*Acta virol.* **28**: 56—58, 1984

# PROTEIN SYNTHESIS IN PIG KIDNEY CELLS INFECTED WITH HERPES SIMPLEX VIRUS TYPE 1

J. MATIS, J. SZÁNTÓ

Institute of Virology, Slovak Academy of Sciences, 817 03 Bratislava, Czechoslovakia

*Received June 14, 1983*

*Summary:* — Two polypeptides of apparent mol. mass 87,000 and 35,000 were identified in pig kidney cells infected with herpes simplex virus type 1 (HSV-1) after reversing the cycloheximide block. The synthesis of the polypeptide 87,000 declined from 22 hr post infection (p.i.). Its production was prevented by actinomycin D added to the infected cells after removal of cycloheximide. Evidence is presented that the polypeptide 35,000 may be of the cellular origin.

*Key words: herpes simplex virus type 1; pig kidney cells; non-structural polypeptides*

## *Introduction*

The replication of HSV-1 in pig kidney (PK) cells is restricted. Using selected HSV-1 strains, persistent infection has been established at multiplicity ranging from 1—4 PFU per cell (Szántó *et al.*, 1980; Leššo *et al.*, 1983). Immunofluorescence staining detected viral antigen in some cells of the infected monolayer situated mainly in the vicinity of the nuclear membrane and in the paranuclear area of the cytoplasm. The ultrastructural studies showed minimal cytopathic changes, lack of budding and nucleocapsid envelopment and at low multiplicity of infection no virus particles were seen either (Čiampor and Szántó, 1982). The synthesis of proteins followed by electrophoresis in polyacrylamide gel (PAGE) in PK cells is described in the present paper.

In preliminary experiments, PK cells were infected with HSV-1 at a multiplicity of 5 PFU/cell, labelled for 1 hr ($^{14}$C-amino acids) at various times postinfections (p.i.) and analysed by electrophoresis. By comparison with mock-infected cells, no synthesis of the viral polypeptides up to 24 hr p.i. was detected (data not published). Therefore, our attention has been focused on the early events of the infection cycle. It has been well established that the polypeptides specified by HSV form at least three groups designated alpha, beta and gama according to the onset and cessation of their synthesis (Honess and Roizman, 1974). Since the alpha-polypeptides, the so called immediate early (IE) proteins, are assumed to play an important role in modu-

lating initial host-virus interactions that may determine the outcome of the infection, their study in non-permissive systems is of special interest. The IE polypeptides do not require preceding viral protein synthesis and are made in only small amounts shortly after infection. However, they are produced in larger amounts after removal of cycloheximide which had been added to the cells at the time of infection (Honess and Roizman, 1974; Roizman *et al.*, 1975). This approach has been utilized to follow the synthesis of early polypeptides in PK cells.

## *Materials and Methods*

*Virus.* The Kupka strain of HSV-1 (isolated by Dr. R. Benda, Prague) was used throughout Stock virus of high titre was prepared as described (Matis and Rajčáni, 1980).

*Cells.* PK cells were grown to confluency in Müller flasks in medium Epl (Michl, 1962) supplemented with 5% inactivated calf serum (ICS). The baby hamster kidney (BHK) cells were grown in Eagle's basal medium (BEM) supplemented with 5% ICS.

*Metabolic inhibitors and radioisotope.* Cycloheximide (Actidione, Fluka AG, Switzerland) was prepared fresh and used at a final concentration of 100 μg/ml of medium; 4 ml per flask. Actinomycin D (BDH Chemical Ltd, England) was used at a final concentration of 2 μg/ml of medium. Cycloheximide was removed from cell cultures by aspirating the inhibitor-containing solution and rinsing the cell layer three times (5 ml/rinse) with phosphate buffered saline (PBS). Radioisotope $^{14}$C-amino acid hydrolysate (specific activity 40 × $10^3$ MBq/g, Prague, Czechoslovakia) was added to the cell cultures in medium BEM/10 (ten times reduced amino acids except arginine) supplemented with 1% ICS.

*Infection and labelling of cells.* Confluent cell monolayers in Müller flasks (approximately 2 × $10^6$ cells per flask) were exposed to 100 PFU of virus per cell in 1.5 ml of cycloheximide-containing medium and incubated at 37 °C for 6 hr. For labelling, the inhibitor-containing medium was replaced with 2 ml of medium BEM/10 containing the radioisotope. At the end of the labelling period the cells were rinsed with ice-cold PBS and harvested immediately (pulse). Specific conditions of labelling varried with the experiment and are described in the legends to Figures.

*Polyacrylamide gel electrophoresis.* At the end of the pulse-labelling period, the cells were scraped off from surface of the flask, solubilized by heating in a boiling water bath for 3 min (Spear and Roizman, 1972) and subjected to slab electrophoresis (8% polyacrylamide with a stacking gel of 3%) as described previously (Matis and Rajčáni, 1980). After electrophoresis, the gels were dried and analysed by contact autoradiography using Medix Rapid X-ray film (Hradec Králové, Czechoslovakia).

## *Results and Discussion*

Four to six IE polypeptides are usually detected in the cells permissive for the herpesviruses. The strain Kupka in BHK cells is capable to induce the production of six IE polypeptides after reversing cycloheximide (Fig. 1), namely IE 175, 136, 110, 87, 68, and 63 (Preston *et al.*, 1978). The numbers indicate apparent mol. mass × $10^{-3}$. We have identified in the infected PK cells after removal of cycloheximide a polypeptide 87 not seen in the mock-infected cells (tracks 1 to 4 in the Fig. 2) furthermore, a polypeptide 35 which had not been found in the mock-infected cells at early times after removal of cycloheximide (Tracks 1 and 2 in the Figs 2 and 3). Therefore, an experiment described in the legend to Fig. 4 was made. It shows that the polypeptide 35 is detectable in PK cells incubated with the virus (tracks 2, 4, and 6) and, moreover, in the cells incubated for 26 hr or 48 hr in the

absence of the virus (tracks 3 and 5). This suggests its cellular origin supposing that it may represent a degradation product of the host cells due to the infection. The absence of polypeptide 87 in this gel supports the suggestion that it is induced by the virus. As shown in Fig. 3, the rate of synthesis of the polypeptide 87 begins to decline between 22 and 28 hr p.i. However, no synthesis of the polypeptide 87 was observed when actinomycin D was present in the infected cells after removal of cycloheximide (track 6 in the Fig. 2). This finding may reflect the functional lability of the transcript coding for the polypeptide 87. Further studies on the biochemical properties of the polypeptide 87 are in progress.

## References

Čiampor, F., and Szántó, J. (1982): Ultrastructural studies on the replication of herpes simplex virus in PK and XTC-2 cells. *Acta virol.* **26**, 67—72.

Honess, R. W., and Roizman, B. (1974): Regulation of herpesvirus macromolecular synthesis. 1. Cascade regulation of the synthesis of three groups of viral proteins. *J. Virol.* **14**, 8—19.

Leššo, J., Szántó, J., and Rajčáni, R. (1983): Restricted replication of herpes simplex virus type 1 in pig kidney cells. *Acta virol.* **27**, 138—146.

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

Michl, J. (1962): Metabolism of cells in tissue culture in vitro. II. Longterm cultivation of the cell strains and cells isolated directly from animals in a stationary culture. *Exp. Cell Res.* **26**, 129—135.

Preston, V. G., Davison, A. J., Marsden, H. S., Timbury, M. C., Subak-Sharpe, J. H., and Wilkie, N. M. (1978): Recombinants between herpes simplex virus types 1 and 2: analyses of genome structure and expression of immediate early polypeptides. *J. Virol.* **28**, 499—517.

Roizman, B., Kozak, M., Honess, R. W., and Hayward, G. (1975): Regulation of herpesvirus macromolecular synthesis: evidence for multilevel regulation of herpes simplex 1 RNA and protein synthesis. *Cold Spring Harb. Symp. quant. Biol.* **39**, 687—701.

Spear, P. G., and Roizman, B. (1972): Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirus. *J. Virol.* **9**, 143—159.

Szántó, J., Leššo, J., and Golais, F. (1980): New model cell systems (PK and XTC-2) for studying acute and persistent infections with herpes simplex and pseudorabies viruses. *Acta virol.* **24**, 244—252.

*Explanation of Figures* (Plates VII-X):

*Fig. 1.* Polypetides synthesized in BHK cells. Cells infected with 5 PFU/cell and labelled (0.2 MBq/ /ml) from 5 to 10 hr p.i. (track 1). Mock-infected (track 2) and infected (100 PFU/cell) cells incubated for 6 hr with cycloheximide and after washing pulse-labelled (0.4 MBq/ml) for 1 hr in the presence (track 3) or absence (track 4) of actinomycin D. Apparent mol. mass $\times 10^{-3}$ of polypeptides are shown on the left.

*Fig. 2.* Polypeptides synthesized in PK cells. Mock-infected (track 1, 3 and 5) and infected (tracks 2, 4, and 6) cells were treated with cycloheximide as described in Fig. 1 and pulse-labelled (0.4 MBq/ml) for 1 hr (tracks 1 and 2) and 13 hr (0.1 MBq/ml) in the absence (tracks 3 and 4) or presence (tracks 5 and 6) of actinomycin D.

*Fig. 3.* Polypeptides synthesized in PK cells. Mock-infected track 1, 3, and 5) and infected (tracks 2, 4, and 6) cells were treated with cycloheximide as described in Fig. 1 and pulse-labelled (0.4 MBq/ml) for 3 hr as follows: tracks 1 and 2 at 6 hr p.i., tracks 3 and 4 at 22 hr p.i., tracks 5 and 6 at 28 hr p.i. In the track 7, infected BHK cells labelled (0.1 MBq/ml) from 4 to 10 hr p.i. are shown.

*Fig. 4.* Effect of infection with 100 PFU/cell on the polypeptides in PK cells. Cells were labelled (0.4 MBq/ml) for a period of 3 hr and then incubated in maintenance medium for 20 hr (track 1), 26 hr (track 3) and 48 hr (track 5) in the absence or presence (tracks 2, 4 and 6) of the virus. Arrow indicates the position of a polypeptide of mol. mass 87,000 absent in the samples.