Evidence that the Major Delayed-Early DNA-binding Proteins of Herpesvirus Saimiri Are Bound to DNA in vivo

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SUMMARY

Associations of herpesvirus saimiri-specified proteins with nuclear fractions from cultures of infected cells were probed by nuclease digestion, detergent extractions and immunofluorescence microscopy using monoclonal antibodies to virus polypeptides. Nuclease digestion selectively released delayed-early polypeptides with apparent mol. wt. of 110000 (110K) and 51000 (51K) from nuclei of infected cultures and the majority of each of these polypeptides partitioned with the insoluble fraction after detergent extraction of such nuclei. However, the nuclease-mediated release of both these proteins was specifically reduced when nuclei were isolated from cultures in which virus DNA synthesis had been inhibited with phosphonoacetic acid (PAA). In addition, the 110K polypeptide partitioned into the soluble fraction when nuclei from PAA-treated cultures were extracted with detergent. Immunofluorescence microscopy revealed characteristic and distinctive subnuclear localizations of the 110K and 51K polypeptides in control cultures and these patterns of subnuclear accumulations were markedly altered in cultures treated with PAA. We conclude that the DNA-binding properties of the delayed-early 110K and 51K proteins of herpesvirus saimiri previously observed in vitro are likely to reflect their functions as DNA-binding proteins in vivo.

We have recently identified and described some properties of more than 30 virus-specific polypeptides in cells productively infected with herpesvirus saimiri (HVS). The synthesis of an early phosphoprotein with an apparent mol. wt. of 52000 (52K) is followed by the synthesis of delayed-early (DE, e.g. 110K, 76K, 51K, 29K) and late (e.g. 160K, 150K, 130K, 38K, 12K) proteins (Randall et al., 1983, 1984a; O'Hare & Honess, 1983a). An essential, phosphonoacetic acid (PAA)-sensitive, DNA polymerase is a member of the DE class and, in the presence of concentrations of PAA sufficient to inhibit virus DNA synthesis, there is a selective reduction in the expression of late gene products (O'Hare & Honess, 1983a, b; Blair & Honess, 1983). As part of the functional characterization of these virus gene products we have shown that a subset of virus proteins, including DE 51K and DE 110K proteins, displayed DNA-binding properties in vitro (Blair & Honess, 1983). Although proteins with stoichiometric DNA-binding functions in vitro normally display DNA-binding properties in vitro, a non-specific affinity for polyanions in vitro may arise as a consequence of charge characteristics of a protein which need not be related

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to a DNA-binding function \textit{in vivo}. For example, studies of the topology of the virion proteins of HVS have shown that the basic 12K polypeptide, which binds to DNA \textit{in vitro} tests (Blair & Honess, 1983), is located outside the nucleocapsid in the virion tegument and is therefore unlikely to have a DNA-binding function in the virus particle (R. Gopal & R. W. Honess, unpublished observations). We therefore wished to obtain additional evidence for the \textit{in vivo} significance of the \textit{in vitro} DNA-binding activities of a subset of HVS polypeptides. Correlations between the phenotypic consequences of conditionally lethal mutations within the structural genes for DNA-binding proteins and the properties of the mutant proteins have provided this evidence for a number of prokaryotic and eukaryotic DNA-binding functions, including the major DNA-binding protein of herpes simplex virus (Littler \textit{et al.}, 1983; Lee & Knipe, 1983; Spang \textit{et al.}, 1983; Weller \textit{et al.}, 1983). However, mutations which prevent the normal functions of a DNA-binding protein \textit{in vivo} need not affect its capacity to bind to DNA \textit{per se}, but may interfere with its associations with other proteins, its transport to the nucleus or its intracellular stability (Kalderon \textit{et al.}, 1984; Vaughan \textit{et al.}, 1984, 1985). The selective release of DNA-binding proteins by nuclease treatment of nuclear fractions from infected cells and the partitioning of DNA-binding proteins during subnuclear fractionations have provided useful data on the functions of herpesvirus DNA-binding proteins (Fenwick \textit{et al.}, 1978; Knipe & Spang, 1982; Quinlan \textit{et al.}, 1984). In this paper, we use these methods and monoclonal antibodies to the major DE DNA-binding proteins of HVS (51K and 110K) to show that these proteins probably have a DNA-binding function within infected cells.

In the first series of experiments, we analysed the virus-specific polypeptides released from nuclei of infected cells by digestion with micrococcal nuclease. Cultures of owl monkey kidney cells (OMK-210 cells) were infected with 5 p.f.u./cell of HVS-1\textsubscript{Onc} and labelled from 12 to 24 h post-infection with 5 ~μCi \textit{z}-[35S]methionine (500 Ci/mmol; Amersham) per ml of medium. At 24 h, cultures were harvested, washed with phosphate-buffered saline, resuspended in ice-cold 10 mM-Tris–HCl pH 7-8, 10 mM-NaCl, 1-5 mM-MgCl\textsubscript{2} (RSB) and disrupted by Dounce homogenization. Nuclear and cytoplasmic fractions were separated by low-speed sedimentation (2000 r.p.m. for 10 min) and the nuclear fractions washed twice by resuspension and sedimentation from RSB containing 1 mM-CaCl\textsubscript{2} and 10~\% sucrose (RSBC) before being resuspended in RSBC at a concentration of 10\textsuperscript{7} to 2 × 10\textsuperscript{7} nuclei/ml. Samples (0-05 to 0-1 ml) were digested with micrococcal nuclease (times and concentrations indicated in Fig. 1) and, after terminating the digestions by addition of 2 mM-EGTA, the soluble or released fractions were separated from the residual nuclear fractions by low-speed sedimentation (2000 r.p.m. for 10 min at 4 °C). Samples of each fraction (equivalent to 10\textsuperscript{6} to 2 × 10\textsuperscript{6} nuclei) were made to 0.5~ sodium deoxycholate and 1 ~ Triton X-100, incubated for...
Fig. 1. Autoradiograms of labelled polypeptides separated by SDS–PAGE from the soluble (sol) and insoluble (pel) fractions produced by micrococcal nuclease digestion of nuclei from HVS-infected cells. Isolated nuclear fractions were digested with (a) 0 to 60 units of micrococcal nuclease per ml for 5 min at 37 °C or (b) with 5 units of the enzyme per ml for 15 to 600 s at 37 °C. Samples of the labelled polypeptides remaining with the nuclear pellets (pel) or released into the soluble fractions (sol) were denatured and separated by electrophoresis through 12% SDS–polyacrylamide gels. Selected host (host 1, host 2, actin) and virus-specific (apparent mol. wt. × 10^{-3}, 28 to 160) polypeptides are annotated as previously described in detail (Randall et al., 1983, 1984a; Blair & Honess, 1984). A sample of the total infected cell lysate (Iv) was separated in the leftmost lane of the gel illustrated in (a).

5 min at 4 °C, and then separated into soluble and insoluble fractions by sedimentation at 2000 r.p.m. for 10 min. Similar samples of labelled nuclei were digested with 40 units/ml DNase I at 20 °C for 20 min, the digestions were terminated by the addition of excess EDTA (5 mM) and the samples were separated into DNase soluble and insoluble fractions by low-speed sedimentation (as above). Aliquots (5 × 10^5 cell equivalents of each fraction) of the total cell suspensions, cytoplasmic fractions and subnuclear fractions were separated on 12% polyacrylamide slab gels. An autoradiogram of the labelled polypeptides is shown in Fig. 2. Detergent and nuclease treatments each revealed differences in the state of the major virus-specified DNA-binding proteins in nuclei from infected cells treated with PAA relative to nuclei from untreated infected cultures (Fig. 2). Densitometry of these autoradiograms and autoradiograms from similar
Fig. 2. Autoradiograms of labelled polypeptides separated by SDS-PAGE from total cell lysates (total cell), cytoplasmic fractions (cyto) and soluble (sol) and insoluble (ins) fractions produced by nuclease (DNase sol, ins) or detergent (DOC sol, ins) treatment of nuclear fractions from uninfected OMK cell cultures (c) and OMK cell cultures infected with HVS and incubated in the presence (a) or absence (b) of PAA. Selected host and virus-specific polypeptides are annotated as for Fig. 1.
Fig. 3. Immunofluorescence microscopy of the reactions of a monoclonal antibody to the 110K protein (a) and an antibody to the 51K protein (b) with nuclei of cells from untreated infected cultures (−PAA) and infected cultures treated with PAA (+PAA) and fixed at 18 or 36 h after infection. The monoclonal antibodies DI (51K) and DC (110K), the methods employed for their use in indirect immunofluorescence and their reactions with untreated infected cultures have been described in detail elsewhere (Randall et al., 1984a, 1985).

experiments showed that the majority (> 70%) of the nuclear content of both the 110K and 51K proteins from control infected cultures partitioned with the detergent-insoluble nuclear fraction, whereas in samples from PAA-treated cultures only 13% of the nuclear content of the 110K protein remained insoluble and 60% of the 51K protein remained insoluble. Nuclease treatment released 40 to 50% of the 110K protein and 25 to 40% of the 51K protein from nuclei of control cultures compared to 10 to 20% of each of these proteins from nuclei of cultures treated with PAA (Fig. 2).

We recently reported the isolation of a panel of monoclonal antibodies to HVS-specified polypeptides, including antibodies to the 51K and 110K DNA-binding proteins [e.g. antibodies DI (51K) and DC (110K); Randall et al., 1984a, b]. Previous immunofluorescence studies with these antibodies also revealed characteristic and distinctive patterns of subnuclear accumulations of the 51K and 110K DNA-binding proteins in cells of untreated infected cultures (Randall et al., 1984a, b). Fig. 3 illustrates a comparison of the nuclear staining patterns
observed at 18 h or 36 h in a control culture and in cultures treated with PAA (200 μg/ml of medium) using the anti-51K and the anti-110K monoclonal antibodies in indirect immunofluorescence reactions against fixed and permeabilized monolayers (Randall et al., 1984a, 1985). In control cultures of infected cells the relatively uniform nuclear distribution of the 110K pattern observed at 18 h changed to give fine, punctate, accumulations by 36 h. Localized accumulations of the 110K protein were observed earlier in PAA-treated cultures and by 36 h there were fewer, larger accumulations than were observed in untreated cultures (Fig. 3a). In control cultures, the 51K protein accumulated in very large subnuclear aggregations by 36 h whereas in the presence of PAA the 51K protein remained evenly dispersed throughout the volume of the nucleus. The uniform distribution of the very early 52K protein (Randall et al., 1984b) was not altered in PAA-treated cultures and these distributions did not reflect accumulations of total cellular DNA as monitored by double-fluorescence with the DNA-binding fluorochrome, 4',6-diamidino-2-phenylindole (not shown, see Randall et al., 1985).

We conclude that the preferential nuclease sensitivity of the 110K and 51K proteins, the evidence that this sensitivity is altered by an inhibitor of virus DNA synthesis and that the intranuclear localization of each of these proteins is altered by preventing virus DNA synthesis are all consistent with these DE polypeptides serving as DNA-binding proteins in vitro as well as in vivo.

REFERENCES


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