is no simple way that such a mechanism can account for the template specificity of the requirement for UL8. In contrast, these data are readily accommodated by the hypothesis that UL8 increases primer stability. In this model, the effect of UL8 should be apparent regardless of the polymerase used to extend primers, and the lack of a requirement for UL8 in assays employing poly(dT) as a template can be explained by the low sequence complexity of poly(dT). Thus, with a template of low sequence complexity, the initial stability of the primer-template association is not critical because the reassociation of primers with template is rapid; in contrast, with a template of relatively high sequence complexity, such as M13 DNA, reassociation of primers is slow and a factor that prevents the dissociation of primers after they are synthesized becomes necessary. Experiments to obtain direct evidence for this model are in progress.

The issue of primer stability has not been explored extensively, but the sizes of the primers synthesized by most known primases suggest that there must be some mechanism to overcome intrinsic primer instability. The requirement for such a function is particularly evident in the case of the bacteriophage T4 primase, for example, which synthesizes primers that are five nucleotides in length (20, 22, 23, 27). Eukaryotic primases synthesize primers that are somewhat longer, ranging from 8 to 14 nucleotides (7, 36, 38); nevertheless, a significant fraction of RNA-DNA duplexes in that size range are likely to be unstable under physiologic conditions. One mechanism for overcoming primer instability is exemplified by the eukaryotic Pol α primase, in which primase activity is tightly associated with a DNA polymerase activity. Thus, in a tightly coupled system, short RNA primers are extended by DNA polymerase before dissociation can occur. Coordination between primase activity and DNA polymerase is likely to be a feature of most DNA replication systems, including those for which there is no evidence of a tight association between primase and DNA polymerase. Nevertheless, there is precedent for the idea that other components of the replication machinery may directly influence primer stability. For example, the T4 helicase-primase is a two-subunit enzyme consisting of a heterodimer of the gene 61 protein and the gene 41 protein. Nossal and Hinton (28) have demonstrated that the 61 protein synthesizes predominately pentanucleotide primers in the presence of the 41 protein but only trace amounts of much longer oligonucleotides (5 to 45 bases) in the absence of 41. Moreover, it is only the minor population of very long primers, which remain stably hybridized to the DNA template, that are elongated by the polymerase in the absence of the 41 protein. These observations are consistent with the idea that the 41 protein serves to stabilize the short pentanucleotide primers that would otherwise dissociate from the template. If the UL8 subunit of the HSV helicase-primase complex proves to act, as we suggest, to increase primer utilization by increasing primer stability, then it will be the first example of a primase subunit that affects primer stability but has no other apparent role in the catalytic activity of the enzyme. Thus, the HSV helicase-primase may prove to be a particularly useful system in which to gain new insights into this aspect of primase function.

Our data provide no new insights into the activities of the other two components of the helicase-primase. Since UL5 contains amino acid sequence motifs that are characteristic of other DNA helicases (26), it seems likely that UL5 contains the helicase catalytic center. By default, then, UL52 is the logical candidate for the primase subunit. There is as yet, however, no direct experimental support for these assignments. Our data, which are in agreement with previous results (1), demonstrate that the isolated UL5 subunit has little enzymatic activity in the absence of UL52, and we have found that UL52 is completely insoluble in the absence of UL5. In this regard, the HSV helicase-primase is similar to other multisubunit helicase-primase complexes. For example, the helicase activity of the phage T4 gene 41 protein and the primase activity of the gene 61 protein are greatly stimulated upon formation of a heterodimer (19, 28, 39). Similarly, the primase activity of the *E. coli* DnaG protein is dependent on the presence of the DnaB helicase, and the helicase activity of the DnaB protein is stimulated by the presence of DnaG (21). It is possible that these observations simply reflect the effect of intersubunit interactions on the folding of each polypeptide. It is also possible, however, that the observed interdependence of helicase and primase activities reflects an underlying control mechanism by which the two activities are regulated at a replication fork. Such regulation may be necessary because the two activities involve translocation of the complex in opposite directions along the same DNA strand and hence cannot occur simultaneously. According to this model, the helicase activity of one subunit of the complex is stimulated by a specific conformation of the primase in which the primase itself is inactive. Conversely, the primase activity would depend on a specific inactive conformation of the helicase subunit. Mutational analyses of each subunit that may shed some light on these ideas are in progress.

Whether or not the HSV primase utilizes specific or preferred sites on a template has yet to be determined. It seems unlikely that primer synthesis is completely dependent on a specific template sequence, since the enzyme has observable activity on homopolymer templates such as

poly(dT). Our experiments involving the extension of primers by the HSV DNA polymerase on single-stranded M13 DNA would appear, at least on the surface, to support the existence of a few preferred sites for primase activity. As seen in Fig. 3 to 5, in addition to observing the formation of fully duplex circular M13 DNA in these experiments, we also observed the formation of three or four discrete products that migrated faster on the gel than did the fully duplex DNA. We initially assumed that these discrete products were the result of elongation of specific primers up to sites of extensive secondary structure in the template. Pausing of the HSV DNA polymerase at sites of secondary structure has been observed previously (16, 18, 29). However, control experiments in which random hexanucleotides were used as primers revealed the same discrete products (35a). Therefore, while we cannot at present rule out the existence of preferred sites for primase activity, we think it more likely that the HSV primase initiates synthesis at random sites. We speculate that the discrete bands of partially duplex DNA seen in our experiments reflect the existence of a few discrete regions of secondary structure on the single-stranded portion of these molecules that leads to sets of molecules in which the electrophoretic mobility is controlled by the location of the duplex region but is relatively insensitive to the size of the duplex region. Other explanations are possible.

In summary, in this study we have used a model system for lagging-strand synthesis to begin to define a role in viral DNA replication for UL8, a subunit of the HSV helicase-primase complex. The lagging-strand model system that we have developed depends on the presence of six of the seven virus-encoded polypeptides that are known to be required in vivo for DNA replication. Continued analysis of this system, therefore, should prove useful in defining the interactions among these polypeptides that lead to the efficient synthesis of viral DNA in lytically infected cells.

ACKNOWLEDGMENTS

We are grateful to Daniel Fierer and Donna Klinedinst for useful discussions and helpful comments on the manuscript.

REFERENCES

1. **Calder, J. M., and N. D. Stow.** 1990. Herpes simplex virus helicase-primase: the UL8 protein is not required for DNA-dependent ATPase and DNA helicase activities. Nucleic Acids Res. **25**:3573–3578.
2. **Carmichael, E. P., M. J. Kosovsky, and S. K. Weller.** 1988. Isolation and characterization of herpes simplex virus type 1 host range mutants defective in viral DNA synthesis. J. Virol. **62**:91–99.
3. **Carmichael, E. P., and S. K. Weller.** 1989. Herpes simplex virus type 1 DNA synthesis requires the product of the UL8 gene: isolation and characterization of an ICP6::*lacZ* insertion mutation. J. Virol. **62**:2970–2977.
4. **Challberg, M. D.** 1986. A method for identifying the viral genes required for herpesvirus DNA replication. Proc. Natl. Acad. Sci. USA **83**:9094–9098.
5. **Chartrand, P., C. S. Crumpacker, P. A. Schaffer, and N. M. Wilkie.** 1980. Physical and genetic analysis of the herpes simplex virus DNA polymerase locus. Virology **103**:311–326.
6. **Coen, D. M., D. P. Aschman, P. T. Gelep, M. J. Retondo, S. K. Weller, and P. A. Schaffer.** 1984. Fine mapping and molecular cloning of mutations in the herpes simplex virus DNA polymerase locus. J. Virol. **49**:236–247.
7. **Conaway, R. C., and I. R. Lehman.** 1982. A DNA primase activity associated with DNA polymerase α from *Drosophila melanogaster* embryos. Proc. Natl. Acad. Sci. USA **79**:2523–2527.
8. **Conley, A. J., D. M. Knipe, P. Jones, and B. Roizman.** 1981. Molecular genetics of herpes simplex virus. VII. Characterization of a temperature-sensitive mutant produced by in vitro mutagenesis and defective in DNA synthesis and accumulation of gamma polypeptides. J. Virol. **37**:191–206.

8a. **Crute, J. J., R. C. Bruckner, M. S. Dodson, and I. R. Lehman.** 1991. Herpes simplex-1 helicase-primase. J. Biol. Chem. **266**: 21252–21256.

9. **Crute, J. J., and I. R. Lehman.** 1991. Herpes simplex virus-1 helicase-primase. Physical and catalytic properties. J. Biol. Chem. **266**:4484–4488.

9a. **Crute, J. J., E. S. Mocarski, and I. R. Lehman.** 1988. A DNA helicase induced by herpes simplex virus type 1. Nucleic Acids Res. **16**:6585–6596.

10. **Crute, J. J., T. Tsurumi, L. Zhu, S. K. Weller, P. D. Olivo, M. D. Challberg, E. S. Mocarski, and I. R. Lehman.** 1989. Herpes simplex virus 1 helicase-primase: a complex of three herpes-encoded gene products. Proc. Natl. Acad. Sci. USA **86**:2186–2189.
11. **Dodson, M. S., J. J. Crute, R. C. Bruckner, and I. R. Lehman.** 1989. Overexpression and assembly of the herpes simplex virus type-1 helicase-primase in insect cells. J. Biol. Chem. **264**: 20835–20838.
12. **Dodson, M. S., and I. R. Lehman.** 1991. Association of DNA helicase and primase activities with a subassembly of the herpes simplex virus 1 helicase-primase composed of the UL5 and UL52 gene products. Proc. Natl. Acad. Sci. USA **88**:1105–1109.
13. **Donis-Keller, H., A. M. Maxam, and W. Gilbert.** 1977. Mapping adenines, guanines, and pyrimidines in RNA. Nucleic Acids Res. **4**:2527–2538.
14. **Gallo, M. L., D. I. Dorsky, C. S. Crumpacker, and D. S. Parris.** 1989. The essential 65-kilodalton DNA-binding protein of herpes simplex virus stimulates the virus-encoded DNA polymerase. J. Virol. **63**:5023–5029.
15. **Gallo, M. L., H. D. Jackwood, M. Murphy, H. S. Marsden, and S. D. Parris.** 1988. Purification of the herpes simplex virus type 1 65-kilodalton DNA-binding protein: properties of the protein and evidence of its association with the virus-encoded DNA polymerase. J. Virol. **62**:2874–2883.
16. **Gottlieb, J., A. I. March, D. M. Coen, and M. D. Challberg.** 1990. The herpes simplex virus type 1 UL42 gene product: a subunit of DNA polymerase that functions to increase processivity. J. Virol. **64**:5976–5987.
17. **Grosse, F., and G. Krauss.** 1985. The primase activity of DNA polymerase α from calf thymus. J. Biol. Chem. **260**:1881–1888.
18. **Hernandez, T. R., and I. R. Lehman.** 1990. Functional interaction between the herpes simplex-1 DNA polymerase and UL42 protein. J. Biol. Chem. **265**:11227–11232.
19. **Hinton, D. M., L. L. Silver, and N. G. Nossal.** 1985. Bacteriophage T4 DNA replication protein 41. Cloning of the gene and purification of the expressed protein. J. Biol. Chem. **260**:12851–12857.
20. **Kurosawa, Y., and T. Okasaki.** 1979. Structure of the RNA portion of the RNA-linked DNA pieces in bacteriophage T4-infected *Escherichia coli* cells. J. Mol. Biol. **135**:841–861.
21. **LeBowitz, J. H., and R. McMacken.** 1986. The *Escherichia coli* dnaB replication protein is a DNA helicase. J. Biol. Chem. **261**:4738–4748.
22. **Liu, C.-C., and B. M. Alberts.** 1980. Pentaribonucleotides of mixed sequence are synthesized and efficiently prime *de novo* DNA chain starts in the T4 bacteriophage DNA replication system. Proc. Natl. Acad. Sci. USA **77**:5698–5702.
23. **Liu, C.-C., and B. M. Alberts.** 1981. Characterization of RNA primer synthesis in the T4 bacteriophage *in vitro* DNA replication system. J. Biol. Chem. **256**:2821–2829.
24. **Marchetti, M. E., C. E. Smith, and P. A. Schaffer.** 1988. A temperature-sensitive mutation in an HSV-1 gene required for viral DNA synthesis maps to coordinates 0.609-0.614 in UL. J. Virol. **62**:715–721.
25. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol.

69:1531–1574.

26. **McGeoch, D. J., M. A. Dalrymple, A. Dolan, D. McNab, L. J. Perry, P. Taylor, and M. D. Challberg.** 1988. Structures of herpes simplex virus type 1 genes required for replication of virus DNA. J. Virol. **62**:444–453.
27. **Nossal, N. G.** 1980. RNA priming of DNA replication by bacteriophage T4 proteins. J. Biol. Chem. **255**:2176–2182.
28. **Nossal, N. G., and D. M. Hinton.** 1987. Bacteriophage T4 DNA primase-helicase: characterization of DNA synthesis primed by the T4 61 protein in the absence of 41 protein. J. Biol. Chem. **262**:10879–10885.
29. **O'Donnell, M. E., P. Elias, and I. R. Lehman.** 1987. Processive replication of single-stranded DNA templates by the herpes simplex virus-induced DNA polymerase. J. Biol. Chem. **262**: 4252–4259.
30. **Olivo, P. D., N. J. Nelson, and M. D. Challberg.** 1988. Herpes simplex virus DNA replication: the UL9 gene encodes an origin binding protein. Proc. Natl. Acad. Sci. USA **85**:5414–5418.
31. **Olivo, P. D., N. J. Nelson, and M. D. Challberg.** 1988. Herpes simplex virus type 1 gene products required for DNA replication: identification and overexpression. J. Virol. **63**:196–204.
32. **Parris, D. S., A. Cross, L. Harr, A. Orr, M. C. Frame, D. J. Murphy, D. J. McGeoch, and H. S. Marsden.** 1988. Identification of the gene encoding the 65-kilodalton DNA-binding protein of herpes simplex virus type 1. J. Virol. **62**:818–825.
33. **Powell, K. L., E. Littler, and D. J. M. Purifoy.** 1981. Nonstructural proteins of herpes simplex virus. II. Major virus-specific DNA-binding protein. J. Virol. **39**:894–902.
34. **Purifoy, D. J. M., R. B. Lewis, and K. L. Powell.** 1977. Identification of the herpes simplex virus DNA polymerase gene. Nature (London) **269**:621–623.
35. **Purifoy, D. J. M., and K. L. Powell.** 1981. Temperature-sensitive mutants in two distinct complementation groups of herpes simplex virus type 1 specify thermolabile DNA polymerase. J. Gen. Virol. **54**:219–222.

35a. **Sherman, G., and M. D. Challberg.** Unpublished data.

36. **Singh, H. J., R. G. Brooke, M. H. Pausch, G. T. Williams, C. Trainor, and L. B. Dumas.** 1986. Yeast DNA primase and DNA polymerase activities. An analysis of RNA priming and its coupling to DNA synthesis. J. Biol. Chem. **261**:8564–8569.
37. **Summers, M. D., and G. E. Smith.** 1987. A manual of methods for baculovirus vectors and insect cell procedures. Texas A&M University, College Station.
38. **Tseng, B. Y., and C. N. Ahlem.** 1983. A DNA primase from mouse cells. J. Biol. Chem. **258**:9845–9849.
39. **Venkatesan, M., L. L. Silver, and N. G. Nossal.** 1982. Bacteriophage T4 gene 41 protein, required for the synthesis of RNA primers, is also a DNA helicase. J. Biol. Chem. **257**:12426–12434.
40. **Weller, S. K., E. P. Carmichael, D. P. Aschman, D. J. Goldstein, and P. A. Schaffer.** 1987. Genetic and phenotypic characterization of mutants in four essential genes that map to the left half of HSV-1 UL DNA. Virology **161**:198–210.
41. **Weller, S. K., K. J. Lee, D. J. Sabourin, and P. A. Schaffer.** 1983. Genetic analysis of temperature-sensitive mutants which define the gene for the major herpes simplex virus type 1 DNA-binding protein. J. Virol. **45**:354–366.
42. **Wu, C. A., N. J. Nelson, D. J. McGeoch, and M. D. Challberg.** 1988. Identification of herpes simplex virus type 1 genes required for origin-dependent DNA synthesis. J. Virol. **62**:435–443.
43. **Zhu, L., and S. K. Weller.** 1988. UL5, a protein required for HSV DNA synthesis: genetic analysis, overexpression in Escherichia coli, and generation of polyclonal antibodies. Virology **166**:366–378.