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Sequences at the C-terminus of the herpes simplex virus type 1 UL30 protein are dispensable for DNA polymerase activity but not for viral origin-dependent DNA replication

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ABSTRACT

The UL30 protein of herpes simplex virus type 1 (HSV-1) is a catalytically active DNA polymerase which is present in virus infected cells in a heterodimeric complex with an accessory subunit, the UL42 polypeptide. Both proteins are essential for viral DNA synthesis but because the UL42 protein is much more abundant it has been difficult to determine whether its role is related to, or independent of, its interaction with the UL30 protein *in vivo*. Since the C-terminal region of UL30 has been shown to be important for interaction with the UL42 protein but dispensable for DNA polymerase activity, a recombinant baculovirus which overexpresses a UL30 protein truncated by 27 amino acids at its C-terminus was constructed and used to assess the significance of the protein-protein interaction. The mutated protein was as active as wild-type (wt) UL30 in a DNA polymerase assay in which activated calf thymus DNA was used as template. However, in contrast to the wt protein, the activity of the truncated polymerase on this template was not stimulated by addition of purified UL42. A monoclonal antibody against the UL42 protein co-precipitated the full length but not truncated polymerase from extracts of cells which had been co-infected with a UL42-expressing recombinant baculovirus. Finally, the truncated protein was not active in a transient assay for HSV-1 origin-dependent DNA replication performed in insect cells in tissue culture. These results indicate that sequences at the C-terminus of the UL30 protein which are dispensable for DNA polymerase activity play essential roles both in viral DNA replication and interaction with the UL42 protein, and strongly suggest that the interaction between the proteins is important *in vivo*.

INTRODUCTION

The genome of herpes simplex virus type 1 (HSV-1) is a linear double-stranded DNA of 152 kbp which encodes approximately 70 distinct polypeptides and contains three elements which function as origins of replication (1). In the absence of a cell-free system capable of carrying out HSV-1 origin-dependent DNA synthesis, studies of the *cis*-acting sequences and virus-encoded products involved in genome replication have relied heavily upon transient assays performed in tissue culture cells. This approach initially led to the identification of the sequences comprising the origins of replication; a single copy of ori_L near the centre of the U_L region and copies of ori_S in the TR_S and IR_S repeats (2–5). It was subsequently shown that the products of seven HSV-1 genes are both necessary and sufficient to activate replication from a cloned viral origin cotransfected into permissive mammalian cells (6–8). The essential requirement for these seven gene products in HSV-1 DNA synthesis is in agreement with studies on virus mutants, and recent investigations have allowed roles in replication to be assigned to each of the proteins (for reviews see 4,5,9). Amongst the activities encoded by the DNA replication genes are a ssDNA-binding protein by gene UL29, a sequence-specific origin-binding protein by gene UL9 and three subunits of a helicase-primase complex by genes UL5, UL8 and UL52. The products of the two remaining genes, UL30 and UL42, are discussed in this paper.

The induction of a novel DNA polymerase activity in HSV-1 infected cells was first observed almost 30 years ago (10,11), and subsequently mutations which conferred thermolability or drug resistance to the polymerase were located within the region of the genome containing the 1235 codon UL30 ORF (12–14). Expression of this ORF by *in vitro* transcription and translation, in yeast and by recombinant baculoviruses has demonstrated that no other viral protein is necessary for DNA polymerase activity as measured on activated calf thymus DNA templates (15–17). Nevertheless, DNA polymerase preparations from HSV infected cells were frequently found to contain not only the UL30 protein but also a polypeptide of apparent M_r 54,000–65,000 (18–21). This protein was identified as the product of gene UL42 (22,23), and it is now recognised that in infected cells the DNA polymerase occurs predominantly as a heterodimer comprising a catalytic UL30 and an accessory UL42 subunit (24,25). The presence of the UL42 protein within the complex has been reported to stimulate DNA polymerase activity on an activated calf thymus DNA template (20), and to increase polymerase processivity on singly primed single-stranded circular templates (25,26). Interestingly, the amount of UL42 protein present in HSV-1 infected cells is at least 20-fold greater than the amount of UL30 (25), and uncomplexed UL42 is able to bind with high affinity to double-stranded DNA in a sequence-independent manner (23,27). It is therefore not clear whether the essential

role of the UL42 protein in viral DNA replication is related to its interaction with the polymerase or to some other function.

In order to investigate whether an interaction of the polymerase with the UL42 protein is important for origin-dependent replication we have employed an assay using recombinant baculoviruses in insect cells. It was previously shown that transfection of *Spodoptera frugiperda* (Sf) cells with a plasmid containing HSV-1 ori_S followed by superinfection with a mixture of baculoviruses which overexpress the seven wt HSV-1 DNA replication proteins resulted in amplification of the input plasmid. The HSV-1 gene products and origin sequences required for plasmid amplification were the same as in permissive mammalian cells, providing an attractive approach for both screening mutated versions of the proteins for ability to participate in origin-dependent DNA replication and producing amounts sufficient for biochemical studies (28). Reports from other laboratories had previously shown that removal of only 89 amino acids from the C-terminus of the UL30 protein was sufficient to abolish interaction with the UL42 protein (29), and that the C-terminal 59 amino acids could be deleted without destroying catalytic activity (30). We therefore expressed a UL30 polypeptide with a short (27 amino acid) truncation at its C-terminus using a recombinant baculovirus and examined its DNA polymerase activity, interaction with the UL42 protein and ability to participate in origin-dependent replication. The results show that although the truncated protein is enzymatically active it is unable to interact with the UL42 protein or to substitute for wt UL30 protein in the replication assay. The interaction between polymerase and UL42 protein thus appears to be important for viral DNA replication.

MATERIALS AND METHODS

Cells and viruses

The growth of Sf cells (strain IPLB-SF-21) and the preparation and titration of stocks of baculovirus recombinants AcUL5, AcUL8, AcUL9, AcUL29, AcUL30, AcUL42 and AcUL52 (which overexpress the HSV-1 UL5, UL8 etc proteins) were carried out as previously described (28). The parental recombinant virus AcRP23*lacZ* (31) which efficiently expresses the *E.coli* β-galactosidase protein was used as a control.

Isolation of AcPOLd1

The wt HSV-1 UL30 gene was previously obtained on an *EarI* fragment (nucleotides 62,759−67,261) which was cloned using *XbaI* linkers (28). To obtain a C-terminal truncation mutant the plasmid was partially digested with *Bsp*EI (cleaves at 63,370, 65,756 and 66,423 within the viral insert), flush ends were produced using T4 DNA polymerase in the presence of all 4 dNTPs and an 8 bp synthetic *XbaI* linker ligated to the ends. The *XbaI* fragment corresponding to nucleotides 62,759−66,423 was cloned in the correct orientation into the previously described *XbaI* site derivative of pAcYM1 (28). The recombinant AcPOLd1 was obtained following recombination of the resulting plasmid with *Bsu*36I cleaved parental baculovirus AcRP23*lacZ* DNA as previously described (32). As illustrated in Figure 1, the product specified by AcPOLd1 consists of the first 1207 amino acids of the wt protein followed by a C-terminal leucine residue.

Preparation of extracts and phosphocellulose chromatography

All operations were performed on ice or at 4°C. High salt extractable total cellular proteins were prepared from approximately 4×10^7 Sf cells infected with AcUL30, AcPOLd1 or AcRP23*lacZ* as described previously (33) except that 0.6M NaCl replaced KCl in buffer C and cells were harvested 70 h p.i. Following centrifugation the supernatant was brought to 50 mM NaCl by dilution with buffer A (20 mM Hepes/NaOH, pH 7.6, 1 mM EDTA, 10% glycerol, 1 mM DTT, 0.5 mM PMSF) and centrifuged at 17,500×g for 20 min in a Sorvall SS34 rotor. The resulting supernatant was applied to a 1 ml bed volume phosphocellulose (P11 cellulose phosphate, Whatman) column previously equilibrated with buffer A containing 50 mM NaCl. The column was washed with 5 ml of this buffer and a 7 ml gradient of 50 mM to 850 mM NaCl in buffer A applied. 0.5 ml fractions were collected. 13 μl samples of gradient fractions were analysed by SDS-PAGE using 9% gels containing 1 part in 40 bisacrylamide, and proteins were stained with Coomassie blue. Protein concentrations were determined using a concentrated dye reagent assay solution (Bio-Rad).

DNA polymerase assays

DNA polymerase activity was assayed as described by Marcy *et al.* (17). Reactions contained, in a final volume of 25 μl, 2.5 μg activated calf thymus DNA (Sigma), 20 mM Tris-HCl, pH7.5, 150 mM $(NH_4)_2SO_4$, 50 μM dATP, 50 μM dGTP, 50 μM TTP, 5 μM dCTP supplemented with approximately 0.5 μCi (α-^{32}P)-dCTP (Amersham, 3,000 Ci/mmol), 5 mM $MgCl_2$, 5 mM DTT, 5% glycerol, 40 μg/ml BSA and 2 μl phosphocellulose column fraction. After 30 min incubation at 37°C 10 μl of 0.25 M EDTA, pH8.0, was added and the reactions spotted onto DE81 discs. The discs were washed four times with 0.5 M sodium phosphate, pH 7.0, twice with 80% ethanol and dried. Filter bound radioactivity was determined by scintillation counting and the incorporation of dCTP into DNA calculated. Stimulation of DNA polymerase activity by the UL42 protein was similarly measured except that the amount of activated DNA was reduced to 0.625 μg/reaction and the appropriate phosphocellulose column fractions were first diluted 10-fold in dilution buffer (20mM Tris-HCl, pH7.5, 100 mM NaCl, 0.5 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, 10% glycerol). Reactions were performed in either the absence or presence of 20 ng UL42 protein, purified from recombinant baculovirus infected insect cells essentially as described by Gottlieb *et al.* (25) and the kind gift of M. Murphy.

Immunoprecipitation

Linbro wells of Sf cells (approximately 3×10^5 cells per well) were either mock-infected or infected with 5 p.f.u./cell of each of the appropriate viruses. Each well was labelled from 25−31 h p.i. by addition of 200 μl TC100 salt solution (33) containing 15 μCi ^{35}S-methionine (Amersham, 1,200 Ci/mmol). The cells were harvested, washed once with Tris-buffered saline and extracted for 1 h on ice with 120 μl extraction buffer (100 mM Tris-HCl, pH8.0, 10% glycerol, 0.5% Nonidet P40, 0.5% sodium deoxycholate, 0.2 mM PMSF; ref. 34). The extract was clarified by centrifugation for 15 min in a microfuge at 4°C. 50 μl samples were incubated with 0.3 μl MAb 6898 against the UL42 protein (22) for 1 h followed by addition of 1 μl sheep anti-mouse IgG (Sigma) for 45 min and 30 μl fixed *Staphylococcus aureus* cells (Immunoprecipitin; Life Sciences) for a further 1 h. The Immunoprecipitin was washed four times with extraction buffer and bound proteins were analysed by SDS-PAGE on a 9% gel containing 1 part in 40 bisacrylamide. Gels were fixed, treated with EnHance (NEN), dried and exposed to autoradiographic film.

DNA replication assay in Sf cells

Replication assays were performed as described previously (28) except that monolayers of Sf cells in Linbro wells (2 cm^2 surface area, 3×10^5 cells/well) were used and the volumes of reagents scaled down appropriately. In summary, each well received 80 ng plasmid pST19 DNA (containing a functional copy of HSV-1 ori_S) using a liposome-mediated transfection procedure. After 4 h the cells were superinfected with a mixture containing 5 p.f.u./cell of each of seven recombinant baculoviruses expressing the HSV-1 DNA replication proteins. Infected cells were harvested 50 h p.i. and total cellular DNA prepared. Samples were cleaved with *Eco*RI and *Dpn*I and examined for the presence of replicated plasmid sequences (*Dpn*I-resistant) by agarose gel electrophoresis followed by Southern blotting and hybridisation to a ^{32}P-labelled pTZ19U vector DNA probe.

RESULTS

Construction of recombinant baculovirus AcPOLd1 expressing a C-terminally truncated HSV-1 DNA polymerase

The construction of the recombinant AcPOLd1 is described in Materials and Methods. This virus specifies a protein consisting of the first 1207 amino acids of the wt UL30 ORF followed by a C-terminal leucine residue (Figure 1). In preliminary experiments the level of expression of the truncated protein by AcPOLd1 was shown to be comparable to that of wt UL30 by the previously characterised recombinant, AcUL30 (28) (data not shown). Although the protein specified by AcPOLd1 is 27 amino acids shorter than the wt polypeptide no difference between their polyacrylamide gel mobilities was apparent.

Phosphocellulose column chromatography of Sf cell extracts

In order to compare the enzymatic properties of the wt and truncated HSV-1 DNA polymerases, extracts were prepared from infected Sf cells and fractionated on small bed volume phosphocellulose columns. A control extract from cells infected with the β-galactosidase expressing parental virus AcRP23*lacZ* was similarly fractionated. Proteins eluted upon application of NaCl gradients to the columns are shown in Figure 2. Large amounts of mutant and wt UL30 proteins were eluted from the columns loaded with the AcPOLd1 and AcUL30 extracts (gels a and b) but a similarly sized protein was absent from the AcRP23*lacZ* column fractions.

DNA polymerase assays on phosphocellulose column fractions

The column fractions analysed in Figure 2 were assayed for DNA polymerase activity as described in Materials and Methods using activated calf thymus DNA as template. The results are shown in Figure 3. Comparison of the panels reveals the presence of a distinct peak of DNA polymerase activity from both the AcPOLd1 (panel a) and AcUL30 (panel b) extracts which was not present in the control AcRP23*lacZ* extract (panel c). The peaks of DNA polymerase activity from the AcPOLd1 and AcUL30 extracts eluted at approximately 290–350 mM NaCl (fractions 5 and 6) in good agreement with a previous report (17). In addition, inspection of Figure 2 revealed that these fractions contained the greatest amounts of HSV-1 UL30 protein. A separate fractionation of extracts from AcPOLd1, AcUL30 or AcRP23*lacZ* infected Sf cells yielded essentially identical results.

The protein concentrations of fractions 5 and 6 from the three gradients shown in Figures 2 and 3 were determined. Values of

(a) CTC CGG ACC ..... TGA
L R T AGFGAVGAGATAEETRRMLHRAFDTLA *

(b) CTC CGG CTC TAG AG
L R L *

Figure 1. (a) Amino acid sequence of the C-teminus of the HSV-1 DNA polymerase. The C-terminal 30 amino acids of the wt UL30 protein (1,206–1,235) are shown together with the DNA sequence surrounding the *Bsp*EI site at position 66,423 (underlined) and the TGA termination codon. (b) The corresponding region of the truncated gene in AcPOLd1 is shown with the *Xba*I linker (which includes an in-frame TAG termination codon) underlined.

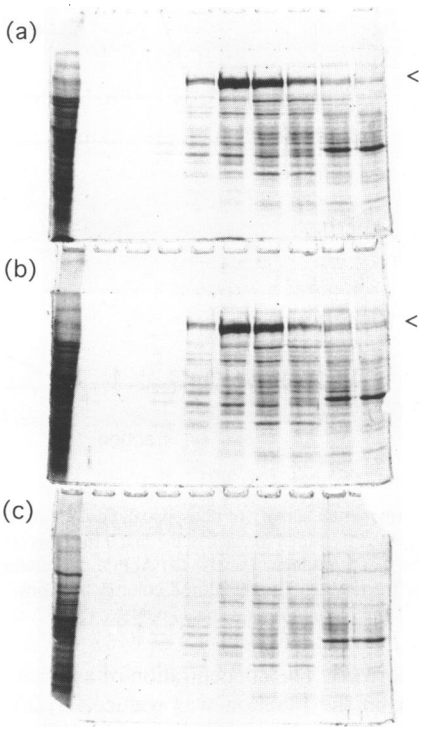

Figure 2. SDS-PAGE analysis of phosphocellulose column fractions. Extracts were prepared from cells infected with AcPOLd1 (gel **a**); AcUL30 (gel **b**); or AcRP23*lacZ* (gel **c**) and proteins were stained with Coomassie blue. In each case the leftmost lane contains the starting extract and the following lanes samples from the first 9 fractions collected after application of the NaCl gradient (corresponding to 50–490 mM NaCl). The positions of the truncated (AcPOLd1) and intact (AcUL30) UL30 polypeptides are indicated by arrowheads.

500 and 452 μg/ml, 397 and 390 μg/ml, and 175 and 208 μg/ml were obtained for the AcPOLd1, AcUL30 and AcRP23*lacZ* column fractions respectively. The higher protein concentrations of the AcPOLd1 fractions appear to correlate with the detection of greater DNA polymerase activity in these fractions than in the corresponding fractions from the AcUL30 gradient. It would appear from these estimates that about half of the total protein present in the peak fractions is HSV-1 UL30 product.

Stimulation of DNA polymerase activity by UL42 protein

To determine whether purified UL42 protein had any stimulatory effect on the above HSV-1 DNA polymerase activities a modified

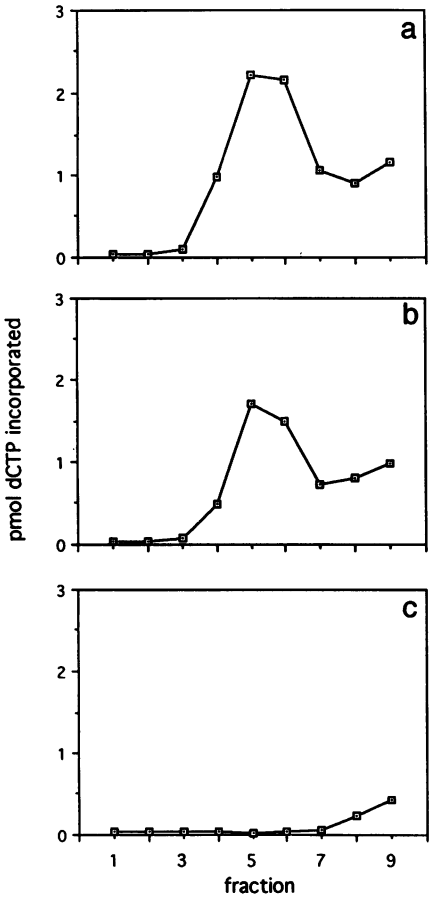

Figure 3. DNA polymerase activity of phosphocellulose column fractions. DNA polymerase activity was measured for each of the first 9 fractions obtained following application of the NaCl gradient. Panels: (**a**) AcPOLd1 column fractions; (**b**) AcUL30 column fractions; (**c**) AcRP23*lacZ* column fractions.

assay was performed. The concentration of activated calf thymus DNA template in the reaction was reduced to 25 μg/ml since a dose response curve (data not shown) had shown that under the assay conditions the activity of the AcUL30 specific HSV-1 polymerase was proportional to DNA concentrations only below 50 μg/ml. In addition the fractions to be tested (fractions 5 and 6 from each gradient) were diluted 10-fold and the activity of 2 μl samples determined in either the absence or presence of 20 ng UL42 protein per reaction. It was estimated that the amount of AcUL30 or AcPOLd1 protein added per reaction was 40–60 ng and that the UL42 and UL30-related proteins were therefore present in approximately equimolar amounts.

The results are shown in Figure 4. In the absence of UL42 protein similar levels of DNA polymerase activity were detected in fractions 5 and 6 from the AcPOLd1 and AcUL30 gradients but little activity was present in the corresponding fractions of the control (AcRP23*lacZ*) gradient. As expected, the incorporation of dCTP was lower than in reactions performed using higher DNA template and DNA polymerase concentrations (c.f. Figure 3). Addition of UL42 protein to the reactions had little effect on the AcPOLd1 activity but markedly stimulated that of the AcUL30 protein. In four independent determinations the presence of UL42 protein resulted in 4.6, 3.9, 3.3 and 3.4 fold stimulation of DNA polymerase activity in fractions containing

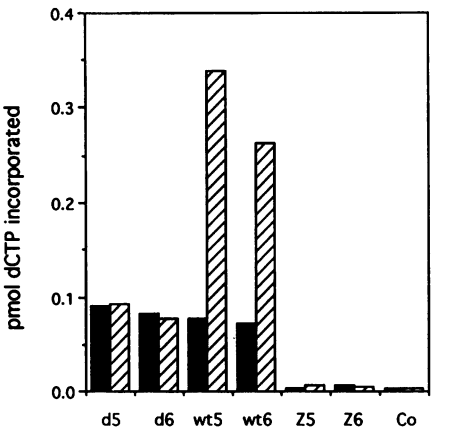

Figure 4. Stimulation of DNA polymerase activity by the UL42 protein. DNA polymerase activity was measured as described in the text either in the absence (solid bars) or presence (hatched bars) of UL42 protein. Fractions 5 and 6 from each set of phosphocellulose column eluates were assayed. d5, d6, fractions from the AcPOLd1 column; wt5, wt6, fractions from the AcUL30 column; Z5, Z6 fractions from the AcRP23*lacZ* column. The control reactions (Co) contained 2 μl of dilution buffer in place of column eluate.

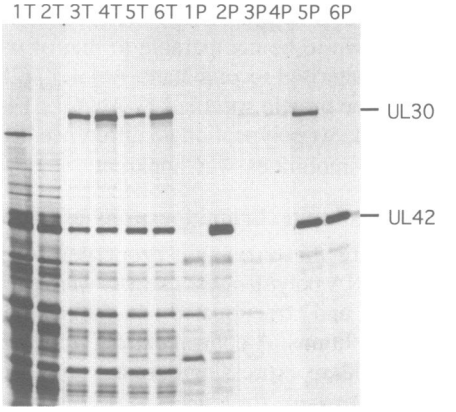

Figure 5. Immunoprecipitation of proteins from Sf cell extracts. ^{35}S-labelled extracts were prepared from Sf cells infected with the following viruses: AcRP23*lacZ* (lanes 1T, 1P); AcUL42 (lanes 2T, 2P); AcUL30 (lanes 3T, 3P); AcPOLd1 (lanes 4T, 4P); AcUL42 plus AcUL30 (lanes 5T, 5P); AcUL42 plus AcPOLd1 (lanes 6T, 6P). Lanes 1T, 2T, 3T, 4T, 5T and 6T contain samples of the starting extract and lanes 1P, 2P, 3P, 4P, 5P and 6P contain proteins precipitated with MAb 6898. The positions of UL30 and UL42 proteins in the gel are indicated.

the AcUL30 protein. Corresponding values of 1.04, 0.93, 0.78 and 0.92 fold stimulation by UL42 were obtained for the AcPOLd1 activity.

In contrast to the wt HSV-1 DNA polymerase, the truncated version specified by AcPOLd1 is therefore unable to respond to the stimulatory activity of the UL42 protein.

Coprecipitation of UL42 and UL30 proteins

Immunoprecipitation experiments were performed to determine whether the wt and truncated HSV-1 DNA polymerases specified by AcUL30 and AcPOLd1 could form complexes with the UL42 protein. ^{35}S-labelled extracts were prepared from singly or mixedly infected Sf cells and reacted with MAb 6898, which

recognises the UL42 protein and is able to bind UL30-UL42 complexes from wt HSV-1 infected cells (23). The results are shown in Figure 5. In control single infections the UL42 protein was efficiently precipitated by MAb 6898 but only small amounts of UL30 protein were detected in the precipitates from AcUL30 or AcPOLd1 infected cells. Similar reactions performed with extracts from cells co-infected with either AcUL30 or AcPOLd1 and AcUL42 revealed that the wt but not the truncated UL30 protein was co-precipitated with the UL42 protein. The C-terminal truncation of the AcPOLd1 polymerase therefore prevents complex formation with UL42 protein.

Activity of the AcUL30 and AcPOLd1 polymerases in origin-dependent DNA synthesis

To determine whether the DNA polymerase specified by AcPOLd1 could function in HSV origin-dependent DNA synthesis transient replication assays were performed in Sf cells as described in Materials and Methods. The results are shown in Figure 6. As previously demonstrated, efficient replication of the ori_S-containing plasmid, pST19, occurred in cells infected with a set of baculovirus recombinants specifying the seven wt HSV-1 DNA replication proteins but not in cells which were mock-infected or infected with a mix of viruses from which AcUL30 had been omitted (28). Replacement of AcUL30 with AcPOLd1 in the virus mix similarly resulted in a failure of pST19 to be replicated. Therefore, although the truncated UL30 protein specified by AcPOLd1 retains DNA polymerase activity, it is not competent to function in HSV-1 origin-dependent DNA replication.

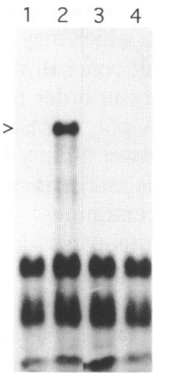

Figure 6. Replicative ability of the AcUL30 and AcPOLd1 proteins. Sf cells were transfected with plasmid pST19 and infected as follows. Lane 1, mock-infected; Lane 2, mix of 7 AcNPV recombinants expressing the wt HSV-1 DNA replication proteins; Lane 3, a similar mix from which AcUL30 had been omitted; Lane 4, a similar mix in which AcUL30 had been substituted by AcPOLd1. DNA was prepared 50 h p.i. and samples corresponding to the yield from 1.5×10^5 cells were cleaved with *Eco*RI plus *Dpn*I. The resulting fragments were separated by agarose gel electrophoresis, transferred to a nylon membrane and hybridized to ^{32}P-labelled pTZ19U DNA. An autoradiograph of the washed filter is shown. The position of replicated input plasmid is indicated by an arrowhead. The smaller fragments are *Dpn*I cleavage products of unreplicated input plasmid DNA.

DISCUSSION

The work presented in this paper aimed to investigate whether physical interactions between the HSV-1 UL30 and UL42 proteins (the catalytic and accessory subunits of the heterodimeric DNA polymerase) were important for viral origin-dependent DNA replication. The experimental approach relied on previously published reports characterising C-terminal truncations of the UL30 protein which demonstrated that amino acids residues near the C-terminus were dispensable for catalytic activity but necessary for interaction with the UL42 protein (29,30). We consequently attempted to overexpress an enzymatically active protein which was unable to complex with the UL42 polypeptide and examine whether it could function in viral origin-dependent replication. Expression of the mutant protein at high levels using a recombinant baculovirus facilitated biochemical analysis and also permitted testing of the functionality of the protein in a transient replication assay in Sf cells.

The finding that removal of amino acids 1208–1235 from the wt protein did not affect catalytic activity is in agreement with the previous findings of Haffey *et al.* (30) who showed that the regions corresponding to residues 1073 to 1144 and 1177 to 1235 were both dispensable.

The failure of the AcPOLd1 protein to be co-precipitated with UL42 by an anti-UL42 antibody, and the unresponsiveness of its enzymatic activity to UL42 stimulation demonstrate an impairment in its interaction with UL42. This finding confirms and extends the earlier work of Digard and Coen (29) who demonstrated that the region of UL30 comprising amino acids 1008–1235 was sufficient for complex formation with UL42, and that sequences essential for the interaction were present within the C-terminal 89 amino acids. It can now be concluded that sequences within the last 28 amino acids must have an important role in this interaction.

Although C-terminal truncations similar to that expressed by AcPOLd1 had been previously constructed no information was available on their ability to participate in viral DNA replication. The results of the transient replication assay (Figure 6) show that the deleted UL30 protein is severely impaired in this function.

The C-terminal region of the UL30 protein responsible for binding to UL42 (residues 1008–1235) appears to represent a discrete functional domain distinct from regions of the protein which have previously been implicated in its catalytic activities (i.e. RNaseH, 3′-5′ exonuclease and DNA polymerase) (29). Since no other function has been attributed to this region our results strongly suggest that the formation of a complex between UL30 and UL42 is essential for viral genome replication, and provide support for utilising this interaction as a possible target for novel antiviral drugs.

The requirement for the UL30-UL42 complex in viral genome replication is very likely to be related to the increase in processivity conferred upon the DNA polymerase by the UL42 subunit (25,26). This property, demonstrated *in vitro* on singly primed, single-stranded DNA templates, may be of considerable importance for continuous leading-strand synthesis at an advancing replication fork. Previous reports have presented conflicting results concerning the ability of UL42 protein to stimulate DNA polymerase activity on an activated calf thymus DNA template. The results shown in Figure 4 are in agreement with the greater than 5-fold stimulation observed by Gallo *et al.* (20). However, Gottlieb *et al.* (25) did not detect any effect, and Marcy *et al.* (17) reported that the specific activity of UL30 alone is very similar to that of the complex. In the present experiments much greater stimulation was detected at a reduced template DNA concentration (25 μg/ml) than at the more commonly employed concentration of 100 μg/ml, and it is possible that the increased processivity conferred by the UL42 protein is reflected in a net increase in DNA synthesis on an activated DNA template when

the number of primer ends is limiting. Hart & Boehme (35) have also recently presented data which may resolve the conflicts. They demonstrated that high salt concentrations (such as used in our experiments) are necessary in order to observe the stimulatory of UL42 protein on DNA polymerase activity, and that this is because UL30 alone has lower affinity for template DNA at high salt concentrations, and is enzymatically more active than the complex at low salt concentrations.

The data presented in this paper do not directly address the question of whether the bulk of the UL42 protein in HSV-1 infected cells which is not complexed with UL30 protein has a separate, essential role in viral DNA synthesis. Although such a role is suggested by the ability of UL42 to bind directly to double-stranded DNA, experiments in which UL42 null mutants are complemented for growth in transformed cell lines expressing the wt protein appear to argue against an independent function (36). In these experiments it was found that wt levels of replication were observed even in cells expressing only 5% the normal amount of UL42 protein. The available data therefore suggest that functional UL42 protein may be required for genome replication solely because of properties it confers upon the heterodimeric DNA holoenzyme.

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