Acta virol 34 : 120— 131, 1990

DETECTION OF VIRAL DNA AND ACTIVATION OF LATENT HERPES SIMPLEX VIRUS IN THE RABBIT NEURAL TISSUE

M. KÚDELOVÁ, J. RAJČÁNI

Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Czechoslovakia

Received May 2, 1989

Summary. — Both trigeminal ganglia, brain stem, and cornea from rabbits with established latent herpes simplex virus type 1 (HSV-1) infection were examined by explantation and by spot blot hybridization using strain 17 *Kpn* I fragments *i*, *d*, and *h* and the DNA extracted from abovementioned tissues. Correlation between positive hybridization and reactivation of infectious virus in the cultured explants was documented by enhanced hybridization with the DNA extracts from explanted ganglion samples. In addition, we found positive hybridization in some noncultured ganglion and brain stem samples which did not yield infectious virus by explantation. Keeping in mind the pitfalls of false positive hybridization, the results may indicate during latency the presence in neural tissues of HSV DNA sequences which did not spontaneously reactivate in culture.

Key words: *herpes simplex virus*; *latent infection*; *DNA*; *trigeminal ganglion*; *spot blot hybridization*; *virus reactivation*

Introduction

Latency established in the mouse and rabbit trigeminal ganglia after peripheral administration of HSV-1 is a nonproductive infection since the ganglion is not infectious at the time of its removal but yields virus in culture (Baringer, 1975; Stevens, 1975; Klein, 1976). The silent HSV genome cannot be eradicated from the ganglion by treating the animals with drugs such as acyclovir (Field *et al.*, 1979), phosphonoacetic acid or phosphonoformate (Svennerholm *et al.*, 1981). Application of hybridization techniques in the investigation of the HSV latency has brought fruitful results. The latent HSV DNA was first demonstrated by reassociation kinetics hybridization using ^{125}I-labelled HSV DNA at a level of 0.11 ± 0.03 genome equivalents per cell (Puga *et al.*, 1978). Later on, using *in situ* hybridization it was shown that RNase treatment of sections reduced the extent and frequency of hybridization probably due to a limited transcription of mRNAs from the persisting genome (Tenser *et al.*, 1982; Galloway *et al.*, 1982). Recently the concept of limited transcription during HSV latency has been investigated in detail by combination of *in situ* hybridization and Northern

blot analysis (Rock *et al.*, 1987) and the latency-related transcripts were recognized. Although translated from the repetitive region flanking the L segment, the latency-associated mRNAs overlap only a part of the ICP0 gene and are antisense to the ICP0 mRNA (Stevens *et al.*, 1987; Spivack and Fraser, 1987); they represent a new subset of transcripts, termed latency-related. This confirms our previous finding (Rajčáni and Matis, 1981) that the ICP0 (110 kD) polypeptide cannot be detected by anticomplement fluorescence in the sections of rabbit ganglia during HSV latency. Southern blot analysis of the latent HSV DNA from mouse ganglia has shown that the *Bam*HI fragments P and S corresponding to the free ends of the genome (Rock and Fraser, 1985; Efstathion *et al.*, 1986) were missing, thus the latent HSV DNA is probably present in a circularized form. To demonstrate the presence of the HSV DNA in the ganglia and brain of mice in experimental latency (Fraser *et al.*, 1986) spot blot hybridization can be also used. Here we describe our experience with this simple technique of HSV-1 DNA detection when latent in the trigeminal ganglia, brain stem, and cornea of rabbits in comparison to the explantation results.

Materials and Methods

Virus and animals. Albino rabbits weighing 3000 g were coming from the breed Velaz. The animals housed under standard conditions were inoculated into the right scarified cornea with $1-2\times10^6$ PFU of the strains Kupka or KOS, respectively (Rajčáni, *et al.*, 1977). After 3 months (or later) post-infection (p.i.), both trigeminal nerves and ganglia, the brain stem at both sides near to the entrance of the trigeminal root, and cornea were removed and immersed into phosphate buffer saline containing 3% foetal calf serum (FCS). The tissue samples were divided into two parts: one part was cultured, while the other one was frozen (at -70 °C) and used for preparing a DNA extract.

Explantation technique. A part of cornea, the half of each ganglion and brain stem specimens were minced and cultured in plastic Petri dishes as described (Rajčáni *et al.*, 1975). The medium (CMRL-1415 supplemented with 10% FCS and antibiotics) was exchanged on days 3, 7, and 10 in culture. By days 7 or 10 in culture, the fragments from the same sample were pooled and used for DNA extraction. In some cases the pooled fragments were homogenized and assayed for HSV in Vero cells. All medium samples were inoculated into Vero cells.

Isolation of the DNA. DNA extracts were prepared from noncultured tissue samples removed at acute and chronic stages p.i., from explanted fragments prepared from tissues removed at the chronic stage and kept for 10 days (or 7 days) in culture, from the noncultured tissues of uninfected rabbits as well as from rabbit embryo fibroblasts (REF) either noninfected or infected with HSV-1 (strain HSZP, multiplicity 2–5 PFU per cell) and harvested by 12 or 20 hr p.i.

Preparation of the DNA extract has been described previously (Kúdelová *et al.*, 1988). Briefly, the tissue samples and their explanted fragments as well as the REF cell suspensions were treated with the lytic buffer (0.2 mol/l TrisHCl pH 7.9 containing 0.5 mol/l EDTA and 0.5% SDS) then with proteinase K (100 μg/ml for 2 hr at 65 °C) and further extracted with phenol-saturated buffer (0.01 mol/l Tris-HCl buffer pH 7.5, 0.15 mol/l NaCl and 1 mmol/l EDTA). The water phase was treated with RNase A(100 μg/ml) for 1 hr at 37 °C then extracted with chloroform-isoamylalcohol (24 : 1) and finally, precipitated with the three-fold volume of ethanol.

HSV-1 DNA was isolated from nucleocapsids sedimented by differential centrifugation from HSV-1-infected quail embryo cells inoculated with the HSV-1 strain HSZP adapted to these cells and to chick embryo cells (Szántó, 1960). Details of nucleocapsid deproteinization and HSV DNA purification were described elsewhere (Kúdelová *et al.*, 1988).

*Kpn*I *fragments of HSV-1 DNA* strain 17 were kindly provided by dr. V. Preston (M.R.C. Virology Unit, Institute of Virology, Glasgow, U.K.). The fragments *Kpn*I *i*, *d*, and *h* cloned

in pAT 153 were isolated as described (Maniatis *et al.*, 1982). The fragment *Kpn*I *i* codes for VP 5 (155 kD) polypeptide, the fragment *Kpn*I *d* contains the gene for gC, while the *Kpn*I *h* contains the genes coding for gD and gE (Fig. 1).

Labelling of DNA probes and hybridization. Nick translation of the total HSV-1 DNA as well as of the cloned fragments was made according to Rigby *et al.* (1977) using α^{32}P-dCTP and α^{32}P-dGTP (specific activity 110 TBq per mmol, IZINTA, Budapest, Hungary); the specific activity ranged from 2 to 4×10^7 cpm per μg DNA, the activity of pAT 153 was 0.5×10^8 cpm per μg.

Spot blot hybridization was performed as described by Fraser *et al.* (1981). The DNA extracted from corneal, ganglion or brain stem specimens, from explanted tissue fragments or from cell suspensions denatured by heating to 95 °C for 5 minutes and then was spotted either on nitrocellulose or on nylon filters (Hybond-N, Amersham). The nitrocellulose filters were rinsed successively 5 min in 0.5 mol/l NaOH, twice 1 min in buffer (1 mol/l Tris-HCl pH 6.8, 0.5 mol/l NaCl), 5 min in buffer (0.5 mol/l Tris-HCl pH 7.4, 1.5 mol/l NaCl) and dried. Then they were floated in ethylalcohol, air dried, rinsed in 0.3 mol/l NaCl, dried and were baken for 2 hr at 80 °C under vacuum. The DNA spotted on nylon filters which were treated according to Amersham protocols in denaturing and neutralizing solutions was cross-linked by UV-irradiation on standard UV transluminator for 5 min. The nitrocellulose filters were prehybridized with denatured salmon sperm DNA (100 μg per ml) overnight at 48 °C (4×SSC, 20% formamide, 0.1% SDS, 10 mmol/l EDTA, 5× Denhardt's solution) (Maniatis *et al.*, 1982) and then hybridized with the hybridization mixture (the same as the prehybridization mixture but containing 50% formamide) in the presence of the denatured nick-translated probe overnight at 48 °C. The filters were washed as follows: in 5×SSC with 0.1% SDS twice for 10 min at 48 °C, in the hybridization mixture not containing the DNA probe for 20 min at 48 °C, in 1× SSC with 0.1% SDS for 20 min at 37 °C, then in the same buffer for 20 min at 64 °C and, finally, in 0.25×SSC with 0.1% SDS at 64 °C. Nylon filters were hybridized in the plastic bags under high stringency conditions recommended by the Amersham protocols. The air dried filters were wrapped to Saran Wrap and autoradiographed with XR film (Medix Rapid) for 2—3 days at — 70 °C.

Results

Hybridization with the labelled HSV-1 DNA probe

Six rabbits with established latent infection infected with the strain Kupka and 4 ones infected with the strain KOS were examined in the first trial. The right trigeminal ganglion (RTG) fragments yielded virus in two out of ten animals (Table 1). Positive hybridization was found with the DNA extract prepared from the other half of 4 RTG samples, i.e. in 3 cases positive hybridization was not associated with virus release from the cultured half of the ganglion. As shown in Fig. 2, in 1 case positive hybridization (confirmed by positive explantation result) was found with the DNA extract of the cultured ganglion only, but not with the noncultured RTG. The results

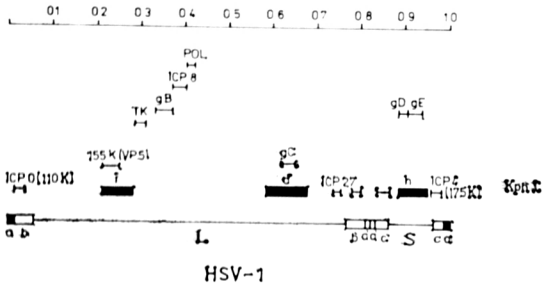

Fig. 1

Assignment of the strain 17 HSV-1 DNA *Kpn*I fragments (according to Preston *et al.*, 1978)

Table 1. Hybridization of HSV DNA probes and of the probes from its KpnI fragments in comparison with the explantation results

Rabbit number	Virus strain	Days p.i.	RTG			LTG			RBS			LBS			RC			Probe	Experiment
			H	He	Ime	H	He	Ime	H	He	Ime	H	He	Ime	H	He	Ime		
5	Kupka	139–177	4	4	1	0	0	0	2	ND	0	ND	ND	ND	ND	ND	ND	Total DNA	I
4	KOS	109–114	0	1	1	0	0	0	4	2	0	ND	ND	ND	ND	ND	ND	Total DNA	
11	Kupka	207–479	8	4*	7	2	1	2	0	0	0	2	0	0	1	0	0	*Kpn*I *i* *Kpn*I *d*+*i* *Kpn*I *h*+*i*	II
1	KOS	482	0	ND	1	0	ND	0	0	ND	0	0	ND	0	0	ND	0	*Kpn*I *h*+*i*	

RTG – right trigeminal ganglion, LTG – left trigeminal ganglion, RSB – right brain stem, LBS – left brain stem, RC – right cornea

H – hybridization, He – hybridization with the extracts of pooled explanted tissue fragments, Ime – isolation of infectious virus from the medium fluid of the explanted tissue between days 7–10 in culture

* out of 8 samples examined (3 samples were positive in infectivity assay see Table 2)

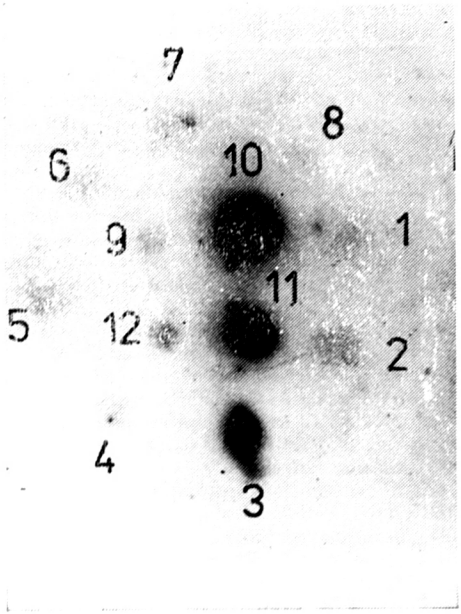

Fig. 2

Hybridization of the DNA extracts from noncultured and cultured ganglia of rabbits No. 71 (1—4) and 72 (5—8) with established latency using the total HSV-1 DNA probe
(1) — RTG — right trigeminal ganglion; (2) — LTG — left trigeminal ganglion; (3) — RTG — cultured for 7 days; (4) — LTG — cultured for 7 days; (5—8) — similar extracts from rabbit No. 72; (9) — noninfected 10^5 REF cells; (10) — DNA extract from 10^6 infected REF cells; (11, 12) — DNA extract from 10^5 and 10^4 infected REF cells, respectively.

in the left (contralateral) ganglia were negative. However, positive hybridization was seen with the extracts of 6 noncultured and 2 cultured brain stem extracts, although none of these yielded infectious virus.

Hybridization with the labelled HSV-1 DNA KpnI fragments

Twelve rabbits were examined in this trial (Exp. II), the majority of which had been infected with the Kupka strain. Explantation of RTG yielded virus in 8 cases; in four of these the cultured RTG fragments were used for DNA extraction, while another four were used for virus isolation. Both, hybridization with the cultured ganglion extract as well as virus isolation from the cultured ganglion homogeneates correlated with virus isolation results from the medium fluid (Table 2). Fig. 3 shows typical relationships between hybridization and explantation results. With the RTG extract of rabbit No. 13 there was an increased positive hybridization after explantation of the other half of the ganglion in culture, which was not the case with the right and left brain stem DNA extracts. This Figure also demonstrates the positive hybridization with practically all examined tissues from rabbit No. 15, which did not yield infectious virus in culture. As seen in the part II of Table 1, positive hybridization of the corneal DNA extract was found in this particular case only.

Experiment II has also shown that in three rabbits infected with the Kupka strain and in 1 animal infected with KOS there was no positive hybridization with the noncultured ganglion tissue, although a clear posi-

Table 2. Comparison of the explantation results by virus isolation and hybridization

Rabbit no.	Virus strain	RTG				LTG				RBS				LBS			
		4*	7*	10*	S	4	7	10	S	4	7	10	S	4	7	10	S
3	Kupka	0	2	3	3 (Is)	0	0	1	1 (Is)	0	0	0	0 (Is)	0	0	0	0 (Is)
1	KOS	0	1	1	1 (Is)	0	0	0	0 (Is)	0	0	0	0 (Is)	0	0	0	0 (Is)
4	Kupka	0	3	4	4 (Hy)	0	0	1	1 (Hy)	0	0	0	0 (Hy)	0	0	0	0 (Hy)

RTG, LTG, RBS, LBS — as in Table 1

* days in culture; on day 10 the fragments from the same tissue were pooled and used either for infectious virus isolation (Is) or for DNA extraction and subsequent hybridization (Hy). S = suspension from the explanted tissue.

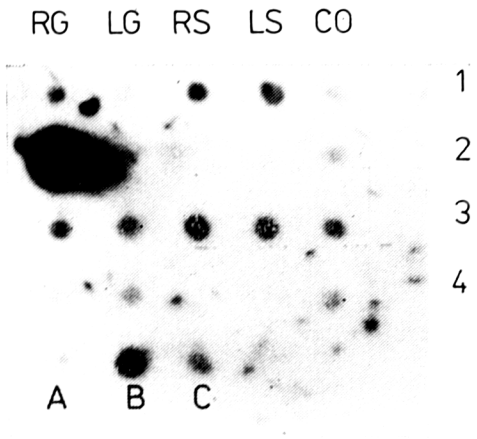

Fig. 3

Hybridization of the HSV-1 DNA fragment *Kpn*I i with noncultured and cultured tissue samples from rabbits Nos. 13 and 15 with established latent infection
Columns: RG — right trigeminal ganglion
LG — left trigeminal ganglion
RS — right brain stem
LS — left brain stem
CO — cornea
Lines: 1 — rabbit No. 13, noncultured tissues
2 — rabbit No. 13, similar tissues cultured for 10 days
3 — rabbit No. 15, noncultured tissues
4 — rabbit No. 15, similar tissues cultured for 10 days
A: extract from control rabbit cells; B: 10^5 infected rabbit cells; C: 10^4 infected cells.

tivity was found with the cultured ganglion extracts (Fig. 4). The positive correlation between enhanced hybridization of the cultured ganglion extracts and virus release from the explanted fragments to medium fluid is shown in Table 2. Regardless whether the suspension of the pooled ganglion explants was examined by infectivity assay or used for DNA extraction, the results were in agreement in each case. The absence of false positivity

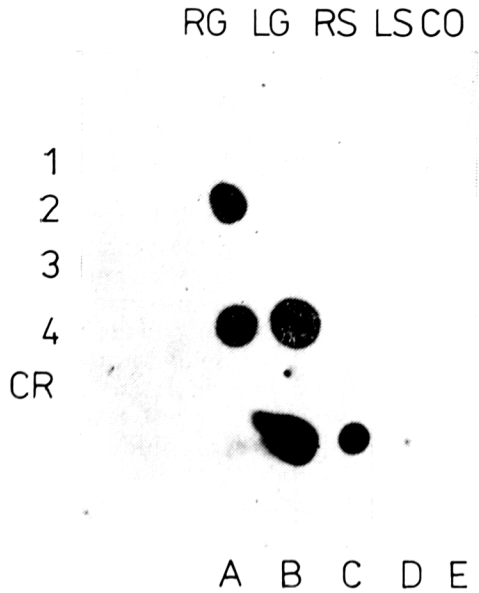

Fig. 4

Spot blot hybridization of the DNA extracts from neural tissues of rabbits with established HSV latency using the labelled *Kpn*I fragments $h+i$
Columns:
RG — right trigeminal ganglion; LG — left trigeminal ganglion; RS — right brain stem; LS — left brain stem; CO — cornea.
Lines:
1 — rabbit No. 19, not cultured;
2 — rabbit No. 19, after 10 days in culture;
3 — rabbit No. 20, not cultured;
4 — rabbit No. 20, after 10 days in culture;
CR — control rabbit tissues
Lines below: A — 10^6 noninfected REF cells; B — 10^6 infected REF cells harvested at 20 hr p.i.; C — 10^5 infected REF cells harvested at 20 hr p.i.; D — 10^4 infected REF cells; E — 10^3 infected REF cells.

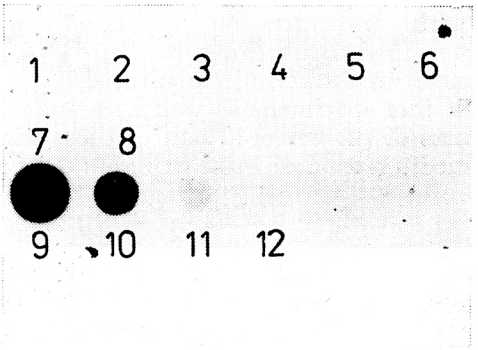

Fig. 5

Spot blot hybridization of the DNA extracts from noninfected rabbit tissues hybridized with the vector pAT 153 probe 1 — RTG; 2 — LTG; 3 — right brain stem; 4 — left brain stem; 5 — cornea; 6 — HSV-1 DNA 0.1μg; 7 — DNA extract from noninfected REF cells (5×10^5); 8 — DNA extract of 5×10^5 KOS-infected REF cells after 20 hr, MOI 3.5 PFU/cell/; 9—12 — 10ng, 1ng, 0.1ng and 0.01ng pAT 153, respectively.

in the case of hybridization with the cultured ganglion and brain stem extracts when hybridized to the cloned *Kpn*I HSV-1 DNA fragments as probes can be clearly deduced from the coincidence with virus infectivity assays. This was further confirmed by using noninfected rabbit tissues and noninfected rabbit cells as controls (Figs. 4 and 6). In addition, noninfected rabbit tissue never hybridized to the labelled vector plasmid pAT 153 DNA (Fig. 5), excluding the vector DNA as source of nonspecific hybridization. It seems, therefore, that the chosen cloned HSV-1 DNA *Kpn*I probes *i*, *h*, and *d* detected HSV-1 DNA in the tissue extracts, at least in those prepared from the explanted tissue fragments.

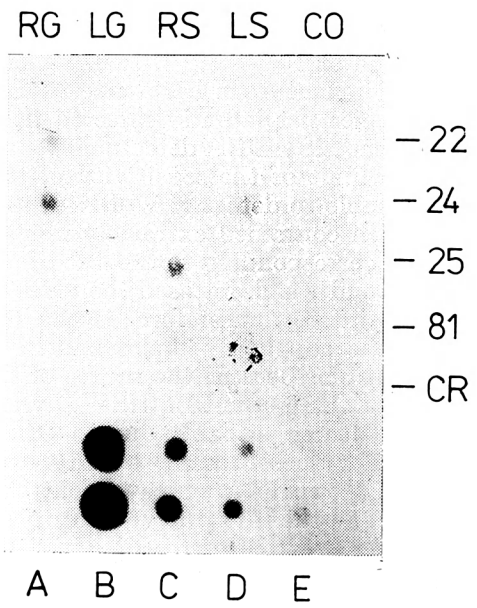

Fig. 6

Spot blot hybridization of the DNA extracts from neural tissues of rabbits with established HSV latency using the mixture of labelled *Kpn*I fragments *h* and *i* Columns: RG — right trigeminal ganglion; LG — left trigeminal ganglion; RS — right brain stem; LS — left brain stem; CO cornea.
rabbits numbers 22, 24, 25, 81;
CR — control rabbit (noninfected)
A control REF cells
B — 10^6 infected REF cells (above: harvested 12 hr p.i.; below: harvested 20 hr p.i.)
C — 10^5 infected REF cells (harvested at 12 hr p.i. or 20 hr p.i.)
D — 10^4 infected REF cells (harvested at 12 hr p.i. or 20 hr p.i.)
E — 10^3 infected REF cells (harvested at 12 hr p.i. or 20 hr p.i.)

Positive hybridization of minimal intensity was found probes with the *Kpn*I HSV-1 DNA probes to the DNA extracts from 4 noncultured RTG, 2 noncultured LTG, 5 noncultured right brain stem, and 2 noncultured left brain stem specimens as well as 1 noncultured corneal specimen. The DNA extracts of the cultured half of these samples showed no hybridization and the medium fluid of these explants did not contain infectious virus.

Discussion

In the first experiment using total HSV-1 DNA for spot blot hybridization we found positive hybridization with 3 RTG and 5 brain stem extracts as well as with the extracts of tissues from control rabbits and from several uninfected cell lines (not shown). We assume that the total HSV DNA probe exerted nonspecific false positive hybridization. Puga *et al.* (1982) and Peden *et al.* (1982) reported that human and/or other mammalian cellular DNA cross-hybridized with the joint and terminal regions of the HSV-1 DNA. Especially a small DNA segment from the inverted repeats and from the central area of the joint region has a composition extremely rich of G+C base pairs. Jones *et al.* (1985) found that the *Eco*RI B HSV-1 DNA fragment showed an authentic base homology to human ribosomal DNA.

We tried to overcome the difficulties with the false positive DNA/DNA hybridization using the cloned *Kpn*I DNA probes. The fragment *Kpn*I "*i*" covers about 5.4% of the genome, the mixture *Kpn*I "*d*+*i*" about 13.4%, and the mixture *Kpn*I "*h*+*i*" about 11.5% of the HSV-1 genome (Fig. 1). The mixtures gave a clear-cut hybridization spot with the DNA extract of 10^3 infected REF cells harvested at 12 hr p.i., but not with 10^2 infected REF cells (Fig. 4), and a slightly positive spot with 10^3 infected cells harvested at 20 hr p.i. (Figs. 4,6). We estimate the minimum sensitivity of our hybridization at 10^5 — 10^6 HSV DNA molecules. Because only a proportion of the ganglion neurons harbours the HSV DNA and because the number of DNA copies per latently infected neuron would hardly exceed 10^1, the amount of the HSV DNA detectable per the half of the rabbit ganglion was just at the limit of the sensitivity of the spot blot hybridization. Indeed, we were unable to detect HSV DNA in some ganglion samples the cultured half of which contained extremely much HSV DNA on days 7—10 in culture (spots corresponding to the size of at least 10^6 or more acutely infected REF cells) and which released the virus to medium fluid.

Of great interest, therefore, is the finding of HSV DNA sequences in a part of the ganglion samples and in brain stem samples which neither yielded virus nor became the source of DNA replication in culture. In contrary, the slight amount of HSV DNA detectable in such samples disappeared in culture, probably due to DNase self digestion. The simplest explantation for these results would be a nonspecific false positive hybridization. However, a series of controls including noninfected rabbit cells and tissues and the pAT 153 vector probe do not confirm this interpretation. Our previous electron microscopic investigations (Rajčáni *et al.*, 1975) have shown that the neurons and especially nonneural satellite cells survived in

culture. Thus, it is unlikely that all cellular DNA, if responsible for the false positive hybridization would disappear from the explanted ganglion or brain stem.

Several experimental data point at the existence of latent HSV which does not spontaneously reactivate in culture. Whitby *et al.* (1987) described the stimulating effect of hypomethylating drugs such as 5-azacytidine on the reactivation rate of the latent HSV infection in the explanted mouse ganglion samples. In our hands, using the SC16 and ANGpath strains of HSV in DBA/2 mice, the reactivation rate of the latent virus was high when the culture fluid contained 5-azacytidine, but it was extremely low in the absence of the inducer (Rajčáni *et al.*, 1990). Brown *et al.* (1979) and Lewis *et al.* (1984) reported that superinfection of explants with a ts mutant at supraoptimal temperature had complemented the defective resident genome and rescued a virus which DNA was distinguished from ts mutant's DNA by restriction enzymes. Thomas *et al.* (1985) found that in mice previously infected with HSV-1 strain F and then superinfected with HSV-2 the ganglia became double infected and that the superinfected HSV-2 DNA acted in trans at rescuing the previously introduced heterotypic HSV-1 genome. Such mechanisms might operate in human brain at inducing reactivation of the spontaneously noninducible resident HSV DNA (Stroop, 1986). HSV DNA was reported in the CNS of mice during latency (Cabrera *et al.*, 1980; Stroop *et al.*, 1984) as well as in the human brain extracts (Fraser *et al.*, 1981). In our previous study (Kúdelová *et al.*, 1988) we found positive hybridization of HSV DNA in the *nc. amygdalae* of 3 out 11 biopsy samples from 10 patients, but no virus had been isolated. Recently using DNA fragments complementary to the latency associated mRNAs, positive hybridization was found in the brain stem of mice and rabbits with established latent infection (Deatly *et al.*, 1988).

We conclude that comparing the explantation and hybridization procedures we found several combinations of positive results: 1. Positive hybridization in the cultured as well as noncultured homolateral sensory ganglion samples associated with virus release into the medium of explants; 2. Negative hybridization with the noncultured ganglion extract followed by positive hybridization of the cultured ganglion extract and with HSV release from the explants; 3. Positive hybridization of the noncultured ganglion and brain stem extracts not followed by viral DNA replication in the explants and with virus reactivation; 4. Slight positive hybridization was detected with a single corneal sample showing negative outcome of explantation. The DNA presence under 3 and 4 may be accounted for false positive. However, a careful analysis of the results based on controls and the data of others allows to assume that the latent HSV genome may not always reactivate during 10 days explantation of the corresponding tissue. Further experiments are being performed to confirm the latter assumption.

Acknowledgements. The authors thank Mrs. L. Vláčiliková and Mrs. E. Srnová for the technical assistance. They also thank dr. O. Kúdela from the Institute of Virology, Bratislava for his help. We feel deeply indebted to dr. V. Preston from the MRC Virology Unit, Institute of Virology, Glasgow, for providing the cloned HSV-1 DNA fragments.

References

Baringer, J. R. (1975): Herpes simplex virus infection of nervous tissue in animals and man *Progr. med. Virol.* **20**, 1—16.

Brown, S. M., Subak-Sharpe, J. H., Warren, K. G., Wroblewska, Z., and Koprowski, H. (1979): Detection by complementation of defective or uninducible herpes simplex type 1 virus genomes latent in human ganglia. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2364—2368.

Cabrera, C., Wohlenberg, Ch., Openshaw, H., Rey-Mendez, M., Puga, A., and Notkins, A. L. (1980): Herpes simplex virus DNA sequences in the CNS of latently infected mice. *Nature (Lond.)* **288**, 288—290.

Deatly, A. M., Spivack, J. D., Lavi, E., O'Boyle, D. R., and Fraser, N. W. (1988): Latent herpes simplex virus type 1 transcripts in peripheral and central nervous system tissues of mice map to similar regions of the viral genome. *J. Virol.* **62**, 749—756.

Efstathion, S., Minson, A. C., Field, H. J., Anderson, J. R., and Wildy, P. (1986): Detection of herpes simplex virus-specific DNA sequences in latently infected mice and in humans. *J. Virol.* **57**, 446—455.

Field, H. J., Bell, S. E., Elion, G. B., Nash, A. A., and Wildy, P. (1979): Effect of acycloguanosine treatment on acute and latent herpes simplex virus infections in mice. *Antimicrob. Agents Chemother.* **15**, 554—561.

Fraser, N. W., Lawrence, W. C., Wroblewska, Z., Gilden, D. H., and Koprowski, H. (1981): Herpes simplex type 1 DNA in human brain tissue. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6461—6465.

Fraser, N. W., Mellerich, D. M., Muggeridge, M. I., and Spivack, J. G. (1986): Molecular biology of latent HSV-1. pp. 39—55. In C. Lopez, B. Roizman (Eds): *Human Herpesvirus Infections: Pathogenesis, Diagnosis, and Treatment*, Raven Press, New York.

Galloway, D. A., Fenoglio, C. M., and McDougall, J. K. (1982): Limited transcription of the herpes simplex virus genome when latent in human sensory ganglion. *J. Virol.* **41**, 686—691.

Jones, T. R., Parks, Ch. L., Spector, D. J., and Hyman, R. W. (1985): Hybridization of herpes simplex virus DNA and human ribosomal DNA and RNA. *Virology* **144**, 387—397.

Klein, R. J. (1976): Pathogenetic mechanisms of recurrent herpes simplex virus infections. *Arch. Virol.* **51**, 1—13.

Kúdelová, M., Rajčáni, J., Pogády, J., and Šramka, M. (1988): Herpes simplex virus DNA in the brain of psychotic patients. *Acta virol.* **32**, 455—459.

Lewis, M. E., Brown, S. M., Warren, K. G., and Subak-Sharpe, J. H. (1984): Recovery of herpes simplex virus genetic information from human trigeminal genglion cells following superinfection with herpes simplex virus type 2 temperature-sensitive mutants. *J. gen. Virol.* **65**, 215—219.

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982): *Molecular Cloning: a Laboratory Manual*, Cold Spring Harbor laboratory, Cold Spring Harbor, N.Y., pp. 88—94.

Peden, K., Mounts, P., and Hayward, G. (1982): Homology between mammalian cell DNA sequences and human herpesvirus genomes detected by a hybridization procedure with high complexity probe. *Cell* **31**, 71—80.

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

Puga, A., Rosenthal, J. D., Openshaw, H., and Notkins, A. L. (1978): Herpes simplex virus DNA and mRNA sequences in acutely and chronically infected trigeminal ganglia of mice. *Virology* **89**, 102—111.

Puga, A., Cantin, E., and Notkins, A. (1982): Homology between murine and human cellular DNA sequences and the terminal repetition of the S component of herpes simplex virus type 1 DNA. *Cell* **31**, 81—87.

Rajčáni, J., Čiampor, F., and Sabó, A. (1975): Experimental latent herpesvirus infection in rabbits, mice, and hamsters: ultrastructure of virus activation in explanted Gasserian ganglia. *Acta virol.* **19**, 19—28.

Rajčáni, J., Čiampor, F., Sabó, A., Libíková, H., and Rosenbergová, M. (1977): Activation of latent herpesvirus hominis in the explants of trigeminal ganglia: The influence of immune serum. *Arch. Virol.* **53**, 55—69.

Rajčáni, J., and Matis, J. (1981): Immediate early and early polypeptides in herpesvirus latency. *Acta virol.* **25**, 371—375.

Rajčáni, J., Herget, U., Košťál, K., and Kaerner, H. C. (1990): Latency competence of herpes simplex virus strains ANG, ANGpath and its gC and gE minus mutants. *Acta virol.* **34**, in press.

Rigby, P., Dickmann, M., Rhodes, C., and Berg, P. (1977): Labelling deoxyribonucleic acid to high specific activity *in vitro* by nicktranslation with DNA polymerase I. *J. mol. Biol.* **113**, 237—251.

Rock, D. L., and Fraser, N. W. (1985): Latent herpes simplex type 1 DNA contains two copies of the virion DNA joint region. *J. Virol.* **55**, 849—852.

Rock, D. L., Nesburn, A. B., Ghiasi, H., Ong, J., Lewis, T. L., Lokensgard, J. R., and Wechsler, S. L. (1987): Detection of latency-related viral RNAs in trigeminal ganglia of rabbits latently infected with herpes simplex virus type 1. *J. Virol.* **61**, 3820—3826.

Spivack, J. G., and Fraser, N. W. (1987): Detection of herpes simplex virus type 1 transcripts during a latent infection in mice. *J. Virol.* **61**, 3841—3847.

Spivack, J. G., and Fraser, N. W. (1988): Expression of herpes simplex virus type 1 latency-associated transcripts in the trigeminal ganglia of mice during acute infection and reactivation of latent infection. *J. Virol.* **62**, 1479—1485.

Stevens, J. G. (1975): Latent herpes simplex virus and the nervous system. *Curr. Top. Microbiol. Immunol.* **70**, 31—50.

Stevens, J. G., Wagner, E. K., Devi-Rao, G. B., Cook, M. L., and Feldman, L. T. (1987): RNA complementary to a herpesvirus α-gene mRNA is prominent in latently infected neurons. *Science* **235**, 1056—1059.

Stroop, W. G., Rock, D. L., and Fraser, N. W. (1984): Localization of herpes simplex virus in the trigeminal and olphactory systems of the mouse central nervous system during acute and latent infections by *in situ* hybridization. *Lab. Invest.* **51**, 27—38.

Stroop, W. G. (1986): Herpes simplex virus encephalitis of the human adult: reactivation of latent brain infection. *Pathol. Immunopath. Res.* **5**, 156—169.

Svennerholm, B., Vahlne, A., and Lycke, E. (1981): Persistent reactivable latent herpes simplex virus infection in trigeminal ganglia of mice treated with antiviral drugs. *Arch. Virol.* **69**, 43—48.

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

Tenser, R. B., Dawson, M., Ressel, S. J., and Dunstan, M. E. (1982): Detection of herpes simplex virus mRNA in latently infected trigeminal ganglion neurons by *in situ* hybridization. *Ann. Neurol.* **11**, 285—291.

Thomas, E., Lycke, E., and Vahlne, A. (1985): Retrieval of latent herpes simplex virus type 1 genetic information from murine trigeminal ganglia by superinfection with heterotypic virus *in vivo*. *J. gen. Virol.* **66**, 1763—1770.

Whitby, A. J., Blyth, W. A., and Hill, T. J. (1987): The effect of DNA hypomethylating agents on the reactivation of herpes simplex virus from latently infected mouse ganglia *in vitro*. *Arch Virol.* **97**, 137—144.