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Molecular Biology of Herpes Simplex Virus Type 1 Latency in the Nervous System

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Contents

Abstract

Herpes simplex virus (HSV) is one of the best studied examples of viral ability to remain latent in the human nervous system and to cause recurrent disease by reactivation. Intensive effort was directed in recent years to unveil the molecular viral mechanisms and the virus-host interactions associated with latent HSV infection. The discovery of the state of the latent viral DNA in nervous tissues and of the presence of latency-associated gene expression during latent infection, both differing from the situation during viral replication, provided impor-

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tant clues relevant to the pathogenesis of latent HSV infection. This review summarizes the current state of knowledge on the site of latent infection, the molecular phenomena of latency, and the mechanisms of the various stages of latency: acute infection, establishment and maintenance of latency, and reactivation. This information paved the way to recent trials aiming to use herpes viruses as vectors to deliver genes into the nervous system, an issue that is also addressed in this review.

Index Entries: Herpes simplex virus; viral pathogenesis; latent viral infection; viral reactivation; viral pathogenesis; nervous system; gene therapy; viral vectors.

Introduction

Two characteristics distinguish herpesviruses from most other virus families: i) they have a very wide host spectrum; indeed, almost every eukaryotic organism examined has been found to have its own particular herpesvirus (for review *see* Roizman and Sears, 1990); ii) and the herpesviruses are capable of establishing and maintaining latent infections in their hosts (Roizman and Sears, 1987; Stevens, 1989). It is this second feature that provides clear biological advantages for the virus and is responsible for, or at least contributes to, the successful survival of herpesviruses in a large spectrum of organisms. Man is afflicted by several herpesviruses that may cause a variety of disorders, including encephalitis, that are associated with considerable human morbidity and mortality. Moreover, herpesviruses are also responsible for diseases in almost every domestic animal, resulting in serious economic consequences. Accordingly, the question of herpesvirus latency has attracted much attention and has become a focus of intensive research.

Among the human herpesviruses, three herpesviruses, herpes simplex virus types 1 and 2 (HSV-1 and 2) and varicella-zoster virus (VZV), are neurotropic, that is, they establish latent infections in the nervous system and induce tissue damage through viral replication and latency in nervous tissue and reactivation from it (Johnson, 1982). HSV-1 is the best studied example in the group. It infects almost every cell in tissue culture, is relatively easy to propagate, and can be used to produce primary as well as latent nervous system infections in a variety of experimental animals. Most of the latter, currently in use for the purpose of studying in vivo HSV-1 infections, are mice and rabbits. The greater volume of the information pertaining to latency of the neurotropic herpesviruses stemmed initially from studies with HSV-1 in experimental animals. Such data was subsequently substantiated on human tissue obtained at post mortem. Findings were also later extrapolated to studies using HSV-2 (Mitchell et al., 1990c), VZV (Croen et al., 1988), and nonhuman neurotropic herpesviruses, such as pseudorabies virus (Cheung, 1989) and bovine herpesvirus (Rock et al., 1986).

Our intention here is to provide an overview of the current state of knowledge of the cellular and molecular biology of HSV-1 latency in the nervous system and to discuss its pathogenesis. These data may be relevant to the possible use of herpesviruses as vectors for delivery of missing and aberrent genes to nervous system tissue, an issue that will be discussed in the last section of this review.

Definitions

Viral latency is defined operationally as absence of infective virus particles in tissues that harbor the viral genome (Stevens, 1989). Under certain circumstances the virus can reactivate, resume replication, and cause recurrent disease in vivo. However, based on recent molecular biology data, this definition is no longer sufficient for HSV-1 latent state. As will be discussed later, both the structure of the viral DNA and the pattern of its gene expression during latent infection in vivo differ from the situation during viral replication in cell culture. If these molecular phenomena are included in the definition criteria of HSV-1 latency, they will call into question the relevance of currently available models of in vitro latency. Therefore, we will focus our discussion

on the situation in vivo and use available data from in vitro studies with caution. We will also provide evidence that extrapolation from in vitro models or from HSV-1 replication in cell culture may not be valid in relation to certain stages of HSV-1 latency.

The process of HSV-1 latency can be thought of as a continuum. At one end the virus replicates at the primary peripheral site of infection, and at the other end it reactivates from the site of latent infection. For methodological purposes, HSV-1 latency is divided into several stages:

1. Viral replication at the peripheral site of infection.
2. Transport of viral particles to the nervous system: to peripheral sensory ganglia (PSG) and to the central nervous system (CNS).
3. Establishment of latent infection.
4. Maintenance of the latent state for the entire life of the host.
5. Reactivation.

Not all stages are currently amenable for in vivo research through animal models. All available models share a similar basic approach (Fraser et al., 1984), namely the establishment of a primary infection at a peripheral site (cornea, pinna of the ear, and so on) with a relatively nonvirulent HSV-1 strain. Following peripheral inoculation, the virus travels to the respective sensory ganglia and the CNS regions (brain or spinal cord) where it usually replicates. When replication ceases and no more infectious viral particles can be detected, the tissues and the animals may then be examined for the various aspects of the latent state at the phenomenological, cellular, and molecular levels.

HSV-1 Genome Organization and Virion Structure

The herpes virion is made of four structural elements (for review *see* Roizman and Sears, 1990): a core that contains the DNA molecule and is surrounded by a capsid composed of 162 identical capsomers (this number is uniform to all herpesviruses), an envelope that is contributed by the nuclear membrane of the host cell, and a tegument layer that is distributed asymmetrically between the capsid and the envelope and contains amorphous material without distinctive morphological features.

The HSV-1 genome is contained within a double-stranded linear DNA molecule, 152 kb long (Fig. 1 A). It consists of two covalently linked components: long and short units, each flanked by repeat regions. The entire viral genome has been sequenced in strain syn 17^+ (McGeoch et al., 1986,1988; Perry and McGeoch, 1988) and analysis of this sequence has yielded at least 70 open reading frames (ORFs), (Roizman and Sears, 1990). Most of the gene products of these ORFs have been identified and their function determined. Mutations within HSV-1 genes have shown that up to half of the genes may be dispensable for viral replication in cell cultures. Nevertheless, wild-type isolates do not resemble any of the laboratory deletion mutants, suggesting that the dispensable genes might in reality be essential for survival and propagation of HSV-1 in vivo.

The genes coded for by the repeat regions within the genome molecule are diploid. The function and importance of duplicate genetic information within the herpetic genome is unclear, but the repeat sequences may be important for the survival, replication, and latency-competence of HSV-1. First, these regions contain sequences that are essential for viral replication and their deletion results in nonviable viral mutants. Second, the five genes that are synthesized first during viral replication, the immediate early (IE) genes, some of which are essential genes, are clustered around the repeat region. One can assume that either two copies of these genes are required, or that a "safety margin" for essential genes enabled this organization of the viral genome to survive. Third, two of the three origins of replication of the virus are located within the repeats of the short unit, sandwiched between the promoters of IE genes. Thus, it seems of importance that the transcriptional activity that is present during HSV-1 latent infection *(see below)* is also diploid and maps within the long and short repeat regions of the viral genome.

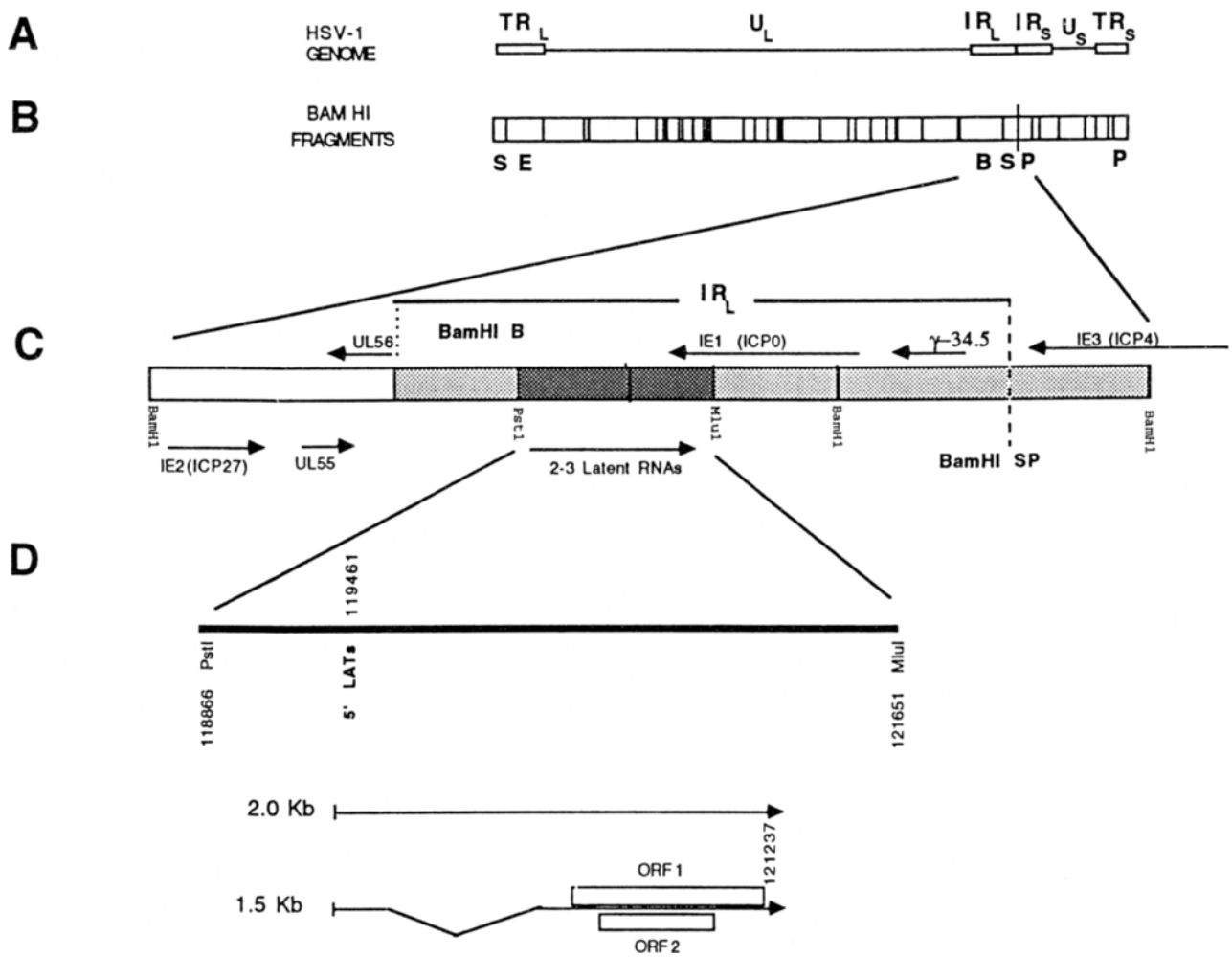

Fig. 1. Herpes simplex virus type 1 (HSV-1) genome organization and location and structure of the viral latency-associated gene expression. **A.** The HSV-1 genome illustrating the unique long (U_L) and unique short (U_S) regions bounded by the terminal and internal repeat regions (TR and IR respectively). **B.** BamHI restriction map of HSV-1. Denoted are the fragments within the repeat regions. **C.** Detailed map of BamHI restriction fragments B and SP. The approximate location of ICP4, γ-34.5, ICP0, UL55, UL56, and ICP27 mRNA are marked by arrows that indicate their transcription orientation. The region of the viral genome that is transcriptionally active during HSV-1 latent infection and is positive by *in situ* hybridization is shaded. The darkly shaded region between PstI and MluI restriction sites hybridized by Northern blot analysis to 2–3 overlapping HSV-1 transcripts that are marked by a single arrow. Also denoted are the location of the internal repeat long (IR_L) and the border between the long and short fragments of the genome. **D.** Detailed map of the PstI-MluI subfragment and the structure of the LATs. The 5' and 3' ends of the LATs and the location of the open reading frames are illustrated.

Acute Infection

The infection of a permissive cell by HSV-1 is initiated by fusion of the viral envelope with the membrane of the host cell and release of viral tegument and capsid into the host's cytoplasm (Fuller and Spear, 1987). Transcription and replication of herpes DNA, as well as the assembly of the viral capsid, take place within the nucleus of the infected cell. The replication cycle of the herpesviruses in cell culture is a highly coordinated process that consists of the temporal sequence of viral gene product synthesis according to function (for reviews, *see* Roizman and Sears, 1987,1990).

The genes encoded within the genome of HSV-1 can be divided roughly into three groups, based on the time of their appearance during viral replication in cells in culture, their function, and the effect of various inhibitory compounds on their expression.

The first group of HSV-1 genes to appear during infection are the IE or α genes, which are expressed prior to viral protein production. Several of the IE genes are essential for viral replication in cell culture. The IE genes are transactivated by a tegument protein, Vmw65, also termed VP16 or alpha transactivating factor—αTIF (Post et al., 1981; Campbell et al., 1984). Vmw65 forms a multiprotein complex that binds and activates a common sequence present within the regulatory elements of all IE genes, the TAATGARAT sequence (R stands for purine) by interacting with cellular proteins, including OCT-1 (O'Hare and Goding, 1988; Preston et al., 1988). The IE genes activate the early (β) genes, many of which encode enzymes involved in nucleotide metabolism. The β genes are dependent on IE gene expression, but do not require prior DNA synthesis. They activate late (γ) genes, which mainly code for the viral structural proteins. Most viral transcripts do not undergo splicing.

When cells in culture are productively infected with HSV-1, the infected cells undergo major structural and biochemical alterations that ultimately culminate in their destruction. Soon after infection, host DNA synthesis is shut off and there is posttranscriptional termination of cellular protein synthesis induced by virus "shut off proteins" (Read and Frenkel, 1983). How far the HSV-1 reproduction cascade can proceed before the process of cell damage becomes irreversible is unclear and may depend on the type of host cell. Although β and γ gene expression seems to cause inevitable host cell death, it has been suggested that the expression of α genes alone may not be deleterious to the host. Thus, manipulation of viral and host factors under experimental tissue culture conditions, e.g., manipulation of temperature or addition of acycloguanisine (acyclovir), may enable viral survival without irreversible cell damage. These conditions, however, do not simulate the normal situation of viral replication in cell culture or in vivo.

Following peripheral inoculation, HSV-1 DNA can be detected in the PSG that innervates the particular peripheral area inoculated. Viral replication at the peripheral site of primary infection is not mandatory for the ability of HSV-1 to reach the PSG since the genomic DNA of mutant viruses lacking the ability to replicate in vivo is also detected in the PSG, and these viral mutants establish latent infection effectively within the ganglia (Steiner et al., 1990; Efstathiou et al., 1989). With or without peripheral replication, the timing of viral DNA appearance at the respective sensory ganglion corresponds with the rate of retrograde axonal flow. It seems, therefore, that following attachment of viral particles to axonal terminals, it is the retrograde axonal flow that mediates viral transport to the nerve cell body (Cook and Stevens, 1973; Kristensson et al., 1986). Other potential routes for HSV spread from the periphery include sequential infection of Schwann cells and lymphatic spread. Sequential infection seems unlikely, since for viral propagation via Schwann cells, replication is mandatory. Schwann cells, however, are resistant to HSV-1 infection (Walz et al., 1976). Moreover, in vivo replication-negative mutants still reach the nervous system. Lymphatic spread seems equally unlikely, since it will not limit establishment of latency to the part of the PSG that innervates the primary site of infection. However, it has been shown that peripheral infection at the territory of the ophthalmic branch of the trigeminal nerve results in detection of viral replication and latency mainly in the section of the trigeminal ganglion (TG) that receives the ophthalmic afferent nerve fibers (Deatly et al., 1988).

Once HSV-1 reaches the peripheral ganglion, it can resume replication. During acute infection it infects neurons as well as satellite cells in the PSG and it appears that many cell types within the ganglion can sustain viral replication. The initiation of replication is dependent, among others, on the presence of sufficient amounts of Vmw65 that reach the ganglion to initiate the replication cascade. This has been demonstrated with the help of viral mutants. The HSV-1 mutant *in*1814 contains a 12-bp insertion into the coding sequence of Vmw65 that abolishes part of its IE transactivating function but does not affect its

structural property as part of the viral tegument (Ace et al., 1989). This mutant is unable to replicate in vivo (Steiner et al., 1990), but maintains, though defectively, its ability to replicate in cell culture (Ace et al., 1989). During viral attachment at the periphery, the envelope of HSV-1 is lost and the remains of the virion, capsid, and tegument enter the axon. As a result, part of the tegument layer, including some Vmw65 proteins, is lost through the transport of the virus to the cell body. The studies with the *in*1814 mutant therefore suggested that only when sufficient amounts of Vmw65 reach the cell body replication ensues in the nucleus, resulting in cell destruction. Lack of Vmw65 aborts replication at a very early stage of infection (Valyi-Nagy et al., 1991a), and enables the infected cell, and therefore the HSV-1 genome within it, to survive.

Following a full replication cycle in PSG neurons, infectious viral particles can leave the neuron to infect satellite cells within the ganglion, and proceed via retrograde axonal transport to the CNS where the same pattern of replication takes place. In the nervous system the virus can also spread transneurally via synapses. The latter feature has been used to map neuronal circuits with the aid of labeled HSV-1 (Kuypers and Ugolini, 1990).

Although the cause of host death during primary infection is poorly understood, mortality in animals that succumb to the primary infection corresponds with the peak of viral replication within the CNS. After a period of 10–14 d following primary infection, no replicating or infectious virus can be detected in the nervous system of the infected animal and the virus enters a latent state.

The Site of Latent Infection

It has been established beyond doubt that HSV-1 can reside in latent form within the nervous system. Long before the infectious agent responsible for cold sores was identified, studies suggested that trigeminal nerve trauma can induce peripheral herpetic vesicles (Cushing, 1905). In 1929, Goodpasture proposed that "...the virus remains in the ganglia in a latent state after the local lesion has healed." Many years later, Stevens and colleagues (Stevens and Cook, 1971; Stevens et al., 1972; Cook and Stevens, 1973) were the first to succeed in isolating HSV from PSG. This was a breakthrough achievement that was since reproduced by other laboratories working on human (Baringer and Swoveland,1973; Bastian et al., 1972; Plummer, 1973; Rodda et al., 1973) and experimental animal (Plummer, 1973) trigeminal and spinal ganglia. The spectrum of nervous tissue that can harbor latent HSV-1 is wider and includes the autonomic nervous system (Price et al., 1975), the brainstem (Deatly et al., 1988; Rock and Fraser, 1983; Dobson et al., 1990), and the cerebral hemispheres (Fraser et al., 1981).

The possibility of extraneural latency has also been addressed. In experimental animals, HSV latency was demonstrated in the skin (Clements and Subak-Sharpe, 1988), cornea (Abgharis and Stulting, 1988), and adrenal medulla (Stevens, 1978). A recent report suggests that HSV-1 may reside in a latent state within human corneas (Kaye et al., 1991). However, it is widely accepted that herpes simplex latency is almost an exclusive feature of nervous tissue and that disorders caused by reactivation result from reactivation from this tissue.

A question equally important to the issue of the type of tissue involved in HSV-1 latency concerns the types of cells within the nervous tissue that can harbor latent viral DNA. In PSG, HSV-1 resides in sensory neurons (Cook et al., 1974; McLennan and Darby, 1980; Kennedy et al., 1983). The type of latently infected cell in the CNS has not yet been characterized, although under experimental conditions it was possible to demonstrate HSV-1 latency in neurons within the CNS trigeminal system (Deatly et al., 1988) or the motor neurons of the hypoglossal nucleus (Dobson et al., 1990). The possibility of latency in nonneuronal cells has also been suggested by a study that demonstrated VZV transcripts in nonneuronal ("satellite") cells of human TG using *in situ* hybridization techniques (Croen et al., 1988). However, *in situ* hybridization of HSV-1 nucleic acids is limited at present to RNA detection *(see below)*. Therefore, this technique

cannot rule out latency in cells that harbor latent viral DNA but do not express viral-specific genes. Future *in situ* hybridization of HSV-1 DNA may help to better characterize the cell type(s) that maintain HSV-1 DNA.

Establishment of Latent Infection

Two avenues are available for the virus to reach the neuronal cell body in order to establish latent infection: It may reach it from the periphery traveling along the neuron's axon, or enter it after replicating in an adjacent cell. Since HSV is a lytic virus, initiation of a replication cycle will eventually shut off cellular protein synthesis and result in cell death. Therefore, by definition, latency cannot be established in cells in which the virus has completed a full replication cycle (Steiner and Kennedy, 1991). Factors, both viral and cellular, that interfere with viral replication will thus favor the establishment of latency. For example, Vmw65 can play a pivotal role in the outcome of the infection for the host cell and the ability of HSV-1 to enter a latent state. As mentioned earlier, during attachment of HSV-1 to the axonal membrane, the virus loses its envelope, and the contents of the tegument as well as the capsid are transported to the neuronal nucleus (Lycke et al., 1984,1988). The amount of Vmw65 that is not lost during this step and is being carried with the virus to the cell nucleus will eventually determine the fate of the infection. If sufficient amounts will reach the neuronal soma, a replication cycle will ensue and hence cell destruction; if the amounts of Vmw65 are insufficient, no replication cascade will begin and latent infection will be formed. Another factor that might influence the ability of HSV-1 to replicate and therefore affect its ability to form a latent infection is the amount of inoculated virus (Fraser et al., 1991), analogous to the importance of the multiplicity of infection (MOI) for the ability of HSV-1 to replicate in cells in culture.

Currently we do not know which viral functions are required for the establishment of latency and if any viral genes are expressed at this stage. The circularization or the concatemerization of the viral DNA (*see* next section) resembles the situation in cell culture immediately following infection. This, however, does not require *de novo* protein synthesis (Poffenberger and Roizman, 1985). Despite previous claims, neither thymidine kinase (Efstathiou et al., 1989; Coen et al., 1989) nor the IE gene, ICPO (Lieb et al., 1989a; Clements and Stow, 1989), are essential for establishment of latency, but the defective replication of mutants with these or similar deletions may render the reactivation incompetent. It has been suggested that ICP4 and ICP27, two IE genes that are essential for viral replication, are mandatory for the establishment of latency (Lieb et al., 1989a). However, this observation is called into question by the ability of the mutant virus *in*1814 with its defective IE expression, to establish latency and reactivate (Steiner et al., 1990; Valyi-Nagy et al., 1991a).

Since latency is established primarily, if not exclusively, in neurons, it appears that some host and cellular properties of the neuron might favor latency by arresting viral replication. Several reports suggested that neurons, at least in culture, express factors that have an inhibitory effect on expression of HSV-1 IE genes (Ash, 1986; Kemp et al., 1990; Wheatley et al., 1991) and therefore arrest HSV-1 replication at an early stage that is not associated with irreversible cell damage.

Molecular Phenomena of Latency: Viral DNA Structure, Location, and Organization

Analysis of HSV-1 DNA extracted from brainstems of latently infected mice by Southern blotting have shown that the entire HSV-1 genome is present (Rock and Fraser, 1983,1985). Thus, the possibility that large mutations within the genome are required for latency appears to have been refuted. Nevertheless, small genomic changes are unlikely to be discovered by this technique. Although the HSV-1 genome is linear, following infection of cells in culture it circularizes and DNA replication culminates in the formation of concatamers (Poffenberger and Roizman, 1985). Southern blot analysis of latent HSV-1

DNA from mice and human TG and from brainstem tissue demonstrated the absence of the ends of the viral genome within the terminal repeats (Rock and Fraser, 1983; Efstathiou et al., 1986). Also, the molarity of the joint repeat fragments of the viral genome during latency has been calculated to be twice the molarity of the fragments within the unique regions (Rock and Fraser, 1983,1985). By the level of detection of this technique it follows that most, if not all, latent HSV-1 DNA is maintained either as a circular molecule or in a concatameric form. Cesium chloride buoyant-density gradient centrifugation permitted the separation of mammalian and viral DNA and led to the conclusion that during latent infection HSV-1 is not integrated into the host cell genome (Mellerick and Fraser, 1987). Partial micrococcal nuclease digestion demonstrated that latent HSV-1 DNA is associated with nucleosomes, similar in pattern to host nuclear chromatin structure (Deshmane and Fraser, 1989). The role of the chromatin formation (which is not present during viral replication in vivo) in the establishment of latent infection and for the long term stability of the viral genome within the nucleus is unclear.

DNA methylation has been correlated with transcriptional inactivity (for review *see* Yisraelli and Szyf, 1984), and is considered one of the mechanisms regulating gene expression in eukaryotic systems. Since most of the HSV-1 genome is transcriptionally silent during latent infection, the extent of methylation of the latent viral DNA might influence or contribute to its transcriptional restriction. Several studies examined the methylation of HSV-1 DNA, but the results from in vitro and in vivo systems are conflicting and the limitations of the techniques do not enable any firm conclusions to be drawn. In an in vitro latency model, HSV-1 DNA was not methylated during the replication phase, but was heavily methylated during the "latent" phase (Youssoufian et al., 1982). On the other hand, DNA extracted from latently infected tissue in vivo was not extensively methylated (Dressler et al., 1987). Nevertheless, the technique used was unable to examine specific regions of the viral genome and the estimate of overall methylation does not necessarily represent subfragments that might be important and contribute to the functions of latency and reactivation.

Attention has been focused on the number of viral copies per latently infected cell. From quantitative blot hybridization on human and mouse nervous tissue, estimates range from 0.01 to 1.0 genomes per cell (Cabrera et al., 1980; Puga et al., 1978; Rock and Fraser, 1983; Efstathiou et al., 1986). However, since neurons comprise only a portion of cells in the nervous tissue, and since probably only some of the neurons contain latent HSV-1, even these are probably underestimates. The importance of the viral DNA copy number per latently infected cell is not clear. It has been suggested (Roizman and Sears, 1987,1990; Fraser et al., 1991) that a smaller copy number is associated with viral inability to initiate replication within the nucleus and a larger copy number per cell will facilitate the viral ability to reactivate. This is an extrapolation from the in vitro situation, where certain mutations (e.g., within Vmw65, [Ace et al., 1989], or α22, [Sears et al., 1985]) render the virus replication deficient, a feature that can be overcome by increasing the MOI.

One of the techniques to identify cells that harbor HSV-1 DNA is *in situ* hybridization for HSV-1 RNA *(see below)*. Noteworthy, however, is the fact that no one has yet succeeded in identifying HSV-1 DNA by this method, apparently because of the high G-C content of the HSV-1 genome, which requires extreme physical conditions for hybridization with resultant destruction of tissue architecture and morphology. Nevertheless, the possibility that the DNA is present in some form that is not readily accessible to this method should also be considered.

Molecular Phenomena of Latency: Gene Expression

No infectious viral particles are present during latent infection. Therefore, early reports suggesting the presence of some viral gene activity during latency (Galloway et al., 1978,1982; Puga et al., 1978) attracted much interest. The consis-

tent evidence for latent phase transcriptional activity has become the focus of much experimental work on HSV-1 latency within recent years. Two main technologies, *in situ* hybridization and RNA (Northern blot) analysis, were used initially, and enabled the characterization and mapping of the region of the viral genome that is transcriptionally active during latency (Fig. 1C). By *in situ* hybridization the viral region that gives rise to a positive signal is located within the repeat regions (Stevens et al., 1987,1988; Deatly et al., 1987; Croen et al., 1987; Steiner et al., 1988; Gordon et al., 1988) within restriction fragments BamHI B and SP (Figs. 1B–C). It comprises a fragment of 10.4 kb and since it is diploid it covers about 13.5% of the entire genome. In PSG, morphological criteria enabled investigators to identify neurons as the type of cell harboring HSV-1 transcripts. When a positive signal is present in other tissues, such as the CNS (Stroop et al., 1984: Deatly et al., 1988), identifying the nature of the positive cell is much more difficult. The intensity of the signal by *in situ* hybridization varies. Part of the positive region, about 2 kb in size, gives a relatively stronger signal than the rest (Fig. 1C). The RNAs with the weaker signal are identified only by *in situ* hybridization and were termed minor hybridization RNAs, minor latency-associated transcripts (Mitchell et al., 1990a), or LAT 2 in contrast to the LATs that were detected also by Northern blot analysis and were termed LAT 1 (Roizman and Sears, 1990). In ganglion cells, most, if not all, of the hybridization signal is identified within and around the cell nucleus. The rest of the viral genome, including the territory coding for Vmw65, is negative by *in situ* hybridization.

Once it had been established that HSV-1 transcriptional activity during latency mapped to the repeat regions of the viral genome, the next step was to attempt to determine the relationship of these transcripts to other genes encoded within the same region on the viral genome, especially the IE genes, and to understand whether these genes are active during viral replication in cell culture or if they are latency-specific. Northern blot hybridization analysis was very useful in addressing these questions in experimental animals (Spivack and Fraser, 1987; Rock et al., 1987) and later in ganglia obtained from humans post mortem (Steiner et al., 1988, Krause et al., 1988). RNA extracted from latently infected TG of mice, guinea pigs, rabbits, and humans subjected to this technique revealed two or three colinear transcripts: a larger, 1.8–2.2 kb in size, and another one, that might in fact be a doublet of approx. 1.5 and 1.45 kb (Fig. 1C–D). These transcripts were termed LATs. They are transcribed in opposite direction to the IE gene ICP0 (Fig. 1C) and overlap the ICP0 message in its 3' end by approx 700 bp. This is not unusual for HSV-1, since genes are coded by its two opposite DNA strands. The other genes within the region positive by *in situ* hybridization, identified and characterized during HSV-1 replication cycle in cultured cells, such as ICP0, ICP27, ICP4, and γ34.5, were not detected. Of the three LATs, only the largest can be identified during the viral replication cycle in cell culture and in much smaller amounts than those present during latency (Spivack and Fraser, 1987; Krause et al., 1988). Moreover, the amounts of the LATs in the tissue were examined for 60 d following acute infection and were found to increase within this time period (Spivack and Fraser, 1988a). Whether this is caused by increased production, decreased degradation, or a combination of both is unclear, but some evidence suggests that the LATs are very stable RNAs. The LATs do not belong to any of the three HSV-1 gene classes: they are expressed in vivo prior to any RNA synthesis (and thus are not β or γ genes), but at least the 2 kb transcript is dependent on HSV-1 DNA and protein synthesis in cell culture, and hence does not belong to the α genes. It was therefore suggested that they are regulated differently than any other HSV-1 genes, and the term δ genes was proposed (Spivack and Fraser, 1988b).

The regulatory elements and the promoter of the LATs were mapped using several in vitro and in vivo approaches, including chloramphenicol acetyltransferase (CAT) assays and insertion of β–galactosidase and β–globin genes into HSV-1 recombinant mutants as well as deletion mutants used to study LATs' function. The LAT promoter was identified at an unusual distance from the 5' end of the 2 kb LAT, 670 bp upstream (Batchelor and O'Hare, 1990; Dobson

et al., 1989; Zwaagstra et al., 1989,1990). This may indicate either another unusual characteristic of the latency associated genes or that the coding start site is located upstream to the LAT 5' end, within or at the beginning of the region positive by *in situ* hybridization and coding for the minor hybridizing RNA part upstream to the LAT. It seems that this promoter controls the entire region transcribed during latency since HSV-1 mutants with deletions that eliminate this promoter do not have any positive signal either by Northern blot analysis or by *in situ* hybridization (Steiner et al., 1989; Mitchell et al., 1990b). The activity of this promoter is increased in neuronal cells in culture (Batchelor and O'Hare, 1990; Zwaagstra et al., 1990; Devi-Rao et al., 1991) and it is repressed by the IE gene ICP4 (Batchelor and O'Hare, 1990).

The 2.0 kb LAT transcript is unspliced, the other 1.5 and 1.45 LAT transcripts are spliced products of the larger transcript (Fig. 1D, Wagner et al., 1988b; Wechsler et al., 1988; Spivack et al., 1991). Using a combined approach of cDNA and RNA polymerase chain reaction (PCR) cloning of RNA obtained from latently infected tissue, the splice acceptor site for a 559 nucleotide intron in HSV-1 strain F was located at about 840 nucleotides downstream to the 2 kb 5' end (Spivack et al., 1991). The 5' end of this intron in HSV-1 strains F, 17^+, and KOS is GC (Spivack et al., 1991) instead of the consensus GT (Mount, 1982), and thus may serve as an inefficient splice signal during viral replication in cells in culture and nonneuronal tissue. This may explain the inability to identify the smaller LATs during HSV-1 replication in cell culture. Since only one LAT intron was identified, the reason for the size difference between the 1.5 and the 1.45 LATs is currently unknown.

It has been suggested that the 2 kb LAT is a stable intron derived from a larger transcript (Farrell et al., 1991; Devi-Rao et al., 1991), probably the RNA that is identified by *in situ* hybridization (the minor hybridizing RNA). This argument is based on the identification of a splice donor site around the 5' end of the 2 kb LAT and by showing that it can be used for splicing in various recombinant transcripts (Farrell et al., 1991), and on the fact that the 2 kb LAT is uncapped (Devi-Rao et al., 1991). Nevertheless, nuclear accumulation of transcripts is not a characteristic of introns, nor is it a feature of HSV-1 introns. It is also very unusual for introns to be further processed, as is the case with the 2 kb transcript, which is processed to form the smaller LATs. It is not clear what accounts for the stability of the LATs but of note is the observation that an HSV-1 construct with a mutation within the 2 kb LAT (Block et al., 1990), which does not abrogate its function *(see below)*, does not result in the accumulation of transcripts during latent infection. It was therefore suggested that the features responsible for LAT stability are mediated by its original sequences.

The significance of the hybridization signal that is identified only by *in situ* hybridization during latent infection and its relationship to LATs is unclear. Several studies suggested the presence of a larger, 8.3–8.5 kb, unstable transcript (Dobson et al., 1989; Zwaagstra et al., 1990; Devi-Rao et al., 1991) that is rapidly processed to form the LATs, as well as an additional 6.5 kb transcript. Therefore these transcripts may be identical to the minor hybridizing RNA (Mitchell et al., 1990a). The LATs and the minor hybridizing RNA differ in their polyadenylation. Although the LATs are mainly present in the unpolyadenelated fraction of RNA obtained from latently infected tissue (Spivack and Fraser, 1987; Wagner et al., 1988a), the unstable larger transcripts, when examined during viral replication in cell culture, are poly $(A)^+$ (Devi-Rao et al., 1991). Accumulating data suggests that the LATs and the minor hybridizing RNA share the same promoter that accounts for all latent-phase transcriptional activity:

1. The nearest consensus upstream LAT promoter is located at the beginning of the minor hybridizing RNA signal (Batchelor and O'Hare, 1990);
2. Mutant viruses defective in this region do not express any transcripts during latency (Lieb et al., 1989b, Mitchell et al., 1990b); and
3. Deletion within the LAT promoter region resulted in absence of latent phase transcription in cell culture (Devi-Rao et al., 1991).

Molecular Phenomena of Latency: Gene Products

Early studies that reported the presence of viral proteins during latent infection, such as ICP4 (Green et al., 1981) or thymidine kinase (Yamamoto et al., 1977), have not been reproduced and attempts to identify HSV-1 gene products during latent infection in vivo have failed. However, the discovery of the latent phase transcriptional activity has stimulated a search for the possible gene products of the latency-associated gene expression. By *in situ* hybridization most of the signal is observed within and around the nucleus of the cell. Therefore, one of the key questions that is yet to be resolved is whether these transcripts leave the nucleus during latent infection and are transported to the cytoplasm (Wagner et al., 1988b) where they might be translated. Since the LATs are involved in the reactivation process *(see later)*, these transcripts might be translated only during reactivation, and therefore no LAT-specific gene products would be present in latently infected tissue. Although it was suggested that the coding sequence of the LATs would not predict the presence of ORFs (Perry and McGeoch, 1988), analysis of the DNA sequence in several HSV-1 strains has revealed at least two potential ORFs (Fig. 1D, Wechsler et al., 1989; Spivack et al., 1991). ORF1 and ORF2 are located at the 3' end of the LATs beyond its presumed intron, which permits their translation. This splicing includes 5 AUG (translation initiation codon) raising the possibility that a potential protein will not be translated from the original 2 kb transcript but from the smaller transcripts after intron removal (Spivack et al., 1991). Another possibility is that a polypeptide is coded for by the LAT intron.

LATs gene products have been examined in vivo and in vitro. Hitherto the in vivo studies have yielded negative results whereas the relevance of the findings in the in vitro systems is questionable. Antibodies raised against synthetic oligopeptides that were generated using the DNA sequence coding for the LATs failed to identify LATs products in latently infected tissue (Wagner et al., 1988a). Moreover, an analysis of the differences in protein profiles between HSV-1 strains capable of expressing the LATs and mutant viruses devoid of it, did not enable the identification of specific LATs-encoded proteins as well (Wroblewska et al., 1991).

Doerig et al. (1991a) looked for LATs gene products in an in vitro latency model that was developed by Wilcox et al. (1987, *see below*) and uses cultures of fetal peripheral sensory neurons treated with acyclovir prior to infection. An 80-kd protein was detected in this system and was presumed to be coded by ORF2 (Fig. 1D), although this ORF would be expected to encode for a 33-kd protein. In vitro translation systems were based on analysis of the sequences coding for the LATs and the two ORFs (Spivack et al., 1991). It was proposed that the intron sequence (located upstream to the ORFs) may inhibit the translation and that its removal facilitates the translation from this region. When the entire LAT coding region was cloned into an in vitro transcription-translation vector, no polypeptides were generated (Spivack et al., 1991) . However, in this in vitro system, a LAT subfragment located downstream to the intron region produced several polypeptides, raising the possibility that the LATs can potentially encode for more than a single gene product.

In conclusion, no latent-phase HSV-1 gene products (LATs-coded or any other) have been identified in vivo, and the in vitro systems findings still await confirmation in animal models. At present, the possibility that no polypeptides are encoded by the latency-associated gene expression seems plausible.

Function and Possible Mechanisms of Action of the Latency-Associated Gene Expression

HSV-1 mutants and HSV-1/HSV-2 intertypic recombinants have been used to examine the function of the LATs and the transcriptional activity of HSV-1 during latent infection (Steiner

et al., 1989; Javier et al., 1988; Ho and Mocarski, 1989; Lieb et al., 1989a and b; Sedarati et al., 1989; Hill et al., 1990; Trousdale et al., 1991). These mutants were also useful tools for mapping the exact location of the regulatory elements of the latent-phase transcriptional activity and the relationship between the LATs and the minor hybridizing RNA.

1704 is an HSV-1 variant (derived from strain 17^+) that has two deletions: In one copy of the LATs the promoter region is missing and in the other the coding sequence of the LATs is missing (MacLean and Brown, 1987; Fazil et al., 1991). This virus does not express the LATs or the minor hybridizing RNAs, and replicates normally in cells in culture. Following peripheral inoculation it is transported to the TG, replicates there like its parental strain 17^+, establishes latent infection effectively in PSG, and maintains it (Steiner et al., 1989). Nevertheless, this virus shows very delayed and asynchronous reactivation kinetics on ganglion explantation (recovery of infectious HSV from explanted tissue) when compared to 17^+ (Steiner et al., 1989) and reactivates poorly in vivo in the rabbit corneal model (Trousdale et al., 1991). Likewise, an HSV-1/HSV-2 recombinant with a deletion within the promoter region had an impaired in vivo reactivation ability (Hill et al., 1990), and a mutant virus generated from HSV-1 strain KOS that lacks the ability to express the LATs had a reduced ability to reactivate following explantation (Lieb et al., 1989b).

Two additional HSV-1 mutants that do not express the LATs and replicate in cell culture and in mice, and that establish latent infections but reactivate with normal kinetics (i.e., similar to parental strains), have been studied. First, RH142 has β-galactosidase gene insertion at nucleotide 136 downstream to the 5' end of the 2-kb LAT in HSV-1 strain F, with a 363-bp deletion that removes the splice donor site of the 2-kb LAT intron, but with the two ORFs intact (Ho and Mocarski, 1989). Second, TB1, generated from HSV-1 strain HFEM (which has a 4.1 deletion in the internal long repeat region that eliminates one of the two LAT copies), contains a replacement with phage λ DNA that removes part of ORF1 downstream to the LAT intron and leaves ORF2 intact (Block et al., 1990). Although TB1 was capable of producing a truncated LAT in cell culture, no transcripts accumulated during latent infection.

Several conclusions can be drawn from the phenotype of these mutants:

1. The LATs participate in reactivation by augmenting the speed of explant reactivation and may be essential for reactivation in vivo.
2. This function is not mediated by the region of the genome located upstream to the intron within the 2 kb LAT.
3. Although LAT stability is controlled by its coding sequence, this stability is not mandatory for LATs function in normal reactivation.
4. If LATs function is mediated via a gene product, it is not encoded by ORF1.

Whether the type of organism or the site of infection play a major role in LATs function is as yet unclear. An HSV-1/HSV-2 recombinant had normal reactivation from mice dorsal root ganglia (Sedarati et al., 1989), and a LAT(-) KOS mutant reactivated normally from lumbosacral ganglia whereas its reactivation from TG was still defective (Sawetell and Thompson, 1992). This study demonstrated more latently infected cells in TG of mice infected with wild-type virus than with the LAT(-) KOS mutant, and suggested that the LATs may also participate in establishment of latency.

Although the general function of latency-associated gene expression has been elucidated, the mechanisms that mediate this function are still unknown. Theoretically, these genes could either act via a gene product or their function could be the outcome of action of a functional RNA. The 3' end of the LATs overlaps with that of ICP0. Therefore, it was suggested that the LATs may act via an antisense mechanism (Stevens et al., 1987). Although this hypothesis could already be discounted on theoretical grounds, results with HSV-1 LAT(-) mutants have excluded this possibility experimentally: These mutants do not differ from parental strains in their ability to replicate in cell culture or in vivo, and they are capable of establishing latent infection. More important, the reactivation kinetics of these mutants is not stimulated as it should have been in the case of the

absent antisense inhibition of ICP0, but on the contrary their reactivation is either impaired or unaffected.

Reactivation

HSV reactivation in humans, with resultant cold sores or genital lesions, can be triggered by conditions that include local stimuli, such as injury to tissues innervated by the neurons harboring latent infection, or by systemic conditions, including exposure to sun, fever, emotional stress, and menstruation (Hill, 1975). It is noteworthy that even after numerous, repeated bouts of reactivation, most patients do not exhibit permanent sensory loss or any other neurological deficit in the affected dermatomes (Gominak et al., 1990). It may therefore be assumed that reactivation is not associated with a significant destruction of latently infected neurons and must differ from the process of HSV-1 lytic replication observed in cell culture systems.

In animal models, too, the triggers culminating in in vivo reactivation consist of either local trauma or systemic manipulations including the administration of various drugs (Harbour et al., 1981; Hill et al., 1975; Shimomura et al., 1983; Willey et al., 1984, and others). However, our understanding of the molecular phenomena associated with the transition from the latent state to reactivation is hampered by the lack of a reproducible and efficient in vivo reactivation model. The two experimental animals in use, the mouse and the rabbit, have several disadvantages, such as spontaneous reactivations in the rabbit and low in vivo reactivation yield in the mouse, which have prevented their use in the study of the molecular concomitants of reactivation (Hill et al., 1978; Nesburn et al., 1967). A key unanswered question from the viral end is how replication begins in the absence of Vmw65 protein to stimulate the early stages of the HSV-1 replication cycle. Such replication does take place, since productive infection can ensue from the HSV-1 genome before the expression of γ genes. Moreover, lack of functional Vmw65 protein does not prevent HSV-1 replication in cell cultures (Ace et al., 1989), or the explant reactivation of *in*1814 from latently infected mouse TG (Steiner et al., 1990). However, it should be emphasized that in vivo reactivation of *in*1814 has not yet been examined. It has been proposed that an increase in the number of HSV-1 genomes per latently infected cell can overcome the lack of Vmw65 in a manner similar to the ability of increased MOI to facilitate replication of *in*1814 in cell culture (Roizman and Sears, 1990). Several compounds, such as hexamethylene bisacetamide and dimethyl sulfoxide (DMSO), are capable of enhancing explant reactivation of HSV-1 (Whitby et al., 1987; Bernstein and Kappes, 1988; Lieb et al., 1989a). These compounds also improve the replication of *in*1814 in cell culture (McFarlane et al., 1992), thus substituting the transactivating function of Vmw65. In other systems, these compounds were shown to stimulate transcription (Campbell et al., 1990), and it was proposed (McFarlane et al., 1992) that their effect on *in*1814 replication is mediated via stimulation of IE transcription. It seems, therefore, that a crucial step in the ability of HSV-1 to reactivate is the presence of some viral or cellular factors that supplement the initial lack of Vmw65 to transactivate IE gene expression.

Most of the work on cellular mechanisms associated with reactivation has been performed in vitro on neuronal cells by using two related approaches: determining the host and environmental factors that enable reactivation by substituting for absent viral functions, such as Vmw65 transinducing function; and utilizing a strategy based on the operational hypothesis that the latent state of the virus within neurons can be viewed as transiently nonpermissive for viral replication (Roizman and Sears, 1987). One can therefore search for cellular factors that might switch a latent cell from a nonpermissive to a permissive state (Wilcox and Johnson, 1987; Valyi-Nagy et al., 1991b; Lieb et al., 1991). Several studies examined two cellular systems:

1. It was suggested that nerve growth factor (NGF) may have a role in rendering a cell nonpermissive for viral replication, since this neurotropic molecule is required for the maintenance of a quasi latent form of HSV in a neuronal cell tissue system (Wilcox and Johnson 1987, *see next*

section). Deprivation of NGF in this model resulted in resumed replication of HSV-1 (Wilcox and Johnson, 1987; Wilcox et al., 1990). In a different system, NGF was able to stimulate expression of chloramphenicol acetyltransferase (CAT) from a construct containing the LAT promoter, which also enhanced explant reactivation (Lieb et al., 1991). NGF message was present in PSG of latently infected mouse ganglia post explantation simultaneously with reactivation (Valyi-Nagy et al., 1991), but the cause and effect relationship between NGF expression and explant reactivation in this observation is open. These conflicting pieces of information were gathered from different systems, and are still awaiting further clarification.

2. Lieb et al. (1991) demonstrated that the promoter region of the LATs contains a cAMP-responsive element and that cAMP accelerated explant reactivation of wild-type viruses, a function that is mediated by a consensus cAMP-responsive sequence located within the LAT promoter region. In this case, extraneural stimuli might trigger HSV reactivation via second messenger signal transduction and cAMP action on LAT regulatory elements.

Information Gathered from In Vitro Latency Systems

There is clear need for an in vitro latency system. However, in view of the discussion presented, two molecular features should be added to the phenotypic definition of latency in order to render any in vitro latency system relevant to the in vivo situation: an episomal viral DNA structure in which the ends of the viral genome are absent, and expression of the LATs. The presence of these two molecular elements in the currently available in vitro latency models is yet to be shown. More importantly, all the in vitro latency systems involve inoculation of cells in culture with HSV under unnatural conditions, such as nonpermissive temperature, and addition of exogenous agents, such as acyclovir, which prevent protein synthesis and viral replication (Wigdahl et al., 1982; Russell and Preston, 1986; Cook and Brown, 1987; Wilcox and Johnson 1987). Thus, data gathered from such in vitro studies and their relevance to the actual situation should be considered with caution.

Recently, the molecular phenomena of several in vitro latency models were examined. In the model developed by Wilcox and colleagues (Wilcox and Johnson, 1987; Wilcox et al., 1990) neuronal cells are obtained from superior cervical or dorsal root ganglia of rats, monkeys, or human fetuses and are treated with acyclovir prior to viral infection. The acyclovir-containing medium is removed 7 d post infection but even then no replicating virus can be isolated. Reactivation was achieved in this model by depriving the cells in culture of their endogenous NGF, suggesting that this compound may inhibit reactivation in vitro (Wilcox and Johnson, 1987). In this system only the 2 kb LAT (and not the 1.5/1.45 transcripts) could be identified (Doerig et al., 1991b), as is the situation during HSV-1 replication in cell cultures. DNA organization in this system was not examined. As already mentioned, however, an antigen that might have been encoded by the LATs was identified (Doerig et al., 1991a).

In a model developed by Preston and coworkers (Russell and Preston, 1988), HSV-2 replication is initially suppressed by infecting the cells in culture at very low MOI and at a supraoptimal temperature of 42°C. The system resembles in part the in vivo condition since HSV-2 DNA is present in the cells at a nonlinear form (Preston and Russell, 1991; Harris and Preston, 1991). However, the LATs were not detected. Based on data derived from studies with this system the possibility was raised that ICP0 is required for reactivation (Russell et al., 1987). However, ICP0 deletion mutants do reactivate from in vivo latency under certain conditions (Lieb et al., 1989a).

Latency Within the Central Nervous System

Although HSV-1 is responsible for the viral encephalitis with the highest fatality rate, more data are available about the molecular biology of latent HSV-1 infection in the peripheral nervous

system than in the CNS, and the nature of the latent state of the virus in CNS tissue is poorly understood. Herpes simplex has also been implicated in the pathogenesis of several other CNS disorders, such as multiple sclerosis (Kastrukoff et al., 1987), Behcet disease (Eglin et al., 1982), and Alzheimer's disease (Ball, 1982), but no conclusive evidence to link HSV with any CNS disease other than encephalitis and Mollaret's meningitis (Yamamoto et al., 1991) has been produced so far.

Following primary infection of TG in experimental animals the virus travels to, and replicates in, the CNS (Cook and Stevens, 1973). In animals that succumb to the primary infection, paralysis develops and the time of death corresponds to the peak of viral replication within the CNS. The organization of the HSV-1 DNA in the brainstem and in the TG is similar (Rock and Fraser, 1983). Restricted gene expression of the viral genome can be detected in the CNS trigeminal system of mice by *in situ* hybridization (Deatly et al., 1988), but these transcripts were not characterized by Northern blot analysis and the extent of similarity between HSV-1 gene expression during latency in the peripheral and central nervous systems is unclear. Some evidence suggests that in experimental animals, the relative amounts of viral DNA and transcripts in the CNS are lower than those in the PNS (Steiner et al., in preparation). Explanted experimental animals brainstem tissues do not reactivate virus. Similarly, despite the presence of HSV-1 nucleic acids in the human brain (Fraser et al., 1981 and our unpublished data) we are unaware of any report of recurrent CNS disease induced by HSV-1, and attempts to reactivate HSV-1 from explanted human CNS tissue have failed. CNS disease as a result of HSV-1 (herpes encephalitis) in immunocompetent individuals is a very rare single event that occurs at a frequency of 1 million fold less than the peripheral disease (Whitley, 1985).

The source of the virus producing herpes encephalitis is not apparent. Not all cases of herpes encephalitis are caused by the same viral strain that is responsible for cold sores in the same individual (Whitley et al., 1982) and it is assumed that in approx half of the patients, HSV-1 encephalitis occurs during the primary viral infection (Whitley, 1990). Moreover, even in the presence of prior HSV-1 infection, a second primary infection with another HSV-1 strain can take place and thus be responsible for the encephalitic infection. In those cases where reactivation might have been responsible for the encephalitis, it was suggested that the reactivated virus did arise from TG (Johnson, 1982). This argument was based on the predilection of the infectious process to involve the cerebral temporal and frontal lobes, brain regions with blood vessels and meninges that derive their sensory innervation from the TG.

Thus, it seems likely that HSV-1 reactivation from the CNS is an extremely rare event. Theoretically, several factors may contribute to the differences between HSV-1 reactivation from the peripheral and the central nervous systems: i) the relative amounts of viral transcripts within the tissues. There is some evidence to suggest that the quantity of the LATs contributes to the efficacy of reactivation. Thus, HFEM, which has only a single LATs copy, has slower explant reactivation kinetics from PSG conpared with wild type strains (Block et al., 1990), and does not reactivate *in vivo* from rabbit TG; ii) still unknown host factors which might inhibit viral reactivation in the CNS.

Use of Herpesvirus as a Vector to Deliver Missing Genes to Tissues

In view of all the currently available knowledge, HSV-1 seems to be a promising and in many ways an ideal vehicle to deliver missing and aberrant genes into nervous system tissue including neurons. Several features of this virus carry obvious advantages for this purpose and may render it a useful vector for gene therapy.

The manipulation of HSV-1 genome has benefited from the following:

1. A large viral genome that has been sequenced and the sequence analyzed for ORFs, location of genes, and their regulatory elements (McGeoch et al., 1988; Perry and McGeoch, 1988).

2. The ability to delete or disrupt a large part of the viral genome that is not strictly essential for viral replication (Roizman and Sears, 1990).
3. The ability to insert into the intact viral genome additional genes, about 10 kb in size, and to package into the virion at least 30 foreign kb after removing from it dispensable sequences (Longnecker et al., 1988).
4. The ability of HSV-1 to infect almost every cell type.
5. The ability of HSV-1 to spread throughout the nervous system, a feature that can be used to direct the virus to reach distant areas via axonal transport and trans-synaptically, thus avoiding the need to induce systemic infection (Kuypers and Ugolini, 1990).
6. The fact that the virus resides in host cells in vivo for the entire life of the host.
7. The ability of the viral genome to remain in an episomal state during latency and not to be integrated into the genome of the host, thus avoiding unforeseen mutations within the host cell genome.
8. Transcriptionally active HSV-1 during latent infection and a viral promoter constantly functioning in the cell harboring latent viral DNA.
9. Neuronal functions and metabolism probably remaining largely unaltered during latent infection in neurons (Stevens, 1989).

Two additional features of HSV-1 that might be problematic for the purpose of gene therapy remain to be overcome:

1. The virus is cytolytic and its full replication results in host cell destruction and death. This can be surmounted by manipulation of the viral genome in order to render it less toxic, i.e., to make it replication incompetent in vivo. Since replication is not a prerequisite for the establishment of latency and for the latent phase transcriptional activity, the use of an HSV-1 "backbone" virus with mutations within genes essential for replication, such as the thymidine kinase (Coen et al., 1989) or the Vmw65 (Steiner et al., 1990) genes, will still enable the virus to reach nervous tissue, establish latent infection there, and express genes without causing cell damage and death. The technical problem of propagating the virus in cell culture depends on the gene affected. For example, the *in*1814 virus, containing a Vmw65 mutation that reduces the expression of several IE genes, does not replicate in vivo, but replicates in cells in culture (Ace et al., 1989), and thymidine kinase mutants are unable to replicate in the adult brain but can replicate in dividing cells in culture (Roizman and Jenkins, 1985).
2. Under certain conditions HSV-1 may reactivate, resume replication, and induce a recurrent disease. This problem can be overcome by deleting the coding sequence of the LATs, leaving its regulatory elements intact. The establishment and maintenance of latency would not be affected, but the ability of the virus to reactivate in vivo would be abolished. Moreover, since the LATs' promoter is active during productive infection in cell culture (Dobson et al., 1989), although to a lesser extent than during latent infection, it will be possible to examine expression of foreign genes combined to the LATs promoter in vitro prior to in vivo studies.

Therefore, by utilizing the ability of HSV-1 to propagate within the nervous system without the need to replicate, and by eliminating its ability to reactivate, it can be used as an innocuous vector targeted to reach nervous tissue and neurons. Several studies have already used the regulatory elements of the latency-associated transcriptional activity of HSV-1 to express foreign and reporter genes in vitro and in vivo. β-globin (Dobson et al., 1989), and *Escherichia coli* lacZ (Ho and Mocarski, 1989) genes were shown to be expressed in vivo for extended periods of time under the regulation of the LATs promoter.

In the light of these various considerations the following requirements have either been met or should be satisfied within the foreseeable future in order to use HSV-1 in gene therapy:

1. The viral construct should be able to reach target cells within the nervous system without being toxic to these cells and without causing tissue destruction on its way.
2. It must retain its ability to establish latent infection within the tissue, and the LATs promoter must remain active.
3. It must reach specific cells that contain the defective genes and are the target for gene therapy. In this respect the question as to whether HSV-1 can establish latency in a wide variety of neuronal and nonneuronal cells, or could be manipulated in a way that will enable the establishment of latency in cell types and tissues of interest under experimental conditions, is still open.

4. The metabolic abnormality that calls for gene therapy of the target cells must be identified and its genetic basis and associated required sequences characterized.
5. It should be in a size that will enable packaging of the required gene into the viral vector.
6. Experimental (in vitro and animal) systems must exist to examine and test the vectors and their effect.

An example of a human condition that may serve as one of the first targets for gene therapy is the Lesch-Nyhan syndrome, which results from the complete deficiency of hypoxanthine-phosphoribosyltransferase (HPRT) activity and is associated with severe CNS neuronal dysfunction (Lesch and Nyhan, 1969). The cDNA sequence of the HPRT gene is known (Brennand et al., 1983), it can be packaged into the HSV-1 genome (Palella et al., 1988), and several animal models with HPRT deficiency are available (Kuehn et al., 1987). However, early studies using HSV-1 vectors containing HPRT gene under the regulatory control of the thymidine kinase gene (Palella et al., 1989) demonstrated some of the future problems in this arena, namely, it is not yet possible to control the level of production of the required gene product, and rendering the virus less pathogenic will limit its spread within the nervous system and thus not enable it to reach a critical number of target cells in order to be effective therapeutically.

Conclusion

During the last few years several molecular phenomena associated with HSV-1 latency within the nervous system were uncovered. The episomal, chromatin-bound state of the viral DNA, its nonlinear, concatameric, or circular organization, and the restricted gene expression have been identified. The latter has been carefully characterized and the regulatory elements of the latent-phase transcriptional activity mapped. Whether this latency-associated transcription codes for gene products is yet unclear, but accumulating evidence suggests that it participates in the reactivation process of HSV-1 from latent infection to cause recurrent disease. The mechanisms by which this transcriptional activity is involved in reactivation is also not yet understood. Not only is this kind of information important for the understanding of the ability of HSV-1 to establish latent infection in the human nervous system, but also the data may enable the use of this virus to function as an ideal vector for gene therapy of disorders caused by missing or aberrant production of nervous system gene products.

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