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THE SYNTHESIS OF THE MAJOR DNA-BINDING PROTEIN (ICP8) IN CELLS INFECTED WITH THE STRAIN HSZP OR KOS OF HERPES SIMPLEX VIRUS TYPE 1

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Summary. - Synthesis of the major DNA-binding protein (ICP8) was investigated in primary rabbit kidney (RK) and Vero cells infected with the syncytial (syn) strain HSZP or with the non-syn strain KOS of herpes simplex virus type 1 (HSV-1). Results showed the following: 1. In contrast to strain KOS, the rate of viral polypeptide synthesis was accelerated in Vero cells infected with strain HSZP. The ICP8 could be detected in the nuclei of cells by one hour post-infection (hr p. i.) where it became associated with the viral DNA (DNase sensitive form). Later on (7 hr p. i.), the synthesis of viral polypeptides decreased and no further translocation of ICP8 from the cytoplasm into the nucleus was observed. 2. Strain HSZP was approx. three times more resistant to the action of phosphonoacetic acid (PAA) than strain KOS. In order to block the synthesis of HSZP gamma-2 polypeptides, a concentration of 600 μg PAA/ml had to be used. Under this condition, the HSZP ICP8 was translocated into the cell nucleus at later interval only (7 hr p. i.), and it was still possible to release this polypeptide from the nucleus by DNase treatment. The failure of the HSZP ICP8 to associate with the nuclear matrix (DNase resistant form) of infected cells in the absence of viral DNA replication may reflect its predominant affinity for the viral DNA which, in turn, may be responsible for the observed accelerated synthesis of the HSZP polypeptides in infected Vero cells. 3. In primary RK cells infected with strain HSZP the ICP8 did not translocate into the cell nucleus. Therefore, no gamma-2 polypeptides were synthesized.

Key words: herpes simplex virus type 1; major DNA-binding protein (ICP8); localization within the cell; phosphonoacetic acid

Introduction

Mammalian cells permisive to herpes simplex virus (HSV) infection express approx. 50 virus induced cell polypeptides (ICPs). The ICPs can be categorized

as alpha, beta, and gamma whose synthesis is coordinately regulated and sequentially ordered in a cascade fashion (Honess and Roizman, 1974; 1975). Immediately after infection alpha polypeptides are made, initiating the synthesis of beta polypeptides. The beta polypeptides, in turn, suppress the continued production of alpha polypeptides and initiate the synthesis of structural gamma polypeptides. These have been divided into two groups, gamma-1 and gamma-2. Transcription of gamma-1 genes occurs at low levels in the absence of viral DNA synthesis but is maximal when progeny viral DNA is synthesized. In contrast, the accumulation of gamma-2 transcripts strictly depends upon viral DNA synthesis (Holland *et al.*, 1980).

The beta polypeptides are synthesized during the peak period of viral DNA synthesis and include the major DNA binding protein. The gene for this protein which was designated ICP8 (relative molecular weight 129 000), has been mapped to approx. 0.40 map units on the HSV genome (Conley *et al.*, 1981; Marsden *et al.*, 1978; Holland *et al.*, 1984). Functional ICP8 is required for DNA replication and affects the level of viral gene expression (Conley *et al.*, 1981; Godowski and Knipe, 1983; Weller *et al.*, 1983). During infection, ICP8 is targeted to two different locations in the nucleus of infected cell as a part of its maturation pathway (Guinlan *et al.*, 1984). ICP8 initially associates with the nuclear matrix and remains there in the absence of viral DNA replication. As viral DNA replication proceeds, ICP8 binds to replicating DNA molecules. Inhibition of DNA replication causes its movement back to the nuclear matrix.

As described (Raučina *et al.*, 1984; Matis and Szántó, 1985; Matis and Krivjanská, 1988), the alpha-mRNA molecules of the HSZP strain of HSV-1 exhibited a pronounced functional stability. The strain is defective with regard to the early shutoff of host protein synthesis and in the HSZP infected cells a truncated glycoprotein C is synthesized. In this communication we have examined the synthesis as well as the localization of the ICP8 in cells infected with the HSZP strain of HSV-1.

Materials and Methods

Cell cultures. Primary rabbit kidney cell cultures (RK) were propagated from 14 to 30-day-old chinchilla or albino rabbits (conventional breed Valez) as described previously (Matis and Szántó, 1985). Rabbit lung (ZP) cells (Szántó, 1960; Szántó *et al.*, 1972) were propagated in Earle's solution with 0.5 % lactalbumin hydrolyzate, 0.1 % yestolate Difco, and 5 % inactivated calf serum (ICS) supplemented with antibiotics. African green monkey kidney (Vero) cells were propagated in Eagle's basal medium (BEM) supplemented with 10 % ICS.

Viruses. HSV-1 strain HSZP (syn strain) propagated in ZP cell line and the prototype strain KOS (a non-syn strain passaged in Vero cells) were used throughout.

Metabolic inhibitors and radioisotope. Cycloheximide (Actidione, Fluka AG. Switzerland) was used at a final concentration of 150 μg/ml of medium and Actinomycin D (Act D, BDH, Chemical Ltd., England) at 2 μg/ml of medium. Phosphonoacetic acid (PAA, Sigma, U.S.A.), inhibitor of viral DNA polymerase, was used at various concentrations (see legend to Figures). Radioisotope ^{35}S-methionine (29.1 GBq/mol) was administered to the cell culture in methionine free medium supplemented with 1 % ICS.

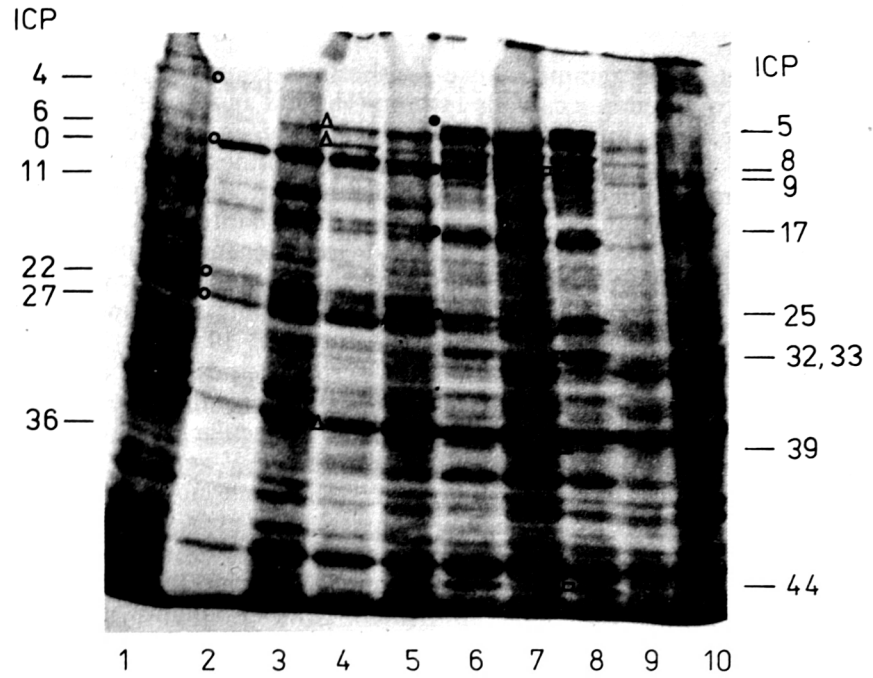

Fig. 1
Expression of the KOS genome of HSV-1 in ZP cells
Cells were infected in the absence (lanes 2,4,6, and 8) or presence (lanes 3,5,7, and 9) of PAA. Cycloheximide was added to the cells 0 (lanes 2 and 3), 1.5 (lanes 4 and 5), 3 (lanes 6 and 7), and 5 hr (lanes 8 and 9) p. i. After its removal, the cells were labelled for 2 hr (0.4 MBq/ml) in the presence of Act D and subjected to electrophoresis. Mock-infected cells incubated in the presence (lane 1) or absence (lane 10) of PAA. ICPs of the alpha (o), beta (△), gamma-1 (●), and gamma-2 (□) kinetic groups are marked.

Fractionation of cells. The nuclear and cytoplasmic fractions of the control and infected Vero cells were prepared according to Brown *et al.* (1983) using 0.1 ml of hypotonic buffer for lysing the cells from one Müller flask (approx. 1 x 10^6 cells).

DNase treatment of nuclei. The procedure of Knipe and Spang (1982) was used. The concentration of DNase I (Calbiochem, U.S.A.) in the incubation mixture was 20 μg/ml.

Infection and labelling of cells. Cell monolayers in Müller flasks were exposed to 20 PFU of virus per cell in 2.0 ml of medium. At various times post infection (p. i.) the cells were rinsed with prewarmed phosphate-buffered saline (PBS) and radioactively labelled. For long-term labelling 2 ml of methionine free medium supplemented with 1 % ICS and containing 0.2 MBq of ^{35}S--methionine was added for indicated intervals. For pulse labelling 1.0 MBq of ^{35}S-methionine was used for 30 - 60 min. Chase conditions were established by adding unlabelled methionine to a concentration of 1 x 10^{-3} mol.dm^3.

Polyacrylamide gel electrophoresis. At the end of the long-term pulse or pulse-chase labelling experiments, the cells were scraped off from the surface of the flask and either directly or after preparation of nuclear and cytoplasmic extracts solubilized for polyacrylamide gel electrophoresis (Matis and Rajčáni, 1980). After electrophoresis the gels were fixed, treated with 1 mol/l sodium salicylate to provide fluorographic enhancement and analysed by contact autoradiography using Medix Rapid X-ray film (Hradec Králové, Czechoslovakia).

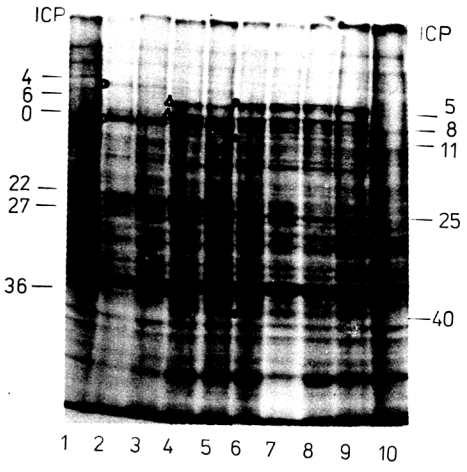

Fig. 2
Expression of the HSZP genome of HSV-1 in RK cells
Cells were infected in the absence (lanes 2,4,6, and 8) or presence (lanes 3,5,7, and 9) of PAA. Cycloheximide was added to the cells 0 (lanes 2 and 3), 1.5 (lanes 4 and 5), 3 (lanes 6 and 7), and 5 hr (lanes 8 and 9) p. i. After its removal, the cells were labelled for 2 hr (0.4 MBq/ml) in the presence of Act D and subjected to electrophoresis. Mock-infected cells incubated in the presence (lane 1) or absence (lane 10) of PAA. ICPs of the alpha (o), beta (△), gamma-1 (●), and gamma-2 (□) kinetic groups are marked.

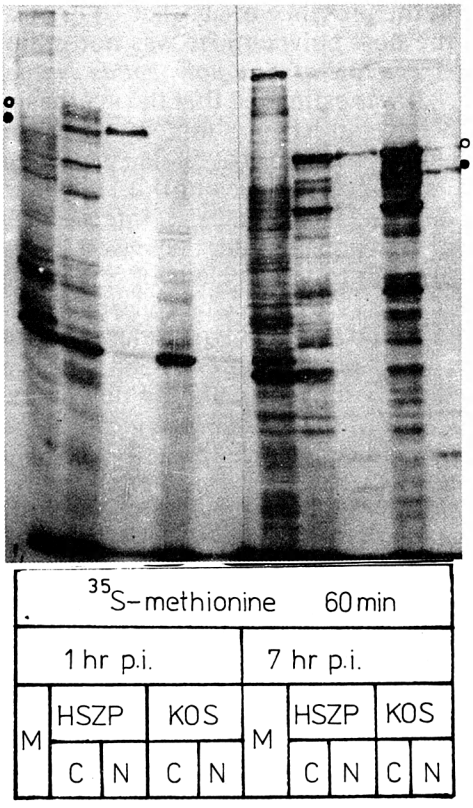

Fig. 3
Polypeptides synthesized in HSZP or KOS infected Vero cells
Mock-infected cells (M); cytoplasmic fraction (C); nuclear fraction (N); ICP5 (○); ICP8 (●).

Results

The effect of phosphonoacetic acid (PAA) on the synthesis of viral polypeptides in HSV-1 infected RK and ZP cells

The cells were infected in the presence or absence of PAA and at various times post infection (p. i.) cycloheximide was added. After its removal, the cells were radioactively labelled in the presence of Act D and subjected to electrophoresis. Such a procedure enables to analyse the electrophoretic profile of polypeptides reflecting the coding capacity of the mRNA molecules transcribed in the cells up to the removal of cycloheximide.

When cycloheximide was added to a permissive virus-cell system (strain KOS - ZP cells) in the times of viral DNA replication (>3 hr p. i.), the greatest differences in the electrophoretic profiles of polypeptides synthesized in the presence or absence of PAA were observed (Fig. 1). In the presence of PAA (non-replicating virus) reduced amounts of beta and gamma-1 polypeptides were synthesized, whereas the productive of gamma-2 polypeptides were practically inhibited. On the other hand, in the RK cells infected with the HSZP strain no differences in the electrophoretic profiles of polypeptides synthesized in the presence or absence of PAA were observed. Moreover, the synthesis of the host polypeptides was not inhibited (Fig. 2).

The rate of viral polypeptide synthesis in the HSZP or KOS infected Vero cells

It was estimated that the synthesis of polypeptides of the HSZP strain in ZP, Vero, and REF (rabbit embryo fibroblasts) cells was accelerated. We studied this property in some detail using Vero cells. As shown in Fig. 3, already by one hr p.i. polypeptides of the HSZP strain were synthesized and ICP8 could be detected in the nuclei of infected Vero cells. Later on (7 hr p. i.), the synthesis of viral polypeptides decreased and no further translocation of ICP8 from cytoplasm into the nucleus of infected cell was observed.

In the KOS infected cells, one hr p. i. no production of viral polypeptides was detected. Viral polypeptides in substantial amounts were synthesized at 7 hr p. i., when transport of ICP8 as well as of ICP5 (major capsid polypeptide) into the cell nucleus could be demonstrated.

The effect of PAA on the localization of ICP8 within the HSZP infected Vero cells

Vero cells were infected with the HSZP strain of HSV-1 in the presence or absence of PAA. Pulse-chase experiments were done at 3 hr p. i. and the labelled polypeptides present in the cytoplasmic as well as nuclear fractions (treated or untreated with DNase) or infected cells were analysed by electrophoresis. The results are shown in Fig. 4. It can be seen that both viral polypeptides (ICP5 and ICP8) which had been synthesized at 3 hr p. i. (pulse) were translocated during the chase period (2 hr) from the cytoplasm into the nucleus of the infected cell. Under the same conditions, but in the presence of PAA, no translocation of both viral polypeptides from the cytoplasm into the cell nucleus was observed. However, small amounts of ICP8 were translocated into the cell nucleus at 7 hr p. i., in a from that DNase treatment was capable of releasing it from the nuclei (Fig. 5).

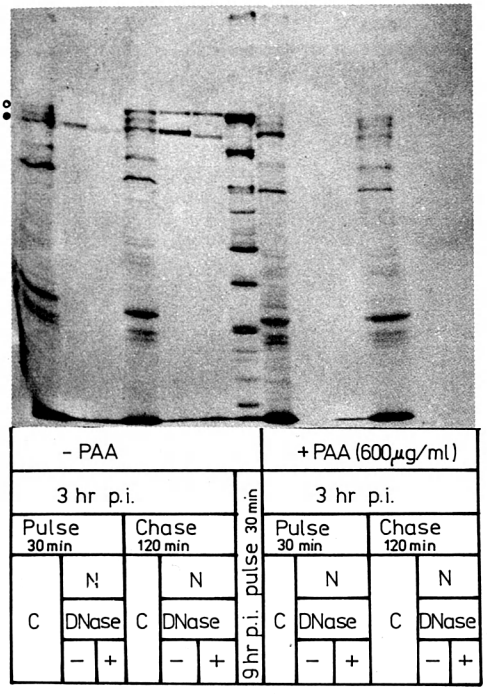

Fig. 4
Effect of PAA on the localization of ICP8 within the HSZP infected Vero cells
For further explanations see Fig. 3.

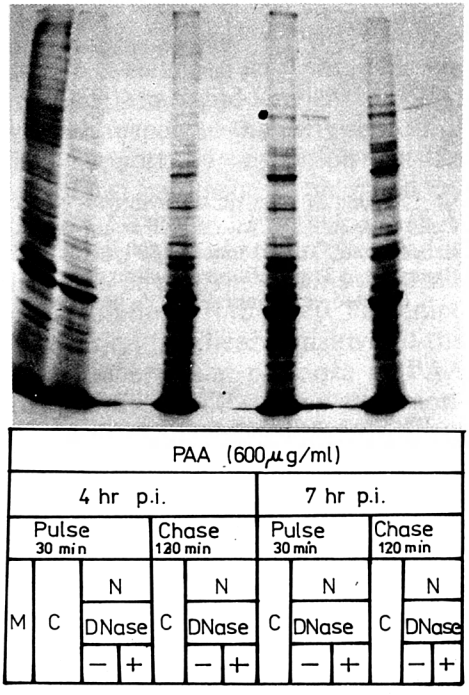

Fig. 5
Localization of ICP8 within the Vero cells infected with the HSZP strain of HSV-1 in the presence of PAA
For further explanations see Fig. 3.

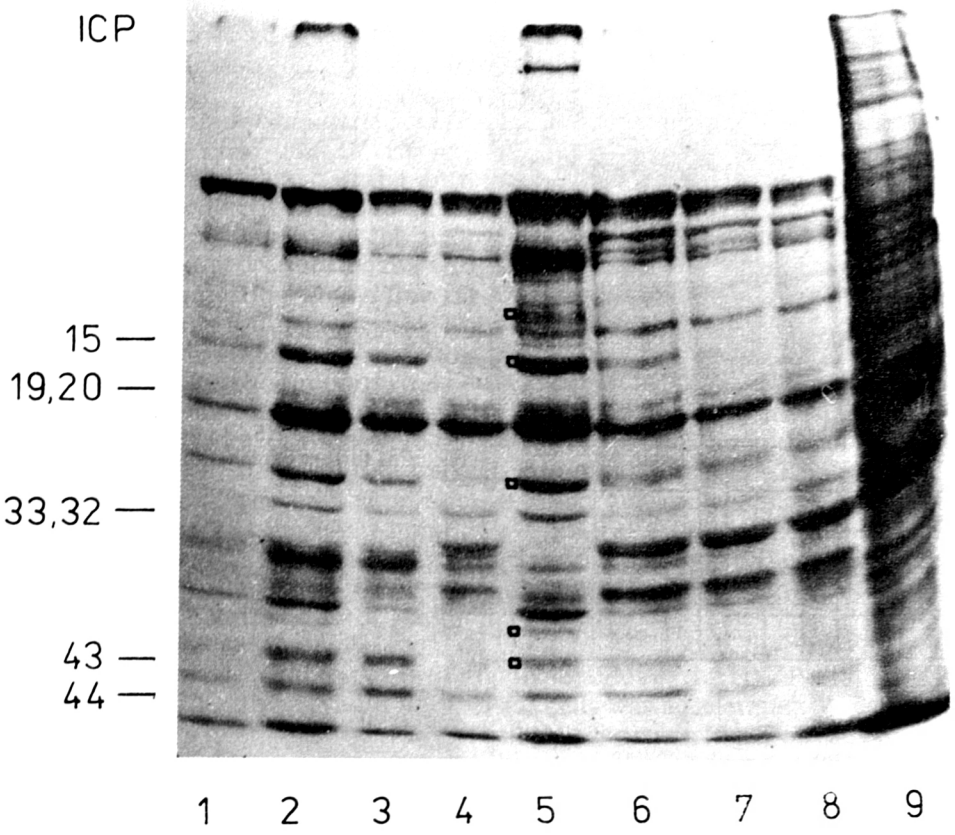

Fig. 6
Effect of PAA on the synthesis of the HSZP and KOS polypeptides in Vero cells
Cells were infected with HSZP strain (lanes 1 to 4) or KOS strain (lanes 5 to 8) in the absence (lanes 1 and 5) or presence of 200 μg (lanes 2 and 6), 400 μg (lanes 3 and 7), and 600 μg PAA/ml (lanes 4 and 8) and after incubation period of 11 hr labelled for 7 hr (0.1 MBq/ml). Mock-infected labelled cells (lane 9). ICPs belonging to the gamma-2 kinetic group are marked (□).

Discussion

The present data demonstrated that the beta polypeptides of the HSZP strain of HSV-1 synthesized in RK cells are functionally impaired. As shown in Fig. 2, they are not capable of switching on the expression of gamma-2 polypeptides. In comparison with the ZP cells, in RK cells no differences in the electrophoretic profiles of the viral polypeptides synthesized in the presence or absence of PAA were detected. These findings may indicate that the genome of the HSZP strain does not replicate in RK cells as the consequence of the inability of ICP8 to translocate into the nuclei (data not shown). Furthermore, in spite of the synthesis of the viral polypeptides belonging to the alpha, beta, and gamma-1

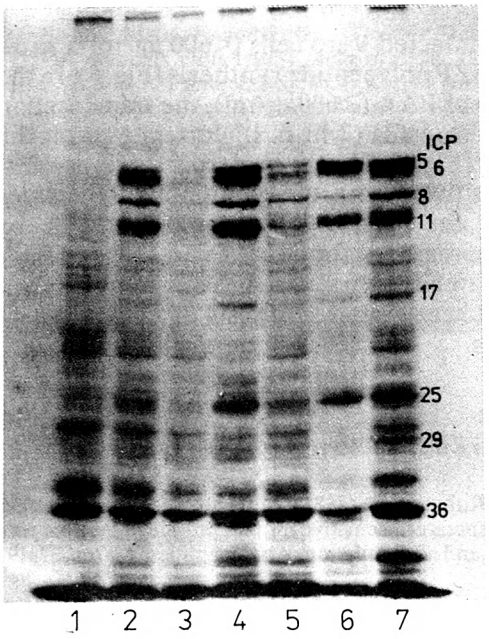

Fig. 7
Polypeptides synthesized in HSZP or KOS infected Vero cells in the presence of PAA
Cells were infected with HSZP strain (lanes 2,4, and 6) or KOS strain (lanes 3,5, and 7) in the presence of PAA (600 μg/ml) and labelled after incubation for 1 hr. Lanes 2 and 3 incubation for 4 hr; lanes 4 and 5 incubation for 7 hr; lanes 6 and 7 incubation for 11 hr. Mock-infected labelled cells (lane 1). ICPs belonging to the beta and gamma-1 kinetic groups are numbered on the right.

kinetic groups, the host protein synthesis in RK cells was not inhibited. We have shown previously (Matis and Szántó, 1985; Matis and Krivjanská, 1988) that the HSZP strain of HSV-1 is defective with respect to the early shutoff of host protein synthesis. Thus, these findings support the suggestion that the secondary („delayed") shutoff of host protein synthesis is mediated by a gamma function(s) (Read and Frenkel, 1983; Strom and Frenkel, 1987).

The infection of other cells with the HSZP strain leads to an accelerated synthesis of viral polypeptides (Fig. 3). The pulse-chase experiments done at 3 hr p. i. revealed that only one of the two conformational forms of ICP8 (Knipe *et al.*, 1982) accumulating during the chase period had been sensitive to the action of DNase (Fig. 4). The experiments done in the presence of PAA revealed that the nuclear association of ICP8 of the HSZP strain was demonstrated only at later times p. i. and that this polypeptide could be released from the nuclei of infected cells with DNase treatment. These results are in contradiction to the findings of other authors who showed that in the presence of PAA the rate of the nuclear association of ICP8 increased and the DNase treatment did not cause its removal from the nuclei (Knipe and Spang, 1982). The observed pronounced resistance of the HSZP strain to the action of PAA may be responsible for this discrepancy. In comparison with the KOS strain of HSV-1, approx. three times higher concentrations of PAA must be used for the inhibition of the HSZP gamma-2 polypeptide synthesis. The presence of

suboptimal concentrations of PAA in infected Vero cells (<600 μg/ml) causes an approx. 10 hr prolongation of the HSZP polypeptide synthesis (Fig. 6). In the presence of inhibitory concentrations of PAA (≧600μg/ml), the translocation of ICP8 into the nuclei of infected Vero cells (7 hr p. i.) correlates with the highest synthesis of viral polypeptides (Fig. 7). However, despite of its association with the viral DNA (DNase sensitive form), the gamma-2 polypeptides were not synthesized. This failure of the HSZP ICP8 to associate with the nuclear matrix of infected cells (DNase resistant form) in the presence of PAA may indicate its predominant affinity for the viral DNA. Such a property of ICP8 might account for the observed accelerated synthesis of viral polypeptides in the HSZP infected Vero cells.

References

Conley, A. J., Knipe, D. M., Jones, P. C., and Roizman, B. (1981): Molecular genetics of herpes simplex virus. VII. Characterization of a temperature-sensitive mutant produced by an *in vitro* mutagenesis and defective in DNA synthesis and accumulation of polypeptides. *J. Virol.* **37**, 191 - 206.

Godowski, P. J., and Knipe, D. M. (1983): Mutations in the major DNA-binding protein gene of herpes simplex virus type 1 results in increased levels of viral gene expression. *J. Virol.* **47**, 478 - 486.

Holland, L. E., Anderson, K. P., Shipman, Jr. C., and Wagner, E. K. (1980): Viral DNA synthesis is required for the efficient expression of specific herpes simplex virus type 1 mRNA species. *Virology* **101**, 10 - 24.

Holland, L. E., Sandri-Goldin, R. M., Goldin, A. L., Glorioso, J. C., and Levine, M. (1984): Transcriptional and genetic analysis of the herpes simplex virus type 1 genome: coordinates 0.29 to 0.45 *J. Virol.* **49**, 947 - 959.

Honess, R. W., and Roizman, B. (1974): Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. *J. Virol.* **14**, 8 - 19.

Honess, R. W., and Roizman, B. (1975): Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral polypeptides. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1276 - 1280.

Knipe, D. M., and Spang, A. E. (1982): Definition of a series of stages in the association of two herpesviral proteins with the cell nucleus. *J. Virol.* **43**, 314 - 324.

Knipe, D. M., Quinlan, M. P., and Spang, A. E. (1982): Characterization of two conformational forms of the major DNA-binding protein encoded by herpes simplex virus 1. *J. Virol* **44**, 736 - 741.

Marsden, H. S., Stow, N. D., Preston, V. G., Timbury, M. C., and Wilkie, N. M. (1978): Physical mapping of herpes simplex virus-induced polypeptides. *J. Virol.* **28**, 624 - 642.

Matis, J., and Rajčáni, J. (1980): Preparation of immune serum to immediate early and early polypeptides specified by herpes simplex virus type 1. *Acta virol.* **24**, 105 - 113.

Matis, J., and Szántó, J. (1985): Host cell and virus strain differences in synthesis of immediate early polypeptides in HSV-1 infected cells. *Acta virol.* **29**, 353 - 361.

Matis, J., and Krivjanská, M. (1988): Herpes simplex virus type (HSV-1) HSZP interferes also heat inactivated with early shutoff of host protein synthesis induced by HSV-1 KOS. *Acta virol.* **32**, 379 - 385.

Quinlan, M. P., Chen, L. B., and Knipe, D. M. (1984): The intranuclear location of a herpes simplex virus DNA-binding protein is determined by the status of viral DNA replication. *Cell* **36**, 857 - 868.

Raučina, J., Matis, J., and Leššo, J. (1984): Defective glycoprotein C of the syncytial strain of herpes simplex virus type 1. *Acta virol.* **28**, 457 - 463.

Read, G. S., and Frenkel, N. (1983): Herpes simplex virus mutants defective in the virion-associated shutoff of host polypeptide synthesis and exhibiting abnormal synthesis of (immediate early) viral polypeptides. *J. Virol.* **46**, 498 - 512.

Strom, T., and Frenkel, N. (1987): Effects of herpes simplex virus on mRNA stability. *J. Virol.* **61**, 2198 - 2207.

Szántó, J. (1960): Stable cell strains from rabbit and rat lung tissue suitable for the propagation of herpes simplex virus. *Acta virol.* **4**, 380 - 382.

Szántó, J., Kleibl, K., Vanková, M., and Rajčáni, J. (1972): Reproduction of freshly isolated and laboratory-maintained strains of human herpesvirus in cell cultures. *Acta virol.* **16**, 449 - 458.

Weller, S. K., Lee, K. J., Sabourin, D. J., and Schaffer, P. A. (1983): Genetic analysis of temperature-sensitive mutants which define the gene for the major herpes simplex virus type 1 DNA-binding proteins. *J. Virol.* **45**, 354 - 366.