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; Dealy 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 relevance 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, Maggoncalda 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 wild-type 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 for 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 promoter 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 (Cenfianto-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.

Figure 1 1–9 correspond to nucleotide locations on Table 1.
HSV DNA polymerase gene, located from \( \approx 62,807 \) 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 \( \approx 125,856 \) to \( 125,112 \) nt (Chou et al., 1990). Two further neurovirulence sequences have been identified and mapped from \( \approx 112,179 \) to \( 113,193 \) nt (Ben-Hur et al., 1987) and from \( \approx 125,213 \) 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 E₂ 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.

### Acknowledgements

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#### Table 1 Nucleotide locations on the herpes simplex virus genome that confer neurovirulence and pathogenicity

<table>
<thead>
<tr>
<th>Corresponding figure</th>
<th>Nucleotide location</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1</td>
<td>~47,602 – 46,674</td>
<td>Becker et al., 1984</td>
</tr>
<tr>
<td>2</td>
<td>~62,807 – 66,517</td>
<td>Day et al., 1988</td>
</tr>
<tr>
<td>3</td>
<td>~107,010 – 130,400</td>
<td>Centifanto-Fitzgerald et al., 1982; Javier et al., 1987</td>
</tr>
<tr>
<td>4</td>
<td>~107,950 – 113,322</td>
<td>Debrov et al., 1985; Ben-Hur et al., 1987</td>
</tr>
<tr>
<td>5</td>
<td>~108,123 – 120,500</td>
<td>Thompson et al., 1983</td>
</tr>
<tr>
<td>6</td>
<td>~112,179 – 113,193</td>
<td>Ben-Hur et al., 1987</td>
</tr>
<tr>
<td>7</td>
<td>~117,007 – 120,398</td>
<td>Becker et al., 1986</td>
</tr>
<tr>
<td>8</td>
<td>~125,213 – 125,972</td>
<td>MacLean et al., 1991</td>
</tr>
<tr>
<td>9</td>
<td>~125,606 – 125,112</td>
<td>Chou et al., 1990</td>
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### References


