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DNA-binding Proteins Specified by Herpesvirus Saimiri

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SUMMARY

Herpesvirus saimiri-specific proteins from the nuclear fractions of productively infected owl monkey kidney cells were dissociated from virus and host DNA by treatment with 2 M-NaCl or separation on Urografin density gradients. Empty virus capsids remained intact and could be separated from major non-structural proteins (110K, 51K and 48K) and from a subset of structural proteins (130K, 29K and 12K), either by Urografin gradient sedimentation or differential centrifugation. The DNA in such soluble extracts of nuclear proteins was efficiently removed by spermine precipitation, together with the host cell histones and large fractions of the 130K and 12K structural proteins. Proteins in the spermine-soluble fraction were analysed by affinity chromatography on columns of single-stranded calf thymus DNA coupled to cellulose. Two major structural proteins (130K and 12K), whose synthesis was sensitive to phosphonoacetic acid (PAA), and one minor PAA-resistant structural protein (29K) bound to DNA-cellulose. The major PAA-resistant 110K non-structural protein and the PAA-resistant non-structural 51K and 48K phosphoproteins were efficiently released into the spermine-soluble fraction and also bound to DNA-cellulose as did the 76K protein and minor species of 42K, 39K, 34K, 25K and 21K. Virus-specific proteins were eluted from such columns by buffers containing 0.4 M-NaCl or by heparin in low-salt buffers. Polypeptides from virus particles, infected cell extracts, or samples of eluates from DNA-cellulose chromatography, were separated by SDS-polyacrylamide gel electrophoresis, transferred onto nitrocellulose filters and probed for their ability to bind labelled polynucleotides. The non-structural 51K phosphoprotein, the 12K and 29K structural proteins and a 100K virion polypeptide all bound labelled DNA. However, the binding activities of the 130K protein from virions or purified by affinity chromatography and of the 110K polypeptide could not be demonstrated reproducibly after transfer from SDS gels to nitrocellulose. Comparisons of the present results on the properties of the herpesvirus saimiri-specified DNA-binding proteins with published accounts of the DNA-binding proteins of other herpesviruses, suggest some striking similarities with the DNA-binding proteins of the Epstein–Barr virus.

INTRODUCTION

The appearance of virus-specific thymidine kinase and DNA polymerase activities and the synthesis of more than 30 virus-specific polypeptides can be detected during the course of a productive cycle of herpesvirus saimiri (HVS) replication in tissue culture (Honess et al., 1982; O'Hare & Honess, 1983a, b; Randall et al., 1983; Modrow & Wolf, 1983; W. Bodemer & B. Fleckenstein, personal communication). In common with the DNA polymerase activities of a number of other herpesviruses, the activity specified by HVS is normally inhibited by phosphonoacetic acid (PAA) (O'Hare & Honess, 1983b). Doses of PAA sufficient to inhibit virus DNA replication inhibit the synthesis of the majority of virus-induced polypeptides [e.g. 250K (250 000 mol. wt.), 160K, 150K, 130K, 117K, 38K, 32K, 28K, 20K, 13–5K and 12K] whilst permitting the synthesis of a subset of early virus-specific polypeptides (i.e. 110K, 76K, 72K, 51K, 48K, 29K to 31K, 24K, 20K to 21K; O'Hare & Honess, 1983a). Of the two major virus-specified phosphoproteins detected in lysates of infected cells (59K and 51K to 48K), the 51K to
48K phosphoprotein is a member of the early PAA-resistant class and is rapidly and efficiently translocated to the nucleus of infected cells in the presence or absence of PAA (Randall et al., 1983; O'Hare & Honess, 1983a; see also below).

The capacity to interact with virus DNA is a property which would be anticipated for an important subset of virus gene products which perform catalytic or regulatory functions in the synthesis and expression of virus DNA or which are involved in the condensation and packaging of progeny DNA into virus particles. There are now abundant precedents, including better characterized members of the herpesviruses (Bayliss et al., 1975; Purifoy & Powell, 1976; Powell et al., 1981; Spang et al., 1983; Dixon et al., 1983; Weller et al., 1983; Littler et al., 1983), for a DNA-binding capacity in vitro correlating with a functional DNA-binding property in vivo. In addition, the ability to bind to DNA provides a powerful method for affinity chromatography and purification of the subset of DNA-binding proteins for subsequent assays of other activities (see e.g. Powell & Purifoy, 1977). We have therefore examined the state of virus-specific polypeptides in lysates of nuclei from infected cells, the partitioning of virus-specified proteins during subnuclear fractionation and the capacity of these proteins to bind to columns of DNA-cellulose in vitro. In addition, we have examined the ability of electrophoretically separated polypeptides transferred from SDS–polyacrylamide gels onto nitrocellulose membranes to bind labelled DNA in vitro.

**METHODS**

*Cells and viruses.* Owl monkey kidney cells (OMK) of the 210 cell line (originally isolated at the New England Regional Primate Center, Boston, Mass., U.S.A.) were used throughout for titration, high and low multiplicity growth of HVS. Vero cells were used for the growth of herpes simplex virus. HVS was the 'oncogenic' strain 11 and its attenuated derivative (i.e. HVS-[11 Onc] and HVS-[11 Att], Falk et al., 1972; Schaffer et al., 1975). Herpes simplex virus type 1 was strain Justin (HSV-[Justin], Frenkel et al., 1975). Procedures for the propagation of infected cells and the growth and titration of low multiplicity stocks of HSV and HVS have been described elsewhere (Honess et al., 1982).

*Conditions for high multiplicity infection and labelling of infected and mock-infected cultures with radioactive precursors.* Methods for high multiplicity infection and for labelling of infected and mock-infected cultures with L-[35S]methionine (500 Ci/mmol), [32P]orthophosphate (carrier-free) and [Me-3H]thymidine (47 Ci/mmol) were as previously described (Randall et al., 1983; Honess et al., 1982), with labelling intervals as specified in the text. All radioisotopes were purchased from Amersham International. Phosphonoacetic acid (ICN Pharmaceuticals Inc., Plainview, N.Y., U.S.A.) was used to inhibit virus DNA synthesis at a final concentration of 200 μg per ml of medium and was present from the time of infection.

*Cell fractionation and preparation of cell extracts for DNA–cellulose chromatography.* Infected and mock-infected monolayer cultures were rinsed thoroughly with ice-cold phosphate-buffered saline (PBS), removed from the surface of the culture vessel with a rubber policeman, sedimented (500 g for 5 min) and resuspended in PBS. All subsequent operations were performed on ice or in a 4 °C cold room. Cell suspensions were disrupted with a Dounce homogenizer and separated into a nuclear fraction and a cytoplasmic fraction by low-speed sedimentation (500 g for 5 min). The nuclear fraction was then resuspended in buffer containing 10 mM-Tris–HCl pH 7.4, 1 mM-phenylmethylsulphonyl fluoride (PMSF, Sigma) and made to 1% Nonidet P40 (NP40). After a 10 min incubation on ice, the detergent-washed nuclear fraction was separated from the NP40-soluble fraction by low-speed sedimentation (14000 g for 1 h). The nuclear fraction was resuspended in buffer containing 20 mM-Tris–HCl pH 7.4, 150 mM-NaCl, 2 mM-PMSF, 5 mM-EDTA, 2 mM-2-mercaptoethanol, and 20% glycerol and lysed by the addition of NaCl (or KCl) to a final concentration of 1.5 to 2.0 M (see text). The lysate was then sonicated with an ultrasonic probe (M.S.E. Instruments, Crawley, Sussex, U.K.: 3 x 15 s, to shear high mol. wt. DNA and reduce the viscosity of the sample) and incubated on ice for 1 h with occasional mixing. High molecular weight complexes were removed by high-speed sedimentation (140000 g for 1 h) and the supernatant made to 5 mM-spermine and dialysed against a buffer containing 5 mM-spermine in 10 mM-Tris–HCl pH 7.4, 50 mM-NaCl (or KCl), 5 mM-EDTA, 1 mM-PMSF, 1 mM-2-mercaptoethanol and 10% glycerol. The spermine–DNA precipitate and reassociated proteins (Hoopes & McClure, 1981; Subirana & Vives, 1981; see text) was removed by sedimentation (11500 g for 15 min) and the supernatant dialysed against buffer containing 10 mM-Tris–HCl pH 7.4, 50 mM-NaCl, 5 mM-EDTA, 1 mM-PMSF, 1 mM-2-mercaptoethanol and 10% glycerol in order to remove spermine. This dialysed spermine-supernatant fraction represented the starting material for DNA–cellulose chromatography.

*Preparation of DNA–cellulose and affinity chromatography of DNA-binding proteins.* Single-stranded DNA was coupled to cellulose (Whatman, CF-11) either by u.v.-irradiation (Litman, 1968) or by chemical coupling to aminothiophenol-derivatized cellulose by a method based on that described by Seed (1982). Coupling efficiencies
and the stability of coupled single-stranded DNA were monitored by addition of a $^{32}$P-labelled DNA tracer. In general, u.v.-crosslinking gave higher average coupling efficiencies, but both methods gave final preparations in which from 6 to 20 mg of single-stranded DNA were coupled via salt- (2 M) and detergent- (2% SDS) stable linkages to each gram of cellulose.

Samples of the dialysed spermine supernatants were applied to columns of single-stranded DNA-cellulose (extracts from 1 $\times$ 10$^8$ to 2 $\times$ 10$^8$ cells applied to a 2-0 ml column containing 0-5 g of DNA-cellulose) or to cellulose which had been u.v.-irradiated or taken through the chemical-coupling procedure in the absence of single-stranded DNA. Unbound material was eluted by passage of 5 column vol. of buffer containing 0-05 M-NaCl and thereafter columns were eluted successively with 2 to 3 column vol. of buffer containing 0-2 M, 0-4 M, 0-7 M, 1-7 M, and 1-7 M NaCl with 2% SDS, or with buffers containing 0-05 M NaCl and 1, 10, 100, and 1000 $\mu$g of heparin per ml. The column eluates were collected in 0-2 to 0-5 ml fractions and their content of labelled protein measured by liquid scintillation spectrophotometry. Fractions comprising each peak were pooled, dialysed into buffer containing 0-05 M-NaCl, made to 50% glycerol and analysed by gel electrophoresis or stored at $-20^\circ$C or $-70^\circ$C.

**Urografin density gradient sedimentation.** Nuclear fractions from infected and mock-infected cells were sonicated in 10 mM-Tris-HCl pH 7-4, 2 mM-EDTA, 2 mM-2-mercaptoethanol with from 0-1 to 0-75 M NaCl and applied to 14 ml gradients of 20 to 80% Urografin-370 (Schering Chemicals Ltd, West Sussex, U.K.); each ml of Urografin contains 0-1 g of sodium diatrizoate and 0-66 g of methylglucamine diatrizoate) which were centrifuged for 12 to 18 h at 140000 g. The gradients were fractionated from the bottom of the tubes and the densities of the fractions monitored by refractometry. In low-salt buffer the density at 20 $^\circ$C ($\rho_{20}$) is related to the refractive index ($n_{20}$) by $\rho_{20} = 3-381 n_{20} - 3-507$ (Russell et al., 1971).

**Virus purification.** Enveloped particles of HSV-1[Justin] and HVS were purified from the medium of infected Vero (HSV-1) or OMK (HVS) cell cultures. Monolayers of Vero or OMK cells in rotating 80 oz Winchester bottles (2 $\times$ 10$^5$ cells/bottle) were infected with 1 to 5 p.f.u. per cell of HSV-1 or HVS and incubated for 30 h (HSV-1) or 50 to 60 h (HVS) at 37 $^\circ$C, by which time the cultures showed an advanced cytopathic effect and the majority of the infectious virus yield (80 to 90%) was present in the culture medium. The culture fluid was decanted, cellular debris removed by low-speed sedimentation (3000 g for 15 min) and the enveloped virus sedimented from the supernatant by high-speed (60000 g for 1 h) centrifugation. The pellets were resuspended in 25 mM-Tris-HCl pH 7-5, 2 mM-EDTA, and 50 mM-NaCl and sedimented on 20 to 40% glycerol gradients (in the same buffer) for 30 min at 60000 g. Enveloped virus particles formed a homogeneous light-scattering band about half-way down the gradient. These bands were removed, diluted to 10% glycerol and the virus sedimented into a pellet (110000 g for 60 min).

**Preparation of labelled polynucleotide probes.** DNA was isolated from purified virions of HSV-1, HVS and adenovirus type 5 (the latter a gift from Mr B. Precious) by solubilization of virus particles with 1% SDS and proteinase K (100 $\mu$g/ml for 2 h at 37 $^\circ$C) in 10 mM-Tris-HCl pH 7-4, 2 mM-EDTA followed by extraction of protein from the digest with phenol/chloroform/isoamyl alcohol (24/24/1) and precipitation of DNA from the aqueous phases by 2 vol. ethanol (15 min at $-20^\circ$C). The DNA was redissolved in 10 mM-Tris-HCl pH 7-4, 1 mM-EDTA and labelled to a specific activity of approximately 10$^6$ ct/min/$\mu$g by nick translation (Kelly et al., 1970; Rigby et al., 1979) using Escherichia coli DNA polymerase I and [$\alpha$-$^32$P]dCTP (3000 Ci/mmol) as the labelled substrate. Poly(dG-dC)-poly(dG-dC) was purchased from P-L Biochemicals (Milwaukee, Wis., U.S.A.) as the sodium salt with an average $s_{20,w}$ of 6 to 9. The double-stranded polymer was dissolved at 2 0 A$^2$600 units per ml in 50 mM-sodium acetate buffer pH 4.5 with 4 M-NaCl. The configuration of the polymer was assessed by measurements of the circular dichroism (C.D.) at wavelengths in the range from 230 to 330 nm. Under the above conditions the polymer adopts the left-handed Z-form and it was stabilized in the Z-form by bromination via the addition of 1 part of bromine-water to 200 parts of solution, followed by incubation at room temperature for 5 min. The solution was then transferred to ice and excess bromine removed with a stream of nitrogen. The brominated polymer was dialysed exhaustively against 10 mM-Tris-HCl pH 7-4, 1 mM-EDTA, 150 mM-NaCl and the retention of the Z-configuration in low-salt buffer (probably due to bromine substitutions on C8 of guanine and C5 of cytosine; Lafer et al., 1981; W. C. Russell & B. Precious, personal communication) confirmed by C.D. spectroscopy. The brominated polymer was dephosphorylated with bacterial alkaline phosphatase and the 5' ends labelled in vitro by phosphate transfer from [$\gamma$-$^32$P]ATP (> 5000 Ci/mmol; Amersham International) with T4 polynucleotide kinase (New England Biolabs). The final specific activities of the 5'-$^32$P-poly(dBrCSC-G-dBrCSC) [abbreviated as 5'-32P-BrdCSC in the text] were $1 \times 10^6$ to $5 \times 10^6$ ct/min/$\mu$g.

**Polyacrylamide gel electrophoresis; electrophoretic transfer of separated polypeptides from polyacrylamide gels onto nitrocellulose membrane filters and use of membrane-bound polypeptides in DNA-binding assays.** Methods for SDS–polyacrylamide gel electrophoresis and the identification and some properties of HVS-specific polypeptides separated from infected cell lysates have been described previously (Heine et al., 1974; Randall et al., 1983; O'Hare & Honess, 1983a). Polypeptides separated on polyacrylamide gels were transferred to sheets of 0.45 $\mu$m nitrocellulose (BA85, Schleicher & Schüll) by transverse electrophoresis essentially by the method of Towbin.
et al. (1979) as described by Russell & Precious (1982). After transfer, the filters, bearing samples of the electrophoretically separated polypeptides, were washed extensively in a buffer containing 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone in 10 mM-Tris–HCl pH 7.4, 1 mM-EDTA, 50 mM-NaCl, and 1 mM-2-mercaptoethanol and equilibrated in the same buffer for 12 h at room temperature. The filters were then incubated with the same buffer containing labelled probes (total vol. 0.1 ml per cm\(^2\) of nitrocellulose containing 10\(^5\) ct/min/80 cm\(^2\)) for 1 h at room temperature and then washed in multiple (3 or 4 times in 1 h) changes of the same buffer and the retained probe detected by autoradiography (Bowen et al., 1980).

**Electron microscopy.** Peak fractions from Urografin or glycerol gradients were diluted in water containing 50 µg of bovine serum albumin per ml, mixed with a calibrated suspension of Dow polystyrene latex spheres (60 nm diam.) and applied as 25 µl drops onto Parafilm. Carbon-stabilized Formvar films on 300- or 400-mesh copper grids were floated on these drops and thereafter transferred to drops of 1% sodium phosphotungstate (pH 7.0). Excess liquid was removed and the dried grids examined in a Philips EM 300 operated at an accelerating voltage of 60 kV and at a final screen magnification of 20000 ×.

**RESULTS**

**Complexes of virus-specified proteins in nuclei of infected cells**

The nuclei of HVS-infected cells accumulate a complex population of virus-specified proteins. In the first series of experiments we examined conditions for the lysis of nuclei that would dissociate the majority of virus-specified proteins from nucleic acids under non-denaturing conditions. Useful separations of structural from non-structural proteins were obtained by lysis of detergent-washed nuclear fractions in high-salt buffers and sedimentation to equilibrium on Urografin density gradients. Results from a typical set of separations on such gradients are illustrated in Fig. 1 to 3. Nuclei were isolated from infected or mock-infected cells labelled for extended periods with \(^{35}\)S)methionine (Fig. 1, 2 and 3) or with \(^{3}\)H)thymidine (Fig. 3). Labelled proteins of nuclear extracts from infected cells were separated into three peaks (P\(_1\), P\(_2\) and P\(_3\)), a poorly resolved pair of peaks at the bottom of the gradients (P\(_1\), P\(_2\)) well separated from a broad peak at the top of the gradient (P\(_3\)). The denser of the two dense peaks (P\(_1\)) corresponded to a diffuse band of coarsely aggregated material which was also present in samples from uninfected nuclei, and P\(_2\) to a hypersharp opalescent band which was not present in extracts from uninfected nuclei. The pattern of bands was relatively little affected by the addition of NaCl at concentrations from 0.1 to 0.75 M. Proteins from nuclei of uninfected cells were distributed differently, with a relatively lower proportion of labelled species banding at high density and with a significant fraction of intermediate density complexes which were dissociated by increasing the salt concentration by the addition of NaCl (compare Un 0.1 M and Un 0.75 M with Inf 0.1 M and Inf 0.75 M in Fig. 1). Electron microscopy of negatively contrasted preparations showed that P\(_1\) from infected cells and the corresponding region of uninfected cells was composed of large aggregates (> 1000 nm) of material with no recognizable regularity of structure; however, P\(_2\) from infected cells contained a high concentration of penetrated virus capsids with little visible contamination with non-capsid structures (not shown). Polyacrylamide gel electrophoresis of proteins from fractions of these gradients (Fig. 2) showed that the labelled polypeptides from nuclei of infected cells were mainly virus-specific and that P\(_2\) contained polypeptides characteristic of the 'empty' HVS nucleocapsid (Fig. 2, fractions 3 and 4; 150K, 38K, 32K and 13-5K polypeptides, R. W. Honess et al., unpublished results). Peak 3 from infected cells (Fig. 2, fractions 7 to 9) contained the entire nuclear content of 130K, 110K, 51K, 48K and 12K polypeptides, the majority of the 29K polypeptide and a small proportion of 150K, 38K and 32K proteins. The 29K and 12K polypeptides are components of nucleocapsids and the 130K protein is a non-capsid component of the virion (R. W. Honess et al., unpublished results; Keil et al., 1980; Randall & Honess, 1980). The 110K, 51K and 48K polypeptides are the major non-structural proteins of the nuclear fractions.

Comparisons of the density distribution of \(^{3}\)H)thymidine-labelled DNA with that of labelled proteins in lysates from infected and uninfected cells (Fig. 3) showed that the density distribution of labelled proteins was not influenced by prior nuclease digestion and that the nucleocapsid peak contained little or no virus or cellular DNA. In addition, although the infected nuclei contained peaks of labelled DNA banding at densities different from those of
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Fig. 1. Comparisons of complexes of [35S]methionine-labelled proteins separated from nuclei of uninfected OMK cells (Un 0.1 M, 0.5 M and 0.75 M) and HVS-[11 Att]-infected OMK cells (Inf 0.1 M, 0.5 M and 0.75 M) by equilibrium sedimentation on gradients of Urografin in the presence of 0.1, 0.5 or 0.75 M-NaCl. Mock-infected OMK cells and cells infected with HVS-[11 Att] were labelled with [35S]methionine from 30 to 60 h after infection and divided into nuclear and cytoplasmic fractions after Dounce homogenization. Nuclei were washed with PBS containing 1% NP40 and the detergent-insoluble nuclear fractions from Un and Inf cells divided into three aliquots, disrupted by ultrasonic oscillation in 10 mM-Tris-HCl pH 7.4, 2 mM-EDTA, 2 mM-2-mercaptoethanol containing NaCl and applied to pre-formed 14 ml gradients of 20 to 80% (v/v) Urografin made to 0.1, 0.5 or 0.75 M-NaCl in the same buffer. Gradients were sedimented at 140000 g for 14 h and thereafter fractionated and the fractions monitored for their content of [35S]methionine-labelled protein (histograms) and refractive index (n, O). P1, P2 and P3 indicate major peaks of labelled proteins separated from nuclei of infected cells (see text).
components in uninfected cells (Fig. 3a, c), the DNA in these components was susceptible to prior nuclease digestion at low-salt concentrations. Without resort to nuclease digestion, the virus proteins of P3 were not separable from some components of host and virus DNA by Urografin density gradient sedimentation.

Partitioning of virus- and host-specific proteins, phosphoproteins and nucleic acids during subfractionation of nuclei

To identify and to isolate those virus-specified proteins with intrinsic affinity for DNA, the ideal fractionation procedure should produce a nucleic acid-free extract of monomeric nuclear proteins in native form and high yield as the starting material for DNA–cellulose affinity chromatography. The protocol we adopted gave a very high yield of non-structural proteins and efficient removal of nucleic acids; however, most structural polypeptides were recovered in low yield. The basic procedure involved (i) disruption of cells by Dounce homogenization, separation of crude nuclear fraction and treatment of crude nuclear fraction with non-ionic detergent (NP40), (ii) lysis of the detergent–washed nuclear fraction by treatment with high concentrations of salt (1.5 to 2.0 M-NaCl or -KCl) and removal of particulate material by high-speed sedimentation, (iii) addition of spermine (5 mM) to the high-speed supernatant and dialysis against low-salt (50 mM-NaCl or -KCl) buffer containing 5 mM-spermine, and (iv) sedimentation (11500 g for 15 min) to remove the spermine–DNA precipitate and dialysis of the DNA-free supernatant against low-salt buffer (50 mM-NaCl or -KCl) to remove spermine. In a
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Fig. 3. Distribution of [35S]methionine-labelled proteins and [3H]thymidine-labelled DNA from high-salt lysates of nuclei from uninfected (a, b) and HVS-infected (c, d) OMK cells separated by equilibrium buoyant density gradient sedimentation on Urografin gradients. Monolayers of OMK cells were mock-infected or were infected with 2 p.f.u./cell of HVS-[11 Att] and labelled from 20 to 60 h after infection or mock infection, with 5 μCi [35S]methionine (◊) and 10 μCi [3H]thymidine (●) per ml of medium (a to d) or from 40 to 60 h after infection, with 10 μCi [3H]thymidine (c, f). Suspensions of NP40-washed nuclei were lysed in buffer containing 0.15 M-NaCl and aliquots sedimented on 20 to 80% Urografin density gradients either without further treatment (a, c) or after DNase digestion (b, d; 50 μg/ml DNase I for 2 h at 20°C). P1, P2 and P3 are shown as in Fig. 1 (and see text).

Fig. 4 shows electrophoretic separations of the total proteins and the corresponding [35S]methionine-labelled polypeptides from stages in the fractionation of mock-infected cells, untreated HVS-infected cells and HVS-infected cells treated with PAA from the time of infection and throughout the labelling interval. Results from an independent experiment comparing the behaviour of phosphorylated polypeptides from untreated infected and typical fractionation of infected and mock-infected OMK cells, from 8 to 20% of the initial [35S]methionine-labelled protein was recovered in the spermine supernatant fraction with from 0.1 to 0.5% of the initial [3H]thymidine-labelled DNA from infected cultures and < 0.1% of initial [3H]thymidine-labelled DNA from mock-infected cultures. The partitioning of virus- and host-specified proteins and phosphoproteins was monitored by gel electrophoresis and typical results are shown in Fig. 4 and 5.

Fig. 5 shows electrophoretic separations of the total proteins and the corresponding [35S]methionine-labelled polypeptides from stages in the fractionation of mock-infected cells, untreated HVS-infected cells and HVS-infected cells treated with PAA from the time of infection and throughout the labelling interval. Results from an independent experiment comparing the behaviour of phosphorylated polypeptides from untreated infected and
Fig. 4. (a) Coomassie Brilliant Blue-stained gel film and (b) the corresponding autoradiogram of polypeptides separated by electrophoresis through a 13% polyacrylamide gel slab from nuclear and subnuclear fractions from mock-infected (M) OMK cells and OMK cells infected at high multiplicity with HVS-[11 Att] and incubated in the presence of 200 μg of PAA per ml (I+) or in the absence of the drug (I). Infected and mock-infected cultures were labelled with 5 μCi [35S]methionine from 20 to 60 h after infection or mock infection and thereafter fractionated as described in the text. Aliquots of each fraction corresponding to about 2 × 10^5 (+ spermine, insoluble) or 1 × 10^5 (other fractions) cell equivalents were subjected to gel electrophoresis. Virus-specific polypeptides are annotated with their apparent mol. wt. (×10^-3) and the positions of host cell histones (h) and actin (a) are also shown.
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Autoradiogram $^{35}$S/$^{32}$P polypeptide fractionation

Fig. 5. Autoradiogram of labelled proteins and phosphoproteins separated on 13% polyacrylamide gel slabs from nuclear and subnuclear fractions of infected (I) or mock-infected (M) OMK cells labelled from 25 to 48 h after infection or mock infection, with 10 μCi $^{35}$S-methionine (IS) or 25 μCi $^{32}$P-orthophosphate (IP, MP) per ml of medium. Labelled cells were fractionated (as described in the text and Fig. 4), and aliquots corresponding to $1 \times 10^5$ (Dounce nuclei, + NP40, soluble and + NP40, nuclear pellet), $2 \times 10^5$ (2 M- NaCl lysate, soluble and insoluble; + spermine, soluble) and $5 \times 10^5$ (+ spermine, insoluble) cell equivalents were separated by SDS-gel electrophoresis. The figure is a composite of autoradiograms from two 13 gels, lanes 1 to 9 being from one gel and lanes 10 to 21 being from the other. Major virus proteins and phosphoproteins are annotated with apparent mol. wt. ($\times 10^{-3}$) and 'hp' indicates major host phosphoproteins of approx. 34K and approx. 40K. The behaviour of polynucleotides can also be assessed from this figure. The $^{32}$P-orthophosphate label in the top 0.5 to 1.0 cm of the gel was susceptible to DNase digestion and partitioned in the same way as $^{3}$H-thymidine-labelled DNA in similar samples assessed either by fluorography of polyacrylamide gels or by scintillation counting (not shown).

uninfected cells with the partitioning of $^{35}$S-methionine-labelled proteins of infected cells are shown in Fig. 5. The identification of the total and PAA-resistant virus-specific polypeptides and their partitioning between nucleus and cytoplasm have been dealt with elsewhere (Randall et al., 1983; O'Hare & Honess, 1983a). The later stages in the fractionation show a number of highly selective differences in the behaviour of different virus-specified polypeptides. Firstly, high-speed sedimentation removed little protein, phosphoprotein or nucleic acid from the high-salt lysates of nuclei from mock-infected cells or cells infected and labelled in the presence of PAA. Consistent with the results from the Urografin gradient analyses, the pellet from infected control cultures consisted almost exclusively of the polypeptides of 'empty' virus nucleocapsids (Fig. 5., i.e. 150K, 38K, 32K, 31–29K and 13.5K). Secondly, spermine precipitation removed virtually all the nucleic acids from infected and mock-infected cells and a large fraction of the phosphorylated proteins (Fig. 5), together with the host cell histones and the majority of the PAA-sensitive 130K and 12K virus-specific polypeptides (Fig. 4 and 5, but see below). Thirdly,
the spermine supernatant fraction was selectively enriched for the early, PAA-resistant, subset of virus gene products relative to total host protein as well as relative to late, PAA-sensitive, virus-specific proteins (Fig. 4). Fourthly, the ratio of label derived from $[^{32}P]$orthophosphate to that incorporated from $[^{35}S]$methionine in the 51K proteins in the spermine-soluble and -insoluble fractions differed, the spermine-insoluble forms being more highly phosphorylated. Finally, the partitioning of virus-specific polypeptides by spermine could be manipulated in a simple and useful way by varying the salt concentration at which spermine was added and the spermine–DNA precipitate and associated proteins removed. Thus, addition of spermine to the high-salt lysates and dialysis against spermine in low salt (0.05 M) in our routine protocol gave high recoveries of the available 160K, 110K, 51K, 48K and 42–34K polypeptides in the supernatant and significant, but variable, minorities of the 130K and 12K polypeptides were also represented in the supernatant. However, if spermine was added to the low-salt dialysis buffer, but not to the high-salt extract prior to dialysis, the majority of 160K and 110K proteins remained soluble with some 50% of the available 29–31K and 48K proteins, but the pellet contained all detectable 130K and 12K polypeptides (>95%) and the majority of the 51K and 42–34K proteins (approx. 70%), results not illustrated.

**DNA–cellulose affinity chromatography**

Samples of the spermine-soluble fractions from mock-infected cells and from infected cells labelled with $[^{35}S]$methionine or $[^{32}P]$orthophosphate in the presence or absence of PAA were applied to affinity chromatography columns of single-stranded DNA coupled to cellulose or applied to columns of cellulose without DNA. Columns were eluted with buffers containing stepwise increases in concentrations of NaCl or KCl (0.2, 0.4, 0.4, 1.7, and 1.7 M + SDS) or heparin (0.1, 1, 10, 100 and 1000 µg/ml). The recovery of applied protein or phosphoproteins was high (>90%) from all columns and from 40 to 80% of applied $[^{35}S]$methionine label from extracts of infected cells and 20 to 50% of labelled proteins in extracts of mock-infected cells or infected cells treated with PAA were retained by DNA–cellulose columns in a form requiring salt for elution. Only 2 to 10% of the applied counts were removed with SDS (see e.g. Fig. 6) from DNA–cellulose columns and whilst columns of cellulose without DNA did not bind a significant fraction of applied protein in a form that could be eluted by salt, some 3 to 5% of applied counts were retained and could be removed with SDS. Comparisons of polypeptides represented in the input, in the unbound fractions and the salt eluates from DNA–cellulose affinity chromatography of samples from uninfected cells and from infected cells labelled with $[^{35}S]$methionine and $[^{32}P]$orthophosphate are shown in Fig. 7 and 8. The results from these and many similar experiments may be summarized as follows.

(i) The 130K, 110K, 51K, 48K, 42K, 39K, and 34K proteins were very efficiently bound to DNA–cellulose and eluted by 0.2 or 0.4 M NaCl. The 160K and 150K polypeptides (if present) were not bound (Fig. 7 and 8). The very efficient binding of this subset of virus-specified proteins contrasted with the behaviour of the majority of proteins of the uninfected cell which did not bind (Fig. 7, compare protein stain and autoradiogram of infected cell sample with autoradiogram of samples from uninfected cells). The small amounts of the 12K polypeptide which remained in the spermine supernatant were efficiently bound to DNA–cellulose and eluted with 0.2 and 0.4 M NaCl (not shown).

(ii) Significant proportions of the 76K, 31K to 29K and 21K proteins present in the input were bound in a form elutable by 0.4 M NaCl, but in a number of experiments these proteins were also enriched in that fraction which required SDS for elution (Fig. 7 and results not shown).

(iii) The 51K, 48K, 42K, 39K and 34K proteins were phosphorylated in vivo and phosphorylated forms bound efficiently to DNA–cellulose. The 59K, PAA-sensitive, phosphoprotein (O'Hare & Honess, 1983a) did not bind efficiently to DNA–cellulose (Fig. 8).

(iv) With the exception of the 130K, 34K and 12K proteins (the synthesis of which is sensitive to PAA), the 0.2 M and 0.4 M eluates from DNA–cellulose columns of extracts from PAA-treated cells could not be differentiated from those from infected control cultures (not shown).

(v) The 48K, 110K and 130K proteins were enriched, relative to 51K and 42–29K proteins, in the 0.2 M-eluates (e.g. Fig. 7). The use of heparin as an eluting solute also differentiated between
Fig. 6. Elution from columns of single-stranded DNA coupled to cellulose of the labelled proteins present in extracts of nuclei from (a) uninfected OMK cell and (b, c) OMK cells infected with HVS and labelled in the absence (b) or in the presence (c) of PAA (200 µg/ml). Cultures were labelled with $[^{35}\text{S}]$methionine (5 µCi per ml of medium) from 20 to 60 h after infection or mock infection and fractionated to obtain spermine-soluble nuclear extracts which were applied to columns of single-stranded calf thymus DNA in 10 mM-Tris-HCl pH 7.4, 0.05 M-NaCl. Columns were eluted successively with the same buffer containing 0.05, 0.2, 0.4 and 1.7 M-NaCl and, finally, with buffer containing 1.7 M-NaCl and 2% SDS (arrows show approximate elution positions of buffers containing the various salt concentrations). At least 90% of input radioactivity was recovered from each column and the percentages of recovered counts eluted at each salt concentration are given next to the respective peaks.

The binding of virus-specified polypeptides. For example, about 50% of the 130K protein could be eluted from DNA–cellulose columns with as little as 10 µg of heparin/ml, together with only 1 or 2% of the 110K and 51K to 29K proteins. The remainder of the 130K and about 90% of the 110K and 60% of the 51K to 29K proteins were eluted by 100 µg/ml and the remainder of the 51K to 29K proteins were eluted with 1000 µg of heparin/ml (not shown).

(vi) Virus-specified polypeptides in cells infected with the ‘oncogenic’ parental strain, HVS-[11 Onc], and its attenuated derivative, HVS-[11 Att], were comparable in number, apparent size, molarity and DNA-binding properties. We have detected no difference in the properties of total virus proteins or DNA-binding proteins which correlates with attenuation in this strain (not shown).

**DNA-binding activities of polypeptides separated on polyacrylamide gels and transferred to nitrocellulose filters**

The fractionation procedure we employed selected against structural proteins of the virus. Thus, of the structural proteins which were present in the high-speed supernatant from the high-
Fig. 7. Comparisons of the total (protein stain) and corresponding [\(^{35}\)S]methionine-labelled (autoradiograms) polypeptides separated on a 10% polyacrylamide gel slab from lysates of detergent-washed nuclei (N), the derived spermine-soluble proteins (I = input for affinity chromatography) and the fractions obtained by DNA-cellulose affinity chromatography of these spermine-soluble proteins of HVS-infected (left, infected) and uninfected OMK cells. Samples of proteins eluted from DNA-cellulose columns with buffers containing 0.05 M-NaCl (i.e. 0.05, unbound fraction) and with 0.2, 0.4, 0.7 M- and with 0.7 M-NaCl with 1% SDS are shown. Approximately equal numbers of cell equivalents of each fraction (1 x 10^5) were loaded onto the gel and the major virus-specific polypeptides are annotated with their apparent mol. wt. (x 10^-3). Minor virus-specific DNA-binding proteins of 42K, 39K and 29K to 31K were also recovered selectively in the 0.4 M eluate. Together with the 34K protein these latter comprise only 3 to 6% of the labelled protein in the 0.4 M eluate and they are not prominent on the autoradiogram shown, which was chosen to give a linear film response for the major polypeptides.

salt extracts of nuclei, the 29K to 31K proteins were present in low concentration and the 130K and 12K proteins were more (12K) or less (130K) efficiently precipitated by spermine. The 12K, 29K and 130K proteins, which were present in a soluble form in the spermine supernatant, bound to DNA-cellulose. We wished to examine the DNA-binding properties of these proteins from virus particles and to monitor other structural and non-structural proteins for DNA-binding activity. We therefore separated the polypeptides from infected or mock-infected cell lysates, the DNA-binding proteins from DNA-cellulose columns and the polypeptides from purified enveloped virus particles of HVS and herpes simplex virus by SDS-polyacrylamide gel electrophoresis and transferred them onto nitrocellulose membranes by transverse electrophoresis. The nitrocellulose membranes bearing separated polypeptides were incubated with labelled DNA ‘probes’ and the selective retention of labelled DNA monitored by autoradiography after washing to remove unbound DNA. A representative set of results which illustrates the main findings from these experiments is shown in Fig. 9.

Extracts of infected and mock-infected cells contained a very large number of proteins active in this DNA-binding assay and, with the exception of histones which are present at high concentration and bind DNA very efficiently, the profiles of DNA-binding activity did not resemble the profiles of total protein. Thus, the assay shows binding by a specific subset of
HVS DNA-binding proteins

Fig. 8. Autoradiograms of labelled proteins and phosphoproteins separated by electrophoresis through 12.5% polyacrylamide gels from fractions (0.25, 0.2, 0.4 and 0.7 M-NaCl eluates) obtained by DNA-cellulose affinity chromatography of spermine-soluble proteins from HVS-infected OMK cells labelled with (a) [35S]methionine (5 μCi/ml; L = column load) or (b) [32P]orthophosphate (20 μCi/ml) from 18 h to 60 h after infection. The 51K and 48K DNA-binding proteins clearly correspond with the 51K to 48K phosphoprotein and the minor 42K, 39K and 34K DNA-binding proteins co-migrate with minor phosphoproteins. The Coomassie Brilliant Blue-stained gel films of the [35S]methionine- and [32P]orthophosphate-labelled samples showed very similar profiles of total proteins in each fraction and similar analyses of double-labelled samples confirm the results illustrated (not shown).

cellular proteins. The only protein which could be demonstrated reproducibly to be specific for total infected cell lysates was the 51K polypeptide, which also showed binding activity when purified by affinity chromatography (Fig. 9a, b). Although polypeptides in the 130K and 110K mol. wt. regions of infected cell extracts showed binding activity in some experiments, they were not reproducibly specific to infected cells and purified preparations of DNA-binding proteins containing the virus-specified 130K and 110K proteins did not show renaturation of their binding activity (e.g. 0.4 M-eluate shown on 10% gel in Fig. 9b). The other proteins present at lower concentrations (e.g. 42 to 29K) would not have been detected due to co-migrating host cell DNA-binding proteins and the binding activity of the 12K polypeptide in total cell extracts could not be assessed because of its proximity to host cell histones. However, the major basic 12K polypeptide from HVS virions and the 29K protein reproducibly showed binding activity with 32P-Br(dG–dC) and with 32P-labelled DNAs from HVS (Fig. 9c), HSV-1 or adenovirus type 5 (not shown). In addition, a 100K virion polypeptide was detected with the 32P-Br(dG–dC), but not with these other labelled DNA in this experiment, but showed activity with natural DNAs in other experiments. The major DNA-binding activity of polypeptides separated from virions of HSV-1 was associated with the major virion phosphoprotein (38K, VP22 of Gibson & Roizman, 1972, 1974; Spear & Roizman, 1972; see also Lemaster & Roizman, 1980; Bayliss et al., 1975; Powell & Purifoy, 1976; Wilcox et al., 1980; Preston et al., 1983).

The correspondence between the other HSV-1 proteins which showed binding activity in these experiments and those identified previously is less certain. The relative and absolute binding efficiencies of various DNA-binding proteins from virus particles or the infected cell
Fig. 9. Autoradiogram of $^{32}$P-labelled DNA bound to polypeptides transferred from polyacrylamide gels to nitrocellulose filters. Samples of unlabelled proteins from a lysate of uninfected OMK cells (U), a lysate of OMK cells taken 60 h after infection with HVS (I), the 0.4 M-NaCl eluate from DNA-cellulose affinity chromatography of a nuclear extract from HVS-infected OMK cells (I, 0.4 M) and from virions of HVS-[11 Att] (HVS) and HSV-1[Justin] (HSV-1) were electrophoresed through (b) 10%, (a) 12% or (c) 13% SDS-polyacrylamide gels. The separated polypeptides were transferred from the polyacrylamide gels by transverse electrophoresis onto nitrocellulose membrane filters and probed with $^{32}$P-Br(dG-dC) (105 ct/min/filter; 106 ct/min/μg) or HVS DNA labelled with $[^{32}$P]dCTP by nick translation (105 ct/min/filter; 106 ct/min/μg). The figure shows a composite of autoradiograms from the washed filters. Positions of the major HVS-specified polypeptides from the total cell extract and the major virion polypeptides of HVS (160K to 12K) and HSV-1 (VP5 etc; Heine et al., 1974) were located by amido black staining of the nitrocellulose filters. Proteins ‘X’ and ‘Y’ in the HSV-1 virion preparations have apparent mol. wt. of 115K and 18K, respectively. The HVS-specified proteins that show reproducible DNA-binding activity in this assay are arrowed (51K in infected cell lysate and 100K, 29K and 12K in virions of HVS).

were comparable whether homologous (i.e. HVS or HSV) or heterologous (e.g. adenovirus type 5 or calf thymus) single- or double-stranded probes were used (not shown). However, the great majority of cell- and virus-specific binding proteins bound the $^{32}$P-Br(dG-dC) probe very much more efficiently than the ‘natural’ DNA probes [note that the filters shown were exposed to equal ct/min of the 5’ end-labelled Br(dG-dC) probe, 106 ct/min/μg, and the nick-translated HVS DNA probe, 108 ct/min/μg]. The high binding efficiency of this probe is not an indication that these proteins bind preferentially to natural left-handed Z-DNA sequences and is probably conferred by the presence of the acidic bromine substituents rather than the polynucleotide configuration. The binding of ‘natural’ DNA probes to virus 51K and to host proteins other than cellular histones on such filters was removed by 0.1 μg of heparin/ml. DNA bound to histone H1 was removed by 1 μg and that bound to all histones was removed by 10 μg of heparin/ml. These
concentrations are significantly less than the 100 to 1000 µg of heparin/ml required to elute the virus 51K DNA-binding protein from DNA-cellulose columns (see above) or to dissociate complexes formed with affinity-purified virus DNA-binding proteins and labelled DNA in solution (not shown).

DISCUSSION

The main findings of the present paper are summarized in Table 1. The 130K, 110K, 51K, 48K, 29K and 12K polypeptides share all known properties with the major co-migrating polypeptides in HVS-infected cells. The major HVS-specific DNA-binding proteins therefore include two PAA-sensitive structural proteins (130K and 12K) and the PAA-resistant non-structural 110K, 51K and 48K proteins. The amount of 48K protein increases relative to the 51K protein at late times in infection or during long chase periods and its relative abundance differs in infections of different cell lines in culture (see e.g. Randall et al., 1983; and Fig. 2). These observations and the similarities between the properties of the 48K and 51K proteins suggest that the 48K protein is a more extensively processed product of the 51K polypeptide. With this exception the other major DNA-binding proteins have distinctive combinations of properties (Table 1) and are likely to be the products of independent genes. The status of the minor proteins (42K, 39K, 34K and also 24K and 21K) is less certain. The 42K, 39K and 34K proteins identified by DNA-cellulose chromatography are phosphorylated in vivo and the 42K and 39K proteins are made in the presence of PAA. Virus-specific proteins of 42K, 39K, 24K and 21K have been recognized as minor gene products in infected cells treated with PAA and a 34K, PAA-sensitive polypeptide has been detected in control infections (O'Hare & Honess, 1983a). However, the phosphorylation status of these minor species was not determined in studies of total cell lysates owing to the presence of host-specified phosphoproteins of similar electrophoretic mobilities. The sum of the [35S]methionine label in all five of these minor proteins is less than 10% of the label in the 51K protein. Despite the reproducible detection of the minor species in similar relative concentrations, we have not eliminated the possibility that one or more of them may arise from a low level of proteolysis of the major DNA-binding proteins. We have used OMK cell cultures throughout this work, since our experience is that virus proteins are normally stable in extracts from these cells. However, in similar experiments with extracts from infected Vero cells, treatment with NP40 results in proteolysis which is not inhibited by PMSF or a range of other protease inhibitors (tosyllysylchloromethyl ketone, tosylphenylalanylchloromethyl ketone, Aprotinin, sodium bisulphite) and the 51K protein is specifically converted to a 42K cleavage product (E. D. Blair, unpublished results). We are currently preparing antisera to the DNA-binding proteins of HVS and these antisera should provide the means to establish the uniqueness or otherwise of the minor DNA-binding proteins detected in extracts of infected OMK cells.

The virus-specified proteins that partition with host cell histones in the DNA-spermine pellet require some comment. The 130K and 12K proteins are selectively removed with the spermine-DNA pellet and this fraction also contains a minority of the 51K protein which is more highly phosphorylated than the 51K protein which remains in the spermine supernatant. We do not know if the highly phosphorylated subclass of the 51K protein has any significance in vivo, but it is clearly desirable to examine the properties of isolated proteins from the fraction. All our experience with the 130K and 12K proteins suggests that they are removed to the spermine-DNA pellet by virtue of their DNA-binding properties and the variable minorities of these proteins in the supernatant bind efficiently to DNA. However, the 130K protein in detergent extracts of infected cells is the least soluble of the major non-capsid proteins at low (<0.2 M) ionic strengths and it is not released efficiently from the spermine pellet by DNase digestion (unpublished results). We cannot exclude the possibility that self-association or association of 130K with other proteins, rather than a direct interaction with DNA, is the reason for its removal with the spermine-DNA pellet. Studies of the binding and condensation of DNA by purified 130K and 12K proteins in the presence or absence of polyamines should enable us to understand and modify their partitioning during the fractionation procedure. Results from such studies should also assist in deducing the roles of these proteins in the virus particle. Further
Table 1. Properties of DNA-binding proteins specified by herpesvirus saimiri

<table>
<thead>
<tr>
<th>Apparent mol. wt. ($\times 10^{-3}$)</th>
<th>Synthesis resistant (R) or sensitive (S) to PAA in vivo</th>
<th>Structural (S) or non-structural (NS)</th>
<th>Phosphorylated in vivo</th>
<th>DNA-binding activity of polypeptide transferred to nitrocellulose</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>130</td>
<td>S</td>
<td>S</td>
<td>–</td>
<td>–</td>
<td>A major protein of infected cell extracts; majority precipitated by spermine.</td>
</tr>
<tr>
<td>110</td>
<td>R</td>
<td>NS</td>
<td>–</td>
<td>–</td>
<td>A major protein, extracted efficiently into the spermine supernatant.</td>
</tr>
<tr>
<td>100</td>
<td>(?)*</td>
<td>S</td>
<td>(?)*</td>
<td>+</td>
<td>* Minor component, only detected in virions.</td>
</tr>
<tr>
<td>76</td>
<td>R</td>
<td>(?)†</td>
<td>–</td>
<td>(?)†</td>
<td>† Variable removal from nuclear fraction in independent fractionations. Infected cell and virions contain other proteins in this size range. Intermediate abundance.</td>
</tr>
<tr>
<td>51</td>
<td>R</td>
<td>NS</td>
<td>+</td>
<td>+</td>
<td>The major NS phosphoprotein, accumulates rapidly in nucleus. Basic pf.</td>
</tr>
<tr>
<td>48</td>
<td>R</td>
<td>NS</td>
<td>+</td>
<td>+</td>
<td>Probable product from 51K protein. Less basic than 51K and with some differences in affinity for DNA.</td>
</tr>
<tr>
<td>42</td>
<td>R</td>
<td>NS</td>
<td>+</td>
<td>(?)†</td>
<td>‡ Minor protein.</td>
</tr>
<tr>
<td>39</td>
<td>R</td>
<td>NS</td>
<td>+</td>
<td>(?)†</td>
<td>‡ Minor protein.</td>
</tr>
<tr>
<td>34</td>
<td>S</td>
<td>(?)§</td>
<td>+</td>
<td>(?)</td>
<td>‡§ Co-migrates with a non-phosphorylated virion polypeptide.</td>
</tr>
<tr>
<td>29</td>
<td>R</td>
<td>S</td>
<td>–</td>
<td>+</td>
<td>Minor protein, probably a processed form of 31K.</td>
</tr>
<tr>
<td>12</td>
<td>S</td>
<td>S</td>
<td>–</td>
<td>+</td>
<td>A major protein of infected cell extracts and virions. Majority precipitated by spermine. Very basic, 'histone-like'.</td>
</tr>
</tbody>
</table>
purification of the DNA-binding proteins isolated by affinity chromatography will also be necessary to associate more specific binding functions or other activites (e.g. DNA polymerase, protein kinase) with particular virus proteins.

The information that is available on DNA-binding activities of the proteins of other herpesviruses is at a relatively superficial level and is restricted to a few members of the group. However, such comparisons as these limited data permit do reveal some striking similarities between our present results for HVS and published accounts of the number, size, relative molarities and phosphorylation status of the DNA-binding proteins of the Epstein–Barr virus (EBV). In contrast, such general comparisons do not show extensive parallels between the DNA-binding proteins of HSV and those of HVS. The major DNA-binding protein (ICP8) of HSV-1 is relatively conserved and has antigenic sites in common with similar proteins in cells infected with equine abortion virus, pseudorabies virus or with bovine mammillitis virus (Yeo et al., 1981) and probably with herpesvirus tamarinus, SA8 (Desrosiers & Falk, 1981) and the other members of the herpes simplex virus 'seron' and 'neutroseron' (Honess & Watson, 1977). However, the HSV protein is an early (β) non-structural protein with an essential role in virus DNA replication and has more properties in common with the 110K early protein of HVS than with the late 130K structural protein of HVS. We have not detected any antigenic sites on HVS polypeptides which are shared with proteins of HSV, and a monospecific polyclonal rabbit antibody to the HSV DNA-binding protein (see Yeo et al., 1981; serum provided by Dr K. L. Powell) did not react with HVS-infected cells in immune precipitation tests (unpublished results). The major DNA-binding protein of the virion of HSV-1 is a 36K to 40K early (β) phosphoprotein which appears to be processed after association with 'full' capsids and acts as a major acceptor in in vitro protein kinase reactions with virions of HSV (Gibson & Roizman, 1972, 1974; Lemaster & Roizman, 1980; Preston et al., 1983; Wong Kai In & R. W. Honess, unpublished observations). The 29K protein of HVS is also associated with capsids under conditions of low ionic strength and is processed from a 31K PAA-resistant precursor in the nucleus of infected cells (Randall et al., 1983; R. W. Honess et al., unpublished results). However, the 29K protein is a minor DNA-binding species and it is not efficiently phosphorylated in vivo or in vitro. The major DNA-binding protein of the HVS virus particle is the PAA-sensitive 12K protein and it has no obvious direct counterpart in the virion of HSV-1. The major non-structural DNA-binding protein of HVS (the 51K phosphoprotein) likewise cannot readily be compared with an HSV-1-specified protein. The EBV-associated 145K and 18K 'late' DNA-binding proteins and the 'early' 120K and 50K to 48K DNA-binding proteins (Sugawara et al., 1982; Kawanishi et al., 1981 a, b; Mueller-Lantzsch et al., 1979) resemble the 130K, 12K and the 110K and 51K to 48K proteins of HVS in relative molarity as well as their relative rates of synthesis in the presence and absence of inhibitors of DNA synthesis. The 152K protein of Roubal et al. (1981) and their 134K and the 55K and 51K EBV-specific DNA-binding proteins bear a similar resemblance to the HVS 130K, 110K and 51K to 48K proteins. These latter authors suggest that 55K and 51K proteins are related as precursor and product and others have shown that EBV-specific polypeptides of similar mol. wt. are phosphorylated in vivo (Feighny et al., 1980; Mueller-Lantzsch & Yamamoto, 1981). We have referred elsewhere to the general resemblance between HVS-specified gene products and those of the EBV (Randall et al., 1983); the present results provide more specific support for this conclusion. It will be of considerable interest to determine if these gross similarities between gene products of the lymphotropic herpesviruses of Old and New World primates are supported by more precise comparisons of gene structure and regulation.

We thank Mr B. Precious and Dr W. C. Russell for gifts of the 5'-32P-end-labelled brominated poly(dG–dC)-poly(dG–dC) probe and purified virions of adenovirus type 5 and for the benefit of their experience in the use of the probe in the detection of DNA-binding activities of polypeptides 'electroblotted' onto nitrocellulose. Dr K. L. Powell kindly provided a rabbit monospecific polyclonal antibody to the major DNA-binding protein of herpes simplex virus. Capable assistance with tissue culture was provided by Mr D. Young and Mrs C. Newman and we gladly acknowledge the secretarial skills of Mrs V. Rogers. E.D.B. was supported by an MRC Studentship for Training in Research Methods.
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HVS DNA-binding proteins


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