Journal of NeuroVirology (1996) 2, 225–229
© 1996 Stockton Press All rights reserved 1355–0284/96 $12.00

Guest editorial

The molecular genetics of herpes simplex virus latency and pathogenesis: a puzzle with many pieces still missing

Until the early 1980s, herpes simplex virus (HSV) latency and pathogenesis was studied mostly in rabbits and mice experimentally infected with various strains of virus. Analysis of human tissue was limited to explantation and cocultivation of latently infected ganglia to rescue virus. In the past 15 years, molecular biologic strategies and techniques have helped researchers tackle the vexing puzzle of herpesvirus latency and pathogenesis. The creation of mutant viruses allowed dissection of genes and sequences that affected the kinetics of reactivation and contributed to neurovirulence. Despite the identification of a unique transcript seen in latently infected human and mouse ganglia, and the identification of multiple regions along the HSV genome that confer neurovirulence, the mechanism by which virus establishes latency, reactivates and produces disease is still unknown. We present a concise review of current features, problems and approaches to solving the riddle of HSV latency and pathogenesis.

Latency

The biologic hallmark of the herpesviruses is the ability to produce both lytic and latent infections. Viral latency can be defined both physically and functionally. Physically, viral latency is characterized by the presence of viral DNA in cells without the production of infectious particles. Functionally, latency entails the potential of virus to reactivate and cause disease. Various model systems have demonstrated that the host immune system (Birmanns *et al*, 1993), viral and host transactivating proteins (Steiner *et al*, 1990; Lillycrop *et al*, 1993), viral transcription (Javier *et al*, 1988b), and cellular growth factors (Wilcox *et al*, 1990) can influence the ability of HSV to establish and reactivate from latency (see Steiner and Kennedy, 1995 for review). Despite these efforts, the host and viral factors required for HSV latency have remained elusive.

The molecular characterization of HSV latency began with the identification of abundant quantities of a single viral transcript in latently infected peripheral sensory ganglia (Stevens *et al*, 1987; Deatly *et al*, 1987). These latency-associated transcripts (LATs) map to a 10.4 kb region internal to the repeat sequences flanking the unique long termini and are transcribed antisense with respect to the ICPO gene, overlapping its 3′-end by 700 nucleotides (nt). The LATs are non-polyadenylated (Spivack and Fraser, 1987) and of varying abundance; the most prevalent species is a 2.0 kb transcript containing two open reading frames. The RNA is localized to the nucleus in latently infected neurons (Stevens *et al*, 1987), but a lower abundance cytoplasmic transcript has been identified in infected tissue culture cells (Spivack and Fraser, 1987; Wagner *et al*, 1988).

Several pieces of evidence suggest that the 2.0 kb LAT is a stable intron derived from a larger primary LAT promoter transcript, but not all data are consistent with this intron hypothesis (see Fraser *et al*, 1992; Nicosia *et al*, 1993). A host of minor LAT species arise from the processing of a primary 8.5 kb LAT promoter transcript (Mitchell *et al*, 1990).

The importance of LATs for the establishment and reactivation of latent herpesvirus infection has been investigated in a number of animal model systems. All LAT mutant viruses have been found to replicate efficiently in tissue culture, and to establish and maintain latent infections *in vivo* (Javier *et al*, 1988b; Lieb *et al*, 1989; Steiner *et al*, 1989). Mutant viruses with deletions involving the LAT promoter and upstream region, however, reactivate slowly in both *in vitro* explant assays and *in vivo* (Lieb *et al*, 1989; Trousdale *et al*, 1991; Block *et al*, 1993; Devi-Rao *et al*, 1994). Deletions downstream of the promoter do not affect reactivation (Izumi *et al*, 1989; Ho and Mocarski, 1989; Block *et al*, 1990; Maggioncalda *et al*, 1994). Despite the distinct slow-reactivation phenotype of certain LAT$^-$ deletion mutants, skepticism still remains regarding interpretation of the data, due to questions concerning the adequacy of the animal hosts and the physiologic relevancy of the reactivation assays.

Sawtell and Thompson (1992) have questioned whether the slow reactivation phenotype of certain mutations is secondary to the establishment of latency in fewer neurons rather than a direct effect on reactivation kinetics. Using a LAT promoter to drive β-galactosidase (β-gal) expression, they showed that a LAT$^-$ mutant reactivation phenotype was coincident with a site-dependent decreased β-gal expression in latently infected ganglia. Hence, LAT expression may be important in the establishment of latency in certain ganglia. Unfortunately, serious questions regarding the relevance and efficiency of the heat stress

reactivation assay make interpretation of these interesting results unclear.

In this issue, Maggioncalda *et al* provide an additional entry to the growing list of deletion mutants in the LAT region. The authors constructed a herpesvirus with a small deletion limited to the region upstream of the promoter. The resulting mutant virus established and reactivated from latency with normal efficiency. The authors studied establishment of latency using a novel *in situ* PCR reaction (Mehta *et al*, 1995) and revealed an identical percentage of neuronal cells harboring mutant viral DNA compared with wild-type virus. Most importantly, their results illustrate that normal reactivation kinetics depend solely on the presence of an intact LAT promoter and questionably proximal downstream sequence. Eliminated from further consideration are the 1.8 and 1.1 kb polyadenylated transcripts encoded upstream of the LAT promoter.

What, then, is the relationship between latency and the LAT locus? The answer to this question will come only after dissection of the relationship between the presence of LATs and LAT promoter activity and the latent state as it is defined functionally. Due to prior technical limitations, it was impossible to qualitatively and quantitatively establish the relationship between LAT expression and the presence of viral DNA *in situ*. Because of this limitation, the molecular definition of latency has become inappropriately entwined with the expression of LATs. Recent *in situ* PCR techniques (Ramakrishnan *et al*, 1994; Mehta *et al*, 1995) have illustrated that in ganglia latently infected with the wildtype HSV, a large majority of neurons containing HSV DNA do not express LATs. While these studies are difficult to interpret clearly due to lingering questions regarding PCR efficiency, technique and primer selection, they introduce an invaluable tool to further study and characterize the LAT region. Similar analyses remain to be performed on 'slow reactivating' mutant viruses.

An important and critical question remains regarding the kinetics of reactivation of LAT^{+}- and LAT^{-}-infected neurons. Analyses to date have not substantiated the hypothesized antisense regulatory role of the LATs. While this consideration has not been entirely eliminated due to the essential nature of the ICPO gene, current studies have indicated that the LAT region and particularly the LAT promotor acts as a positive regulator of virus reactivation. This activity of the LAT promoter may reside solely in the ability of local transcriptional activating sequences to permit unwinding of DNA, change DNA conformation, and dictate protein-DNA and protein-protein interactions. Such phenomena have been noted for SV40 DNA transcription and replication (Guo and DePamphilis, 1992; Gruskin and Rich, 1993; Kilwinski *et al*, 1995). The effects of substituting an alternative constitutively active promoter for the LAT promoter may prove quite elucidating!

Pathogenesis

Equally perplexing are neurovirulence and pathogenicity produced by HSV. Like latency, these features appear to depend on many variables. In experimentally infected mice, CNS infection and disease are affected by the strain of HSV, (Stroop and Schaefer, 1987), the route of inoculation (Stroop and Baringer, 1982), the age (Ben-Hur *et al*, 1983) and strain of the mouse, and even the state of cells into which virus is injected. For example, HSV is more virulent and pathogenic when injected into the ear epidermis rather than into the footpad of mice (Sprecher and Becker, 1987).

Neurovirulence does not always correlate with the ability of virus to replicate within the brain, and that further complicates pathogenesis studies. Some HSV strains of low neurovirulence replicate to high titers in the brain, yet do not produce high mortality (Dix *et al*, 1983; Javier *et al*, 1988a).

The use of molecular genetics has not identified a simple specific neurovirulence marker. Multiple regions of the HSV genome contain sequences conferring neurovirulence (Figure 1). Neurovirulence markers have been found between ~107 010 and 130 400 nucleotides (nt) of the viral genome (Centifanto-Fitzgerald *et al*, 1982; Javier *et al*, 1987). Within ~108 123 nt and 120 500 nt is a region which, when deleted, results in a 10 million-fold decrease in HSV neurovirulence (Thompson *et al*, 1983).

In adult mice, expression of the HSV thymidine kinase (TK) gene (Becker *et al*, 1984), located between ~47 802 nt and 46 674, is also important for neurovirulence (Field and Wildy, 1978; Hay *et al*, 1995). Within the HpaI-P fragment of HSV DNA, located at 117 007 to 120 298, is a region that determines intraperitoneal virulence, but does not affect pathogenicity by intracerebral inoculation (Becker *et al*, 1986). The BamHI-L fragment of HSV DNA, located at 107 950 to 113 322 nt, contains sequences that confer neurovirulence for mice by intracerebral inoculation, and within the same region are sequences that encode a virus cell fusion gene (Debroy *et al*, 1985; Ben-Hur *et al*, 1987). Thus, the neurovirulence function within BamHI-L is closely linked to the HSV gene for cell fusion. The

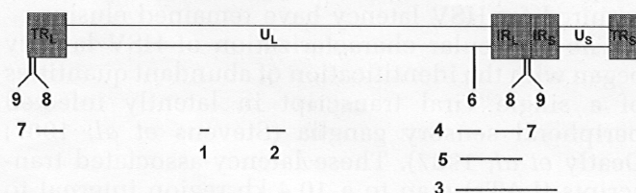

Figure 1 1–9 correspond to nucleotide locations on Table 1.

HSV DNA polymerase gene, located from ~62 807 to 66 517 nt, helps virus to spread in the CNS (Day *et al*, 1988), and to kill after intracerebral inoculation of mice (Field and Coen, 1986). Another region that allows HSV to replicate in the CNS and kill has been mapped between ~125 856 to 125 112 nt (Chou *et al*, 1990). Two further neurovirulence sequences have been identified and map from ~112 179 to 113 193 nt (Ben-Hur *et al*, 1987) and from ~125 213 to 125 972 (MacLean *et al*, 1991). Table 1 summarizes the nt regions of neurovirulence sequences on the HSV genome.

The study of HSV neurovirulence genes now extends to humans. In this issue, Rozenberg and Lebon report results of analysis of HSV glycoprotein (g) D nucleotide sequences in HSV DNA amplified from the CSF of humans with HSV encephalitis. They found 22 mutations among a total of 6580 base pairs (bp) analyzed over a portion of 1000 bp fragments of the HSV gD gene: 20 mutations were silent, but two mutations produced amino acid substitutions; one missense mutation surfaced in two CSF samples as well as in two control laboratory strains. Overall, the results argue against the role of gD in neurovirulence in humans. Of interest is that previous studies in experimentally infected mice have shown that the HSV gD gene increases the ability of virus to gain access to the CNS, but does not affect its ability to produce CNS disease (Izumi and Stevens, 1990).

This issue also contains a study by Ben-Hur *et al* suggesting that sequences within HSV gene 53 enhance virus-induced brain IL-1β gene expression, increase brain prostaglandin E_2 synthesis, and promote hypersecretion of hypothalamic-pituitary-adrenocortical axis function. Together these virus-induced brain-derived cytokines and prostaglandins may contribute to clinical features of acute HSV encephalitis at early stages of the disease before virus burden and inflammation increase. Thus, not only must we take viral genetic factors into consideration regarding production of neurovirulence and pathogenicity, but also we must examine the influence of viral-specific genes on factors released from brain cells.

The plot thickens.

Table I Nucletide locations on the herpes simplex virus genome that confer neurovirulence and pathogenicity

Corresponding figure 1#	*Nucleotide location*	*Reference*
1	~47 802–46 674	Becker *et al*, 1984
2	~62 807–66 517	Day *et al*, 1988
3	~107 010–130 400	Centifanto-Fitzgerald *et al*, 1982; Javier *et al*, 1987
4	~107 950–113 322	Debroy *et al*, 1985; Ben-Hur *et al*, 1987
5	~108 123–120 500	Thompson *et al*, 1983
6	~112 179–113 193	Ben-Hur *et al*, 1987
7	~117 007–120 298	Becker *et al*, 1986
8	~125 213–125 972	MacLean *et al*, 1991
9	~125 856–125 112	Chou *et al*, 1990

Acknowledgements

We thank Dr Randall J. Cohrs for preparing the figure, Dr Ravi Mahalingam for helping to coordinate HSV neurovirulence gene markers, Mary Devlin and Lisa Schneck for editorial review, and Cathy Allen for preparing the manuscript. DHG is supported in part by Public Health Service Grants AG 06127 and NS 32623 from the National Institutes of Health.

Jeffrey L Bennett and Donald H Gilden
Department of Neurology, Box B-182,
University of Colorado Health Sciences Center,
4200 East 9th Avenue, Denver,
Colorado 80262, USA

References

Becker Y, Gilden DH, Shtram Y, Asher Y, Tabor E, Wellish M, Devlin M, Snipper D, Hadar J, Gordon Y (1984). Herpes simplex virus type I thymidine kinase gene activity controls virus latency and neurovirulence in mice. In: *Latent Herpesviruses Infections in Veterinary Medicine.* Wittman G, Gaskell RM, Rziha HJ (eds). Martinus Nijhoff Publishers: Boston, pp 3–19.

Becker Y, Hadar J, Tabor E, Ben-Hur T, Raibstein I, Rösen A, Darai G (1986). A sequence in Hpal-P fragment of herpes simplex virus-1 DNA determines intraperitoneal virulence in mice. *Virology* **149:** 255–259.

Ben-Hur T, Hadar J, Shtram Y, Gilden DH, Becker Y (1983). Neurovirulence of herpes simplex virus type I depends on age in mice and thymidine kinase expression. *Arch Virol* **78:** 303–308.

Ben-Hur T, Asher Y, Tabor E, Darai G, Becker Y (1987). HSV-1 virulence for mice by the intracerebral route is encoded by the BamHI-L DNA fragment containing the cell fusion gene. *Arch Virol* **96:** 117–122.

Ben-Hur T, Rosenthal J, Itzik A, Weidenfeld J (1996). Rescue of HSV-1 neurovirulence is associated with induction of brain interleukin-1 expression, prostaglandin synthesis and neuroendocrine responses. *J NeuroVirol* **2** 279–288.

Birmanns B, Reibstein I, Steiner I (1993). Characterization of an in vivo reactivation model of herpes simplex virus from mice trigeminal ganglia. *J Gen Virol* **74**: 2487–2491.

Block TM, Spivack JG, Steiner I, Deshmane S, McIntosh MT, Lirette RP, Fraser NW (1990). A herpes simplex virus type 1 latency-associated transcript mutant reactivates with normal kinetics from latent infection. *J Virol* **64**: 3417–3426.

Block TM, Deshmane S, Masonis J, Maggioncalda J, Valyi-Nagi T, Fraser NW (1993). An HSV LAT null mutant reactivates slowly from latent infection and makes small plaques on CV-1 monolayers. *Virology* **192**: 618–630.

Centifanto-Fitzgerald YM, Yamaguchi T, Kaufman HE, Tognon M, Roizman B (1982). Ocular disease pattern induced by herpes simplex is genetically determined by a specific region of viral DNA. *J Exp Med* **155**: 475–489.

Chou J, Kern ER, Whitley RJ, Roizman B (1990). Mapping of herpes simplex virus-1 neurovirulence to $\gamma_1$34.5, a gene nonessential for growth in culture. *Science* **250**: 1262–1266.

Day SP, Lausch RN, Oakes JE (1988). Evidence that the gene for herpes simplex virus type 1 DNA polymerase accounts for the capacity of an intertypic recombinant to spread from eye to central nervous system. *Virology* **163**: 166–173.

Deatly AM, Spivack JG, Lavi E, Fraser NW (1987). RNA from an immediate early region of the type 1 herpes simplex virus genome is present in the trigeminal ganglia of latently infected mice. *Proc Natl Acad Sci USA* **84**: 3204–3208.

Debroy C, Pederson N, Person S (1985). Nucleotide sequence of a herpes simplex virus type 1 gene that causes cell fusion. *Virology* **145**: 36–48.

Devi-Rao GB, Bloom DC, Stevens JG, Wagner EK (1994). Herpes simplex virus type 1 DNA replication and gene expression during explant-induced reactivation of latently infected murine sensory ganglia. *J Virol* **68**: 1271–1282.

Dix RD, McKendall RR, Baringer JR (1983). Comparative neurovirulence of herpes simplex virus type 1 strain after peripheral or intracerebral inoculation of BALB/c mice. *Infect Immun* **40**: 103–112.

Field HJ, Coen DM (1986). Pathogenicity of herpes simplex virus mutants containing drug resistance mutations in the viral DNA polymerase gene. *J Virol* **60**: 286–289.

Field HJ, Wildy P (1978). The pathogenicity of thymidine kinase-deficient mutants of herpes simplex virus in mice. *J Hyg (Camb)* **81**: 267–277.

Fraser NW, Block TM, Spivack JG (1992). The latency-associated transcripts of herpes simplex virus: RNA in search of function. [Review]. *Virology* **191**: 1–8.

Gruskin EA, Rich A (1993). B-DNA to Z-DNA structural transitions in the SV40 enhancer: stabilization of Z-DNA in negatively supercoiled DNA minicircles. *Biochemistry* **32**: 2167–2176.

Guo ZS, DePamphilis ML (1992). Specific transcription factors stimulate simian virus 40 and polyomavirus origins of DNA replication. *Mol Cell Biol* **12**: 2514–2524.

Hay KA, Gaydos A, Tenser RB (1995). The role of herpes simplex thymidine kinase expression in neurovirulence and latency in newborn vs. adult mice. *J Neuroimmunol* **61**: 41–52.

Ho DY, Mocarski ES (1989). Herpes simplex virus latent RNA (LAT) is not required for latent infection in the mouse. *Proc Natl Acad Sci USA* **86**: 7596–7600.

Izumi KM, McKelvey AM, Devi-Rao G, Wagner EK, Stevens JG (1989). Molecular and biological characterization of a type 1 herpes simplex virus (HSV-1) specifically deleted for expression of the latency-associated transcript (LAT). *Microb Pathog* **7**: 121–134.

Izumi KM, Stevens JG (1990). Molecular and biological characterization of a herpes simplex virus type 1 (HSV-1) neuroinvasiveness gene. *J Exp Med* **172**: 487–496.

Javier RT, Thompson RL, Stevens JG (1987). Genetic and biological analyses of a herpes simplex virus intertypic recombinant reduced specifically for neurovirulence. *J Virol* **61**: 1978–1984.

Javier RT, Izumi KM, Stevens JG (1988a). Localization of a herpes simplex virus neurovirulence gene dissociated from high-titer virus replication in the brain. *J Virol* **62**: 1381–1387.

Javier RT, Stevens JG, Dissette VB, Wagner EK (1988b). A herpes simplex virus transcript abundant in latently infected neurons is dispensable for establishment of the latent state. *Virology* **166**: 254–257.

Kilwinski J, Baack M, Heiland S, Knippers R (1995). Transcription factor Oct1 binds to the AT-rich segment of the simian virus 40 replication origin. *J Virol* **69**: 575–578.

Lieb DA, Bogard CL, Kosz-Vnenchak M, Hicks KA, Coen DM, Knipe DM, Schaffer PA (1989). A deletion mutant of the latency-associated transcript of herpes simplex virus type 1 reactivates from the latent state with reduced frequency. *J Virol* **63**: 2893–2900.

Lillycrop KA, Estridge JK, Latchman DS (1993). The octamer binding protein Oct-2 inhibits transactivation of the herpes simplex virus immediate-early genes by the virion protein Vmw65. *Virology* **196**: 888–891.

MacLean AR, Ul-Fareed M, Robertson L, Harland J, Brown SM (1991). Herpes simplex virus type 1 deletion variants 1714 and 1716 pinpoint neurovirulence-related sequences in Glasgow strain 17⁺ between immediate early gene 1 and the 'a' sequence. *J Gen Virol* **72**: 631–639.

Maggioncalda J, Mehta A, Fraser NW, Block TM (1994). Analysis of a herpes simplex virus type 1 LAT mutant with a deletion between the putative promoter and the 5′ end of the 2.0-kilobase transcript. *J Virol* **68**: 7816–7824.

Maggioncalda J, Mehta A, Bagasra O, Fraser NW, Block TM (1996). A Herpes simplex virus type 1 mutant with a deletion immediately upstream of the LAT locus establishes latency and reactivates from latently infected mice with normal kinetics. *J NeuroVirol* **2** 268–278.

Mehta A, Maggioncalda J, Bagasra O, Thikkavarapu S, Saikumari P, Valyi-Nagy T, Fraser NW, Block TM (1995). In situ DNA PCR and RNA hybridization detection of herpes simplex virus sequences in trigeminal ganglia of latently infected mice. *Virology* **206:** 633–640.

Mitchell WJ, Lirette RP, Fraser NW (1990). Mapping of low abundance latency-associated RNA in the trigeminal ganglia of mice latently infected with herpes simplex virus type 1. *J Gen Virol* **71:** 125–132.

Nicosia M, Deshmane SL, Zabolotny JM, Valyi-Nagy T, Fraser NW (1993). Herpes simplex virus type 1 latency-associated transcript (LAT) promoter deletion mutants can express a 2-kilobase transcript mapping to the LAT region. *J Virol* **67:** 7276–7283.

Ramakrishnan R, Levine M, Fink DJ (1994). PCR-based analysis of herpes simplex virus type 1 latency in the rat trigeminal ganglion established with a ribonucleotide reductase-deficient mutant. *J Virol* **68:** 7083–7091.

Rozenberg F, Lebon P (1996). Analysis of herpes simplex virus type 1 glycoprotein D nucleotide sequence in human herpes simplex encephalitis. *J NeuroVirol* **2** (this issue).

Sawtell NM, Thompson RL (1992). Herpes simplex virus type 1 latency-associated transcription unit promotes anatomical site-dependent establishment and reactivation from latency. *J Virol* **66:** 2157–2169.

Sprecher E, Becker Y (1987). Herpes simplex virus type 1 pathogenicity in footpad and ear skin of mice depends on Langerhans cell density, mouse genetics, and virus strain. *J Virol* **61:** 2515–2522.

Spivack JG, Fraser NW (1987). Detection of herpes simplex virus type 1 transcripts during latent infection in mice [published erratum appears in J Virol 1988 Feb; 62 (2): 663]. *J Virol* **61:** 3841–3847.

Steiner I, Spivack JG, Lirette RP, Brown SM, MacLean AR, Subak-Sharpe JH, Fraser NW (1989). Herpes simplex virus type 1 latency-associated transcripts are evidently not essential for latent infection. *EMBO J* **8:** 505–511.

Steiner I, Spivack JG, Deshmane SL, Ace CI, Preston CM, Fraser NW (1990). A herpes simplex virus type 1 mutant containing a nontransinducing Vmw65 protein establishes latent infection in vivo in the absence of viral replication and reactivates efficiently from explanted trigeminal ganglia. *J Virol* **64:** 1630–1638.

Steiner I, Kennedy PG (1995). Herpes simplex virus latent infection in the nervous system. *J NeuroVirol* **1:** 19–29.

Stevens JG, Wagner EK, Devi-Rao GB, Cook ML, Feldman LT (1987). RNA complementary to a herpesvirus alpha gene mRNA is prominent in latently infected neurons. *Science* **235:** 1056–1059.

Stroop WG, Baringer JR (1982). Persistent, slow and latent viral infections. *Prog Med Virol* **28:** 1–43.

Stroop WG, Schaefer DC (1987). Severity of experimentally reactivated herpetic eye disease is related to the neurovirulence of the latent virus. *Invest Ophthalmol Vis Sci* **28:** 229–237.

Thompson RL, Wagner EK, Stevens JG (1983). Physical location of a herpes simplex virus type 1 function(s) specifically involved with a 10 million-fold increase in HSV neurovirulence. *Virology* **131:** 180–192.

Trousdale MD, Steiner I, Spivack JG, Deshmane SL, Brown SM, MacLean AR, Subak-Sharpe JH, Fraser NW (1991). In vivo and in vitro reactivation impairment of a herpes simplex virus type 1 latency-associated transcript variant in a rabbit eye model. *J Virol* **65:** 6989–6993.

Wagner EK, Devi-Rao G, Feldman LT, Dobson AT, Zhang YF, Flanagan WM, Stevens JG (1988). Physical characterization of the herpes simplex virus latency-associated transcript in neurons. *J Virol* **62:** 1194–1202.

Wilcox CL, Smith RL, Freed CR, Johnson E Jr. (1990). Nerve growth factor-dependence of herpes simplex virus latency in peripheral sympathetic and sensory neurons in vitro. *J Neurosci* **10:** 1268–1275.