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DNA REGIONS AND GENES DETERMINING THE VIRULENCE OF HERPES SIMPLEX VIRUS

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Summary. - The outcome of virus-host interaction after peripheral inoculation of herpes simplex virus (HSV) depends on virus replication at the portal of entry, the ability of the virus to invade nerve endings and capillary endothelium cells and, the rate of virus replication in neurons and nonneural cells of the nervous system. Functions involved are the activity of viral thymidine kinase, DNA polymerase, immediate early transactivator proteins, the transcription initiation protein, the envelope protein(s) governing virus penetration, syncytium formation and natural killing of infected cells as well as some other regulatory DNA sequences.

Key words: herpes simplex virus; pathogenicity; deletion mutants; molecular and genetic mechanisms

Introduction

The outcome of infection with herpes simplex virus (HSV) in various experimental animals depends on the virus dose, the inoculation route, the susceptibility of the given animal species, and on the properties of the inoculated virus in general termed pathogenicity and/or virulence. Using the same strain of inbred mice, virus dose and inoculation route, the pathogenicity of different viruses may vary depending on the virus serotype (Nahmias and Dowdle, 1968; Plummer *et al.*, 1968) and on the passage history (Rajčáni *et al.*, 1973). The molecular basis of virulence may be assessed using pathogenic and nonpathogenic derivatives of the same virus strain, i.g. ANG and ANGpath (Kümel *et al.*, 1986) and/or comparing the behaviour of certain deletion mutants with the parent virus strain. This review summarizes the recent results dealing with the possible role of deleted viral genes or recombinant viral DNA regions for the spread of various mutants in the host.

To assess the behaviour and spread of the inoculated viruses it seems reasonable to use a standardized nomenclature of pathogenicity in comparative studies. Based on the comparative analyses of the outcome of infection in Balb/c mice, Dix *et al.* (1983) classified the HSV strains as follows: class I strains highly virulent by peripheral as well as by intracerebral inoculations,

class II strains virulent after i.c. inoculation only, class III strains nonvirulent by either inoculation routes. Because of the latent outcome of the host interaction with nonpathogenic viruses, the behaviour of various HSV strains after peripheral inoculation can be differentiated as follows: 1. Local replication followed by efficient virus spread by various routes and extensive growth in the CNS as target tissue (lethal outcome); 2. Local virus replication with limited virus spread followed by moderate replication in the target tissues such as the regional sensory or vegetative ganglion (nonlethal outcome); 3. Minimal local virus replication followed by axonal transport to the regional ganglion and establishment of latency; 4. Minimal local virus replication with establishment of nonreactivable latency as evidenced by the presence of noninducible HSV DNA in the ganglion; 5. Absence of any virus spread from the inoculation site.

The thymidine kinase gene

With respect to HSV pathogenicity and neuroinvasivity the most thoroughly investigated virus function is that of the thymidinekinase (TK) gene. The TK gene is located in the *Bam*HI *p* fragment (0.29 MU - 0.35 MU), i.e. in the ORF UL 23 (McKnight, 1980) encoding the beta polypeptide of M_r 43 kD (Summers *et al.,* 1975) which displays thymidine and deoxycitidine kinase activities (Jamieson *et al.,* 1974). A TK minus mutant can be prepared by propagation of the virus in the presence of base analogues 5-bromo-2'-deoxyuridine (Kit and Dubs, 1963), 5-bromo-2'-deoxycitidine (Brown and Jamieson, 1978), arabinofuranosilthymine (Tenser and Dunstan, 1979) and/or acycloguanosine (Field *et al.,* 1980). The TK expression was found to be necessary for HSV replication in nondividing cells, especially in the neurons of sensory ganglia, which in adults do not express their own TK. When inoculated onto scarified cornea, the TK negative HSV mutants multiply in the cornea but not in the trigeminal ganglion, while the w.t. virus multiplies in the cornea as well as in the ganglion (Tenser *et al.,* 1981). Intermediate TK± mutants with low levels of TK expression showed some minor replication in the ganglia, which was correlated with the extent of viral TK activity. The w.t. NIH strain of HSV-1 when inoculated to scarified cornea in a dose of 10^6–10^7 PFU was pathogenic for outbred white mice of different age groups ranging from 4 to 28 days. The intermediate TK± mutant (25 % TK activity) was pathogenic for mice up to 10 days of age, similarly as the TK negative mutant expressing no TK activity. The latter mutant, however, was apathogenic for mice older than 10 days, which do not express TK activity in the brain, while the growth of the intermediate TK± mutant in the ganglion tissue of this age group was dose dependent (Ben-Hur *et al.,* 1983). It seems that the extent to which the HSV strains express their TK activity is decisive for their growth in the neural tissue.

This statement would be fully correct if the nervous system would consist of a single cell type (neurons) only. When inoculated into brain, the TK negative HSV-1 mutant failed to grow to titres as high as did the w.t. virus (the LD_{50} for the TK negative mutant was 10 000 times higher). If the same TK^- mutant was

given to immunosuppressed mice, the LD_{50} dose decreased about 2000 times (Gordon et al., 1984). This indicates that the host immune response also contributed to limited replication and spread of the TK^- mutant to surrounding permissive glial cells.

Nevertheless, it is widely accepted that after peripheral inoculation the growth of HSV TK^- mutants in the regional sensory ganglion is highly restricted. Another question is, whether latency can be established in the absence of productive virus replication. Indeed, Field and Wildy (1978) reported that after inoculation into the ear the virus could be recovered from cultured cervical dorsal root ganglia of mice in which latency had been established with a TK^- HSV mutant. On the other hand, Gordon *et al.* (1983) but not the TK^- mutant found that the w.t. NIH strain and the „low level" TK mutant could be recovered from the ganglia during latency. The latter finding was questioned by Sears *et al.* (1985) who prepared HSV recombinants in which low TK expression was achieved by insertion of an alpha or a gamma-2 gene promoter between the TK gene promoter and the TK coding sequences. Some TK gene chimeras regulated by the alpha promoter underwent deletions in the TK gene so that these recombinants expressed extremely low amounts of TK activity. Though classified as TK negative, the latter still were able to establish latency at high frequencies. Thus rearrangements of the TK gene by insertion and deletion do not seriously impair the ability of HSV to establish latent infection in the regional sensory ganglion.

Based on these results it can be suggested that TK expression is important rather for virus replication in neurons and for reactivation of the latent HSV genome than for the establishment of latency. Tenser *et al.* (1989) investigated the presence of latency-associated transcripts (LATs) (see later) in sensory ganglion neurons of mice after inoculation with TK mutants of HSV. In contrast to the w.t. virus-infected mice, no virus was isolated from the mutant -infected mice ganglion explants. Although reactivable virus was not detected, LATs were commonly present implicating some role for TK expression in the reactivation process. In guinea pigs intravaginal inoculation of TK deficient HSV type 2 resulted in similar vaginal virus titres as the w.t. strain, but only low virus titres were noted in the spinal cord homogenates. Latent infection and its reactivation were noticed in the regional sensory ganglia (Stanberry *et al.*, 1985). However, it may be reasoned that TK minus mutants used by different investigators variably express at least minimal amounts of the enzyme. This was detected by Tenser and Edris (1987) who used a mixture of w.t. and TK negative virus in various ratios to demonstrate the possible *in vivo* complementation of the TK^- mutant by small amounts of TK expressing virus within the ganglion.

Most recently Fridrich *et al.* (1990) investigated virus reactivation in cultured ganglions by spot blot hybridization and polymerase chain reaction (PCR) of viral DNA. At late intervals post-infection with HSV-1 and HSV-2 TK^- strains, the virus did not reactivate in cultured ganglions; the positive results of

dot blot hybridizations could be confirmed by PCR using primers flanking a region of the *Bam*HI restriction fragment *b* comprising the ICPO gene sequences overlapping the sequences of the LATs ORF. This could mean that the establishment of latency by axonal transport of the viral core-DNA complex at one hand and the reactivation of latency and productive virus replication in the ganglion cells on the other hand are different functions, but only the latter is affected by the absence of TK expression.

The HFEM deletion

Interesting results concerning a DNA region related to neurovirulence came from the comparison of two HSV-1 strains F and its derivative HFEM. The former is highly pathogenic for Balb/c mice and tree shrews, while the latter is apathogenic for these animals. In addition, HFEM has been found unable to colonize the sensory ganglia of tree shrews and at late intervals post-infection it could be recovered from spleens only. One of the recombinants of HFEM carrying the strain F *Bam*HI fragment *b* caused generalized and lethal herpes virus infection in juvenile and adult tupaias (Rösen *et al.*, 1985). This recombinant R-MLC1 was pathogenic for the tree shrew and slightly virulent for mice (Rösen *et al.*, 1986). Genome analysis of the HFEM DNA showed a deletion in the genome at coordinates 0.762-0.789 as compared to strain F. The deletion was mapped by maker rescue in that sequences of the *Ml*uI subfragment of *Bam*HI *b* strain F were inserted into the HFEM genome fragment *Hpa*I *p* (0.761-0.796) which was then used in cotransfection experiments to prepare the recombinant MLC1. In contrast to the peripharally (i.p.) nonpathogenic strain HFEM, which did not invade the adrenal glands of mice after intraperitoneal inoculation, the complemented MLC1 virus was able to invade the adrenal glands but did not penetrate their spinal cord (Peles *et al.*, 1990).

The ORF's flanking the delected region are these of the ICP27 gene (IE 63 kD) and the ICPO gene (IE 110 kD). When sequences close to the left terminus of *Bam*HI fragment *b* of strain F were used as a hybridization probe to characterize RNA transcripts, a novel mRNA (1.5 kb) was found in all virus strains tested, except of strain HFEM (Rösen-Wolff *et al.*, 1988). The corresponding protein encoded by this transcript could not be identified, but it appears likely that this mRNA is transcribed from UL 55 (or UL 56?). The 1.5 mRNA was missing in organ and cell extracts in which the HFEM strain multiplied. It was further absent in the spleen of infected tree shrews, where the HFEM virus persisted, but it was present in the ganglion extracts of the tree shrews infected with the F strain colonizing the sensory ganglia (Rösen-Wolff *et al.*, 1989). The HFEM deletion covers furthermore a great part of the latency-associated transcript region. Indeed the HFEM recombinant R-MLC1 was able to establish and reactivate latency in the dorsal root ganglion of the intraperitoneally infected tree shrews.

The immediate early genes

The immediate early HSV genes are transcribed by the unmodified host cell

RNA polymerase; the corresponding proteins, the IE3 (175 kDa, ICP4) and the IE1 (110 kDa, ICPO) are regulatory transactivator proteins, which in association with the host cell transcription factor SP1 bind to the specific sequence elements in the β- and β/γ gene promoter regions (Everett, 1987). ICPO deletion mutants located in the inverted repeats flanking the U_L region, especially one with a 700 bp deletion in both gene copies and another one with a large 3.1 kb deletion in both copies (dlx3.1) derived from strain KOS grew in all cells tested, however, reaching considerably lower titres (Sachs and Schaffer, 1987). The polypeptide profiles generated by these mutants were similar to that of the w.t. strain. The dlx3.1 mutant was significantly less pathogenic when inoculated into scarified cornea of mice and/or rabbits in comparison to strain KOS as judged either by lower virus titres in the eye secretions and in the trigeminal ganglion, or by host survival (Gordon *et al.*, 1990*a*). Thus ICPO expression plays an important role for efficient replication of the virus in the portal of entry and subsequent replication of the virus in the regional sensory ganglion after corneal inoculation. Establishment of latency was demonstrated both in mice and in rabbits by cocultivation and DNA hybridization, although the frequency of ganglionic latency was lower than that established by the w.t. virus (Gordon *et al.*, 1990*b*). In contrast to all others, the ICPO mutant was sponteneously shed from eyes of rabbits with latent ganglionic infection indicating that the mutant ICPO might have easier escaped the latency control than the w.t. strain KOS. The latter apparently is restricted in the host cell. Leib *et al.* (1989) used nonsense and deletion mutants of HSV-1 of three immediate early proteins, ICPO, ICP4, and ICP27 (IE2, 63 kDa). Mutants with lesions in genes ICP4 and ICP27 did not replicate in the cornea and ganglia of CD-1 mice. They also failed to establish reactivable latency as judged by virus reactivation in culture. The ICPO deletion mutants varied in their ability to establish latency and virus reactivation from the latent state. This suggested that ICPO is at least required for the efficient establishment of latent ganglionic infection. In the case of the ICPO mutants viral DNA could be detected in ganglion extracts by spot-blot hybridization.

Transcription of the IE genes is initiated by a virus-encoded structural protein designated as α-TIF (transinducing factor, VP16, M_r 65 kDa), which in association with cellular transcription factors (C-jun, c-fos, AP1) interacts with the IE regulatory TAATGAAT sequence in the α-gene promoter (O'Hare and Goding, 1988; McKnight *et al.*, 1987; Preston *et al.*, 1988; Gerster and Roeder, 1988). After entering the nerve endings and following uncoating of the virions, the viral DNA-containing core reaches the nucleus of the pseudounipolar sensory neurons by quick axonal transport sooner than α-TIF (Roizman, 1990). The α-TIF deficient insertion mutant 1814 derived from strain 17 of HSV-1 did not replicate in the regional sensory ganglion but quickly established latency within 24-48 hr post infection (Steiner *et al.*, 1990). Thus latency may be established in neurons in the absence of α-TIF and acute replication of the virus in the ganglion is no prerequisite for the establishment of latent infection. Presu-

mably latent and lytic pathways of HSV-host cell interaction are different as already postulated in earlier studies (Rajčáni and Čiampor, 1978; McLennan and Darby, 1980). The differential transport of the HSV DNA and α-TIF to the nucleus of neuron facilitates the block of α-gene promoters by methylation and other mechanisms preventing the virus to enter a lytic cycle, but an expression of this protein is not needed for the establishment latency.

The latency-associated transcription of HSV-1

The idea of limited transcription during latency with HSV was first supported by the finding that RNase-treated ganglion sections showed only weak signals by *in-situ* hybridization as compared to those not treated with the enzyme, suggesting the presence of viral in RNAs (Tenser *et al.*, 1982). Preliminary experiments with different HSV DNA fragments showed abundant hybridization using the *Hind*III restriction fragment *b* and various other probes mapping in the left hand part of the viral genome (Galloway *et al.*, 1982). In other approaches *in situ* hybridization and Northern blotting of the ganglion -extracted RNA demonstrated that the transcripts present during latency hybridize to a region flanking the right end of U_L and neighbouring internal repeat IR_L (Rock *et al.*, 1987; Spivack and Fraser, 1987; Stevens *et al.*, 1987). These transcripts were found to be antisense to the ICPO mRNA; they are evidently transcribed from the opposite DNA strand and partially overlap the ORF's of IE3 and ICPO. Three sets of viral transcripts could be detected in the extracts from ganglia of Balb/c mice previously infected to scarified cornea: 1.45 kb, 1.5 kb, and 2.0 kb, the latter being the most abundant (Spivack and Fraser, 1988*a*). This report apparently support our earlier finding that the ICPO /110 kD/ protein could not be detected in the ganglion sections by anticomplement fluorescence (Rajčáni and Matis, 1981). More recently Gordon *et al.* (1988) demonstrated the absence of ICPO mRNAs in the sensory ganglia during HSV latency, although the same ganglia hybridized with the RNA probe designed to detect the antisense transcripts (LATs) in the nuclei of neurons in 46 % of human trigeminal ganglia.

The HFEM strain of HSV expresses the LATs mapping in the region of the restriction fragment *Bam*HI *e* because the 4.1 kb HFEM deletion located between genes ICPO and ICP27 affects the LAT ORF in *Bam*HI *b*. Nevertheless, LATs are synthesized in a lower but still sufficient amounts (Spivack and Fraser, 1988*b*). HSV-1 KOS was deleted in LAT ORF at 0.778-0.784 MU (*Pst-Sal*I subfragment of *Bam*HI *b)* and in LAT ORF at 0.042-0.036 MU (*BseL*II subfragment of *Bam*HI *e)* and designated KOS 8117. Both KOS 8117 and the restored recombinant KOS D362 (by using the deleted 960 bp) sequence cloned in a plasmid established latency in murine sensory neurons. Thus, there was no difference between viruses expressing LATs (w.t. KOS and KOS D362) and the deletion mutant KOS 8117 not expressing LATs with respect to the amount of HSV DNA present in the ganglion extracts for an 11 month latency period (Sedarati *et al.*, 1989). The strain 17 mutant 1704

prepared by Steiner *et al.* (1989) had two deletions: a 3.8 kb deletion at the IR_L/U_L transition from ORF UL55 to the 5' end of LAT-coding sequences (represented by the *Hpa*I *a* and *v* subfragments from *Bam*HI *b)* and a second 1.2 kb deletion in TR_L (*Bam*HI *e)* which affects the promoter of the LAT gene 150 bp upstream from the 5' end of this gene. Neither of these deletions influenced the expression of IE1 ICPO protein. Both, strain 17 and the LAT^- deletion mutant replicated in the trigeminal ganglion and established latent infection but only the parental virus was capable to reactivate in culture within a 7 days cultivation period. Reactivation of the strain 1704 in cultured explants was considerably delayed as long as 30 days. The data suggest a certain role for the LATs during the reactivation of latent virus. Similar findings were reported of the KOS-derived LAT^- deletion mutant dl LAT 1.8 (Leib *et al.*, 1989).

Another insertion mutant was derived from the HFEM virus (see above) in which a short *Hpa*I-*Hpa*I sequence from *Bam*HI *b* between nucleotides 120 301 and 120 469 and a similar sequence from *Bam*HI *e* between nucleotides 6070 and 5902 was replaced by a 440 bp *Hpa*I fragment of lambda phage DNA. This mutant, TB 1 does not express full length 2.0 and 1.5 kb LATs, but did express a truncated 0.7 to 0.8 kb fragment TB1 which established latency in mice after ocular infection as efficiently as its parent and reactivated from explanted culture at normal kinetics. It was suggested from these results that the first 838 bp of LATs are not involved in the biological function of this transcript, especially not in the reactivation process (Block *et al.*, 1990). The KOS 8117 LAT deficient mutant was furthermore used to establish latency by footpad inoculation in Swiss mice (Izumi *et al.*, 1989). Following reactivation of this mutant in the cultures of dorsal root ganglia no considerable delay was found in comparison to the parental strain KOS. Since the viral LATs were not present in the ganglia of mice latently infected with KOS 8117, the maintenance of the latent state in neurons seems to be a cell-associated function rather than governed by a virus-encoded gene product. Up to now no protein was identified due to translation of the LATs. It remains to be investigated whether the LAT coding ORF has a function similar to BZLF1 gene of EB virus, which codes for the regulatory ZEBRA protein activating the expression of early proteins.

The DNA polymerase complex

Growth of the HSV-1 strain 17 is 100-fold greater in the trigeminal ganglion after corneal inoculation than the growth of the low-invasive HSV-2 strain 186. This difference is not due to lower TK activity of the latter virus, which replicates well in the mouse brain after intracerebral inoculation (Oakes *et al.*, 1986). The virulent phenotype of the PAA resistant SC16 mutant RSC26 could be restored after cotransfection with the *Bam*HI fragment *r* (MU 0.413-0.434); this confirmed the role of DNA polymerase (Larder *et al.*, 1986). Three intertypic recombinants were constructed containing HSV-1 DNA inserts of different lengths within the HSV-2 genome: HSV-R (B1E) containing a strain 17 19.5 kb DNA sequence between MU 0.30-0.44, HSV-R(R3B) with a strain 17

6.0 kb DNA sequence between 0.4–0.44 and finally, the HSV-R(D) having a 2.0 kb strain 17 DNA sequence at MU 0.413–0.426. Following ocular infection of mice, the recombinants B1E and R3B spread into the CNS similarly as did the HSV-1 strain 17 indicating that the region between 0.40–0.44 of HSV-1 DNA contains the capacity of these recombinants to spread from infected corneas along nerves (Day *et al.*, 1987). The DNA sequence in question includes the HSV-1 strain 17 Ori_L, the DNA polymerase gene and a portion of the ICP8 ORF. Because the HSV-1 (D1) recombinant showed the same neuroinvasive properties in SJL/J mice it was concluded that the essential sequences accounting for neurovirulence map in the 0.413–0.426 MU region encoding the DNA polymerase (Day *et al.*, 1988). Since this region codes for the N-terminal domain of the DNA pol molecule (Gibbs *et al.*, 1985), it seems reasonable to assume that this region plays some important role for the capacity of the virus to replicate in sensory ganglia and spread to CNS causing encephalitis.

The possible role of the envelope glycoproteins

In elucidating the molecular basis for virulence, surprisingly few attention has been paid to the role of genes coding for envelope glycoproteins. Some hints came from the finding that the DNA region at 0.31–0.44 MU governs virulence (Oakes, *et al.*, 1986; Thompson *et al.*, 1986). At least two *Eco*RI fragments, namely the fragment *f* (0.32–0.42 MU) and the fragment *a* (0.49–0.64) were found to transfer virulence (Goodman *et al.*, 1989). The former 0.32–0.42 MU sequence designated *inv*II (inasive region II) codes also for gB and is clearly associated with the spread of virus to CNS (in addition to the data mentioned above). We tested the recombinants between the strain KOS which is nonpathogenic for mice and the pathogenic strain ANGpath (Kaerner *et al.*, 1983). Recombinants were prepared in which the fragment *Bam*HI *g* in ANGpath was replaced by the corresponding fragment of KOS, or in which smaller subfragments coding for parts of gB were exchanged. These were the 0.8 kb fragment *Bam*HI-*Sst*I at 0.345–0.351 MU encoding the cytoplasmic C-terminal domain of gly B and carrying the syn3 locus and a 2.7 kb 0.351–0.368 MU *Sst*I-*Sst*I subfragment from *Bam*HI *g* encoding the surface domains of gB including the B6 marker (the monoclonal antibody B6 reacts exclusively with gB KOS). Strain ANGpath has the phenotype syn in contrast to strain KOS. Strain ANGpath and the recombinant ANGpath/$B6^{KOS}$ replicated extensively at the portal of entry and spread both by haematogenic and neural routes. In contrast, ANGpath/syn^+ $B6^{KOS}$ and ANGpath/syn^+ recombinants were not pathogenic for mice and the virulence of the former was considerably decreased in rabbits (Košťál *et al.*, 1992). From these experiments it was concluded that gB may be important for HSV virulence in mice and rabbits but has no influence on the latency competence of the virus. The critical DNA region is presumably located between 0.345–0.359 MU (between *Bam*H1 site and the *Sal*I site of *Bam*H1 *g*) encoding the cytoplasmic C-terminal domain and

a part of the surface domain of gB. The former region contains the syn 3 locus, but its mutation does not solely determine the difference between gB ANGpath and gB KOS. The experiments in question also showed that the B6 locus located near to the NH_2 terminus of gB molecule does not influence the pathogenicity of ANGpath.

Interesting results were obtained by transferring the gD^{path} gene (*Bam*H1 *j*) fragment of ANGpath DNA) to the nonpathogenic ANG and/or by replacing the gD gene in pathogenic ANGpath by gD^{KOS} (*Bam*H1 fragment *j* of KOS DNA). The details of these experiments will be published elsewhere (Herget *et al.*, 1991; Herget personnal communication). Using these recombinants we have shown that the pathogenicity of ANGpath (gD^- lac Z^+)/gD^{KOS} decreased, while the pathogenicity of the ANG (gD^- lac Z^+)/ gD^{path} was similar to ANGpath w. t. (Rajčáni *et al.*, manuscript in preparation).

Using the deletion mutants ANGpathI2-4 (Neidhardt *et al.*, 1987) and ANGpathgCI-8 (Schranz *et al.*, 1989) we found that the gE negative mutants were pathogenic neither for mice (Rajčáni *et al.*, 1990*a*) nor for rabbits (Kúdelová *et al.*, 1991). The gE deletion mutants did not spread from the portal of entry by neural route and their haematogenic spread was extremely limited (Rajčáni *et al.*, 1990*a*). When testing the quick axonal transport by explanting the trigeminal ganglion within 22 hr after corneal/lip inoculations the gE deleted mutants did not reach the ganglion although the spread of a similar inoculum of ANGpath w.t. to the trigeminal ganglion could be clearly assessed. It seems that the neuritic uptake of the gE^- mutant was impaired in addition to the more limited replication of this mutant in the portal of entry. Because gE is a part of the Fc receptor, when expressed at the surface of HSV-infected cells, gE interferes with cytotoxic and natural killer immune destruction of these cells (Adler *et al.*, 1978; Courtney *et al.*, 1984). In rabbits the gE deletion mutants were nonpathogenic as well (Kúdelová *et al.*, 1991). The axonal spread of the ANGpathI2-4 mutant DNA to the trigeminal ganglion was highly limited as judged by *in situ* hybridization (positive signals in 1 out of 10 ganglia only) and tissue explantation showed no virus reactivation in either of the ganglia examined. It should be mentioned in this context, that direct intracerebral inoculation of the gE negative mutants resulted in encephalitis although there was no spread to the CNS upon peripheral inoculation.

The gC defective mutant ANGpathgC18 (Weise *et al.*, 1987) showed a prolonged but slightly limited viraemic spread and minimal invasion of the CNS (Rajčáni *et al.*, 1990*a*) when inoculated to DBA-2 mice by the i.p. route. The latency competence in DBA-2 mice as well as the axonal transport of two gC other mutants ANGpathgC18 and of KOSgC39 (Homa *et al.*, 1986) appeared to be unchanged or just slightly reduced as compared with the parental strains (Rajčáni *et al.*, 1990*b*). Mannini-Palenzona *et al.* (1988) found no restriction of the capacity of the gC^- mutant of HFEM strain to spread along nerves and to establish latency. Furthermore, acute replication along nerves and to establish latency. Furthermore, acute replication and spread of the

Table 1. List of genes and DNA regions associated with the pathogenicity and virulence of the herpes simplex virus

Location of the region (MU)	Coding capacity for	Function	Possible role for pathogenicity
0.29–0.31 (UL23)	thymidine kinase	thymidine kinase	replication in neurons
0.762–0.789	1.5 kb mRNA	unknown (HFEM del)	colonization of the sensory ganglion, replication in adrenals
0.02–0.04	ICPO (IE3)	transactivator	efficient replication
0.79–0.81	110 kD protein	protein	in the portal of entry
0.835–0.86	ICP4 (IE1)	transactivator	essential for replication
0.96–0.98	175 kD protein	protein	in the portal of entry and for establishment of latency
0.74–0.75 (UL54)	ICP27 (IE2) 63 kD		
0.00–0.02	no ORF	regulatory	invasion of CNS after
0.81–0.83	identified	DNA sequence	peripheral inoculation
0.675–0.690 (UL48)	α-TIF (VP16) 65 kD protein	transinducing factor, initiation of α-gene transcription	replication in neurons
0.036–0.042	2.0 and 1.5 kb	latency-associated	reactivation of latency
0.778–0.784	mRNAs	transcription, protein unknown	
0.413–0.426 (UL30)	DNA polymerase Ori_L	replication of viral DNA	growth at the portal of entry, in neurons and in nonneural cells
0.34–0.37 (UL27)	glycoprotein B (VP7) (C-terminal domain)	penetration to cells	efficient replication at portal of entry and in target tissues
0.93–0.945 (US8)	glycoprotein E(VP12)	Fc receptor preventing natural killing	virus clearance at portal of entry, neuritic uptake
0.906–0.926 (US6)	glycoprotein D	virus neutralization epitope, adsorption and penetration	the ability to invade spinal nerves

HSV-2 gC$^-$ mutant was unaffected after intravaginal inoculation of mice (Johnson *et al.*, 1986) the gC acts *in vivo* as an inhibitor of the alternative complement casçade, namely by interfering with the C3b and by accelerated decay of C3b5b complexes (Fries *et al.*, 1986). Assuming that gC is a C3b receptor for human complement, its affinity to mouse C3b might be different, thus the interaction of gC with the mouse complement system may not be of the same importance as in man. Indeed, in rabbits all ANGpath gC$^-$ mutants inoculated into the cornea showed the same virulence as the parental ANGpath strain (Kúdelová *et al.*, 1991).

The role of the syn loci in invitro cytopathology and pathogenicity

It seems reasonable to assume that polycaryocyte formation would facilitate cell to cell spread of HSV both *in vitro* and *in vivo*, i.e. giant cell formation and neurovirulence should be correlated to each other. Yamada *et al.* (1986) reported that out of 5 variants of the HSV-1 Miyama strain 3 were syn (+GC) and 2 were syn+ (-GC). When comparing plaque size and virulence after i.c. and i.p. inoculations in 3 mouse lines, the variant +GC (LPV) exhibited the largest syncytia and was the most virulent by either inoculation route. This variant lacked the ability to produce gC, an observation being in agreement with the findings of different investigators who examined the gC negative virus mutants. Upon infection by the intranasal route the +GC (LPV) variant spread to the olfactory bulb by the olfactory pathway and along the trigeminal nerve pathway. From the olfactory bulb the virus quickly reached *nc. amygdalae, nc. accumbens,* lateral and frontal pyriform cortices and hippocampus on one hand, and several brain stem nuclei on the other hand (Stroop and Schaeffer, 1989).

A mutation termed syn 1 was located to MU 0.735-0.74 (Bond and Person, 1984), another locus designated syn2 maps close but is clearly distinct of these coordinates. Another syn locus was mapped in the gB gene, close to the C-terminal cytoplasmatic domain (DeLuca *et al.*, 1982; Kousoulas *et al.*, 1984).

Other HSV DNA regions involved in neuropathogenicity

A deletion mutant of HSV-2 strain HG52 termed JH2604 carrying a 15 kb deletion within both inverted repeats flanking the long unique sequence (located at MU 0-0.02 and at 0.81-0.83) which corresponds to the fragment *Bam*HI *v* of HSV-2 (Taha *et al.*, 1989). An additional function spanning from the joint to the ICP4 gene was identified by means of the avirulent recombinant RE6. Neurovirulence could be restored by marker rescue using a fragment mapping from 0.818-0.832 MU. No ORF was assigned to this region between the ICPO and ICP4 genes in the internal repeats on both sides of the L-S joint. This could indicate that this region has a control function.

Table 2. List of some virus strains and their mutants (recombinants) mentioned in this review

Virus strain	Phenotype	Comment
NIH	wild type	pathogenic for mice
F	wild type	pathogenic, widely used in many studies
HFEM	egg and mouse adapted F; deletion at MU 0.762–0.789	nonpathogenic for mice
R-MLC1	HFEM complemented with the *Mlu*I subfragment of *Bam*H1 *b* fragment from F DNA	pathogenicity restored (at least partially, by achieving adrenal gland colonization)
KOS	wild type	moderatly pathogenic for rabbits, nonpathogenic for mice after peripheral inoculation
d1x3.1	ICPO gene deletion	decreased replication in rabbit tissues
KOS 8117 lat	defective in the LAT region	
TB1	LAT defective	
SC16 (Bristol strain)	wild type	highly pathogenic for mice and rabbits
RSC26	deletion at MU 0.413–0.434 in SC16 DNA	pathogenicity decreased
ANG	wild type	nonpathogenic for mice after peripheral inoculation
ANGpath	ANG-derived variant by serial mouse brain passages	pathogenic after peripheral inoculation to mice
Miyama	wild type	pathogenic for mice

Conclusions

Table 1 summarizes DNA regions which encode functions possibly associated with pathogenicity and virulence *in vivo* of HSV. Such functions are the activity of thymidine kinase (TK gene), immediate early transactivator proteins (IE proteins), the initiation protein of transcription (α-TIF), functions associated with replication of viral DNA, functions involved in adsorption and penetration of virions into cells (gB, syn3), interference with virus clearance by natural killing (gE), and some unknown regulatory DNA sequences (between the genes IE3 and IE1). They influence the behaviour of virus at the portal of entry, virus penetration to nerve endings and neuritic uptake, the growth of virus in neurons and nonneural cells of peripheral nerves, and in central nervous system. Presumably this list is not final. Nevertheless, the mentioned functions determining virulence of HSV show some similarities to those of other large enveloped viruses, for example those determining initiation of transcription, viral nucleic acid replication, and the envelope glycoproteins. Table 2 summarizes the mutants most frequently mentioned in this review.

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REPORT UPON THE APPLICATION OF DISCOVERY

Within the framework of proceeding pursuant to the Act of Discoveries, Inventions, Improvement Suggestion and Industrial Patterns No. 84 / 1972 there had been accomplished the state examining procedure concerning the Applications of the Discovery Serial No. PO 20-90 under the title "New Species of Herpesvirus Murium" by the Authors Dionýz Blaškovič, Marta Stančeková, Jarmila Svobodová, Jela Mistríková from the Department of General and Applied Virology, Faculty of Natural Sciences, Comenius University Bratislava on 25th July 1990 with priority from 31st December 1980.

The publication of this Application of Discovery is being performed under the provisions of the Paragraph 17, Section 4 of the aforementioned Act.

1. The object of Discovery is the existence of a new species of the Herpesviridae family, which was called Herpesvirus murium.

2. The subject of Discovery. The properties of the new virus given in the point 1. have been specified by the antigenic differences from all herpesviruses of animals and by differences of their genome structure.

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The report in the Bulletin of the Office of Inventions and Discoveries will be carried out in accordance with the previsions of the Paragraph 17, Section 4 of the said Act on March 18, 1992 Bulletin of the Office of Inventions and Discoveries No. 3/92.

Under the provisions of the Paragraph 18 of the cited Act, everybody is entitled to raise objections to the Diploma on Discovery intended to be granted at the Office within a year of the date of the publication of this Report in the Bulletin of the Office of Inventions and Discoveries, i.e. until the day .....