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Guest editorial

Herpes simplex virus replication hits a nerve

In this issue of *JNV*, two papers deal with two distinct aspects of the life cycle of herpes simplex cirus (HSV). In one (Deshmane *et al*, 1995), viral DNA replication, *in vitro*, is studied. In the other (Henken *et al*, 1995), the impact of HSV upon host gene expression, *in vivo*, is examined. Both make valuable contributions and are worthy of attention.

All herpes viruses — from channel cat fish to HSV — are characterized by the ability to establish latent infections in their natural host (Stevens, 1994). Latency is defined operationally as the ability to persist in the host in the absence of clinical manifestations (Fraser *et al*, 1992). The viral and host factors which influence latency have been the subject of intense research.

HSV contains a genome of approximately 150 kb of double stranded DNA which replicates and buds from the nucleus (Roizman, 1989). The genome structure is unusual, in that it contains terminal and internal redundancies. The chromosome can be thought of as two discreet and unique coding regions: a 125 kb long (U_L) and 25 kb short region (U_S), separated by internal and terminal repeat sequences of less than 10 kb (reviewed in Roizman, 1989). Moreover, as isolated from purified virions, the virus genomes actually exist in four equimolar genomic isomers (Sheldrick and Berthelot, 1975; Hayward *et al*, 1975). That is, as a consequence of replication or recombination, the U_L and U_S regions 'invert' with respect to each other, meaning that in some chromosomal isomeric forms two different genes are closer to each other (and possibly promoters) than in others. Only one isomeric form is believed to be replication competent and this dramatic inversion property of the genome is believed to be related to the replication strategy of the virus. Moreover, since only circular (or other endless forms) of HSV 1 genomes are found latently in cells of the central and peripheral nervous system (Mellerick and Fraser, 1987, Rock & Fraser, 1983; Efstathiou *et al*, 1986), the kinetics and maintenance of a specific genome structure with a specific isomeric configuration may have implications for pathogenesis.

The availability of the complete DNA sequence of the viral chromosome (McGeoch *et al*, 1988) and the innovative uses of transfection, expression and genetic systems, has permitted considerable progress in the elucidation of the various viral gene products involved in the viral replication process (eg Mocarski and Roizman, 1982 and reviewed in Chalberg, 1991).

Despite impressive progress in the dissection of the various viral gene products involved in mediating origin dependent viral DNA replication, many questions regarding the physical dynamics of replication are unresolved. More than 15 years ago, it was hypothesized that HSV replicates via a rolling circle mechanism (Becker *et al*, 1978; Jacob *et al*, 1979). More physical support for this model has now been generated. High molecular weight viral DNA, some of which appears to have head to tail concatemers, has been detected within the productively infected cell, and pulse experiments suggest a precursor–cleavage relationship between the high molecular weight and monomer genome length DNA (Jacob and Roizman, 1979; Jacob *et al*, 1979; Jongeneel and Bachenheimer, 1981). However, the exact intermediates expected for a rolling circle mechanism have only recently been detected, and it is in this area that the work of Deshmane *et al* (1995) make the greatest impact. In the rolling circle mechanism, viral DNA which has circularized, supports initiation of replication from a nick followed by the movement of the growing replication fork around the circle to produce head to tail concatomers. These concatomers must then be processed (by endonucleolytic cleavage) into monomer chromosomes and packaged into viral capsids. The length of the concatomers and other details of the process have, until recently, only been hypothesized.

The development of methods for the separation of very large DNA molecules (eg between 100 and 200 kb) from each other by pulse field gel electrophoresis now permit the taking of biochemical 'snap shots' of the process. Garber *et al* (1993) recently reported that the viral chromosome, linear within the virion, quickly circularizes after entering the cell. Several groups have detected the presence of very large HSV DNA containing molecules within the infected cell (eg Severini *et al*, 1994; Zhang *et al*, 1994). The nature of this superstructure, and its probable relationship to the generation of mature, unit length genomes, is only now becoming clear. These super structures appear to be entangled networks, probably reflecting the recombination intermediates which are responsible for production of the chromosomal isomers (Severini *et al*, 1994).

However, Deshmane *et al* (1995) show that the

super structures are probably not very long concatomers, as previously expected. Rather, fine restriction enzyme analysis and quantification of the termini of the high molecular weight structures retained within or near the wells of the gel of the field inversion electrophoresis were performed. The authors have concluded that the amount of precursor (replication intermediate) is, at most, only one to two genome lengths in size. Moreover, only one end is free, providing information about the asymmetric direction of the genome growth or packaging: first the L and then the S end, which is consistent with the mechanisms proposed by Varmuza SL *et al* (1985), Diess and Frankel (1986) and Bataille and Epstein (1994). First the left, then the right foot. Thus, it appears that the conventional wisdom in which long concatomers are systematically processed into multiple unit length molecules, is not correct. Rather, newly replicated and presumably single length genomes are rapidly packaged into capsids, right off the rolling circle. This new information predicts a close temporal and physical coupling of DNA replication and packaging. Perhaps replication during latent infection, if it occurs at all, involves an uncoupling from packaging. In addition, since the L segment appears to be packaged first, during productive infection, perhaps distinct origins of replication are favored during productive and latent phase viral replication. Such possibilities remain to be examined.

The manuscript by Henken *et al* (1995) takes a very different perspective on herpes simplex virus infection. By reporting that HSV-2 infection of peripheral nervous tissue, *in vivo*, results in a significant induction of the neuronal growth associated protein (GAP43), we are reminded that HSV infection is a two way street: as the virus is replicating and executing its own plans, the host is also mounting a response which is a combination of physiological and immunological activities. Thus, although the virus is usually destructive to tissue culture cells, the outcome *in vivo* is more favorable, if not more complex, to the host.

GAP43 has several alias: neuromodulin/pp46/F1/B-50 (Skene, 1989). It is a membrane phosphoprotein associated with synaptic turnover and axonal outgrowth, particularly with regard to regeneration and injury response. Although GAP43 may be expressed in non-neuronal support cells of the CNS as well as the PNS, it is a good marker of neuronal sprouting and is generally located at or near the damaged axonal tip (Woolf *et al*, 1992).

Using a mouse foot pad model, Henken *et al* report that the GAP43 response to HSV-2 infection is first apparent 2 weeks after infection — well after the virus has launched a vigorous productive infection. The GAP43 response is, however, sustained, lasting at least a month after infection. This is a time at which the productive phase of infection has subsided, and latency may have been established. Since host factors probably contribute to the virus' ability to establish latency, one wonders as to the role, if any, of GAP43 in influencing viral pathogenesis.

However, perhaps the most clinically relevant aspect of this work relates to the association of a herpes virus infection with a sprouting response and the possibility of explaining post herpetic neuralgia (PHN) — the haunting pain which often accompanies adult episodes of varicella zoster virus, the herpesvirus cousin of HSV which also makes the PNS its home. Non-viral causes of peripheral nerve injury have been associated with central sprouting (Woolf *et al*, 1992), and it has been hypothesized that regeneration and sprouting of nerves might result in occasional maladaptive placement of fibres that may account for some of the severe pain associated with post herpetic neuralgia. It would be interesting to know if VZV, the agent associated with the most intractable PHN, is also an inducer of GAP43.

In any event, the notion that herpes virus infections should be studied in context is a good one. Thus, although there are many useful models of productive HSV infection, *in vitro*, it is also a good idea to study the non-cytopathic systems, in which both the virus and the host have a chance (Block *et al*, 1994).

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