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Strength in diversity: Understanding the pathways to herpes simplex virus reactivation

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ABSTRACT

Herpes simplex virus (HSV) establishes a latent infection in peripheral neurons and can periodically reactivate to cause disease. Reactivation can be triggered by a variety of stimuli that activate different cellular processes to result in increased HSV lytic gene expression and production of infectious virus. The use of model systems has contributed significantly to our understanding of how reactivation of the virus is triggered by different physiological stimuli that are correlated with recrudescence of human disease. Furthermore, these models have led to the identification of both common and distinct mechanisms of different HSV reactivation pathways. Here, we summarize how the use of these diverse model systems has led to a better understanding of the complexities of HSV reactivation, and we present potential models linking cellular signaling pathways to changes in viral gene expression.

1. Introduction

Herpes simplex virus (HSV) 1 and 2 are ubiquitous pathogens that persist for the life of infected individuals. The ability of these viruses to develop lifelong infections is due to the presence of a latent pool of virus in terminally differentiated neurons, most commonly in the peripheral ganglia. It is estimated that approximately 90% of individuals worldwide are infected with HSV-1, HSV-2 or both of the viruses (Arvin et al., 2007). In the United States, the estimated prevalence rate of HSV-1 and HSV-2 in people aged 14–49 is 47.8% and 11.9% respectively, with higher prevalence in women and Mexican-American and non-Hispanic black persons (McQuillan et al., 2018). HSV infection is often clinically silent. However, HSV periodically re-enters a lytic replication cycle in a process known as reactivation. In immunocompetent persons, reactivation events result in replication at the body surface that can give rise to recurrent blisters or sores, which are typically self-limiting and resolve rapidly (Roizman et al., 2013). These lesions most commonly occur at oral, nasal or ocular sites with HSV-1 infection and at the genital skin and mucosa with HSV-2 infection. HSV reactivation can also lead to significant morbidity and mortality in immunocompromised individuals, and, in rare cases, infection of the central nervous system can lead to acute viral encephalitis or a recurrent lymphocytic meningitis.

The ability of HSV to reactivate from latency and re-enter a productive replication cycle significantly contributes to its ability to cause lifelong disease that results in recurrent infection, viral shedding and transmission to new hosts. However, the molecular events that underlie the switch from latent to productive infection are not well understood. This review will examine our current understanding of how different reactivation stimuli may trigger this switch in the HSV viral life cycle and illuminate some of the remaining questions in the field.

2. Lytic replication and latency: contrasting viral life-cycles

The ability of HSV to establish a lifelong infection can be attributed to its capacity to undergo contrasting infectious events, termed the lytic and latent stages. Primary infection at the body surface results in productive replication in epithelial cells (Roizman et al., 2013). During this initial lytic stage of infection, over 80 viral gene products are expressed in a cascade-dependent manner. Initial viral gene expression is enhanced by delivery of viral tegument proteins, including the viral transactivator, VP16, into the nucleus. This potent transcriptional activator induces the formation of a transcriptional regulatory complex with multiple cellular co-activators to promote transcription of the viral immediate early (IE) or α genes. Products of the IE genes include proteins required for transcription of the early (E) or β genes, which encode proteins required for viral DNA replication. The final group of genes is the late (L) or γ genes, whose expression is dependent on viral DNA replication. L genes encode structural proteins required for assembly, egress and release of the infectious HSV particle.

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During primary infection, HSV is able to enter the terminal axons of neurons that innervate tissue at the initial site of infection. Although viral genomes are most frequently detected in sensory ganglia, particularly the trigeminal ganglia (HSV-1) and lumbar-sacral ganglia (HSV-2), viral DNA and reactivation-competent virus can also be isolated from sympathetic and parasympathetic neurons (Baringer and Pisani, 1994; Baringer and Swoveland, 1973; Richter et al., 2009; Warren et al., 1978). Furthermore, HSV DNA can be detected in the central nervous system, with the frequency of detection increasing with age (Beffert et al., 1998; Fraser et al., 1981; Gordon et al., 1996). Following neuronal infection, the virus can enter latent infection. However, neurons can also support lytic replication, which may be associated with neuronal death (Thompson and Sawtell, 2001). There is also evidence of prior lytic promoter activity in latently infected neurons (Proenca et al., 2008). The mechanisms that regulate entry into lytic replication versus latent infection in neurons remain largely undefined.

HSV latency is defined as the persistence of viral DNA in the absence of detectable infectious virus that retains the ability to reactivate following an appropriate stimulus. While expression of the viral lytic genes is largely repressed during latent infection, there is active transcription of the latency-associated transcript (LAT), which is composed of a primary 8.3 kb unstable transcript that is spliced to give rise to a stable intron of approximately 2 kb in length and multiple miRNAs (Kramer et al., 2011; Stevens et al., 1987; Umbach et al., 2008). Latency is usually defined at the level of the ganglia, but within a ganglion only a sub-population of latently infected cells will reactivate at any one time (Sawtell and Thompson, 2004). In addition, there is evidence that different stimuli can result in reactivation from different subtypes of neurons (Yanez et al., 2017). Therefore, the definition of a “latently” infected neuron may depend not only on the neuronal subtype, but also on the nature of the reactivating trigger.

3. Modeling HSV latency

A strength of the HSV field is the diversity of the model systems used to investigate the pathways to reactivation. Although there may be differences in the interpretation of the data based on the system or stimuli, all these systems will ultimately have relevance to basic science and human health. To elucidate the cellular signaling pathways involved in the reactivation processes, models of latency that allow faithful establishment of latency, robust reactivation and easy manipulation of signal transduction pathways are required. The most commonly used model organism in HSV research is the mouse. Infection of mice with HSV-1 results in initial lytic replication at the body surface and entry of the virus into innervating sensory and autonomic neurons. Following an initial period of acute replication in the ganglia, HSV establishes latency, and reactivation can be triggered by explant of the ganglia (Sawtell and Thompson, 2004), hyperthermic stress (Sawtell and Thompson, 1992), UV irradiation (Shimeld et al., 1996) or hormone treatment (Cook et al., 1991; Vicetti Miguel et al., 2010). Overall, in vivo models of reactivation have the advantage of more accurately recapitulating the natural course of infection and incorporate host antiviral responses that may impact the state and population of latent genomes or modulate viral reactivation. However, the manipulation of cellular pathways in vivo can be challenging.

To elucidate the molecular pathways involved in HSV reactivation, in vitro models have proven to be invaluable (Thellman and Triezenberg, 2017; Wilson and Mohr, 2012). The optimal model system would utilize mature, human neurons. However, while sensory neurons can be isolated and maintained from human donors (Valtcheva et al., 2016), access to this material is limited, consistency is difficult to achieve, and the tissue may already be latently infected with HSV or varicella zoster virus (VZV). Human sensory neurons differentiated from embryonic stem cells have also been used to investigate latency and reactivation for both HSV and VZV (Markus et al., 2015; Pourchet et al., 2017). A recent study utilizing human differentiated neurons achieved latently infected cultures that could be reactivated with sodium butyrate, a histone deacetylase inhibitor (Pourchet et al., 2017). There is also an emerging interest in human cell lines that can be easily differentiated into neurons, as shown using the HD10.6 cell line, which can be differentiated into sensory neurons with nociceptive properties (Raymon et al., 1999; Thellman et al., 2017). Quiescent infection can be established in the presence of acyclovir, and reactivation can be triggered from a sub-population of neurons following depletion of nerve growth factor (Thellman et al., 2017).

Perhaps one of the best characterized in vitro systems to study HSV reactivation utilizes primary sensory or sympathetic neurons isolated from the peripheral ganglia of pre-natal rats and post-natal or adult mice (Camarena et al., 2010; Cliffe et al., 2015; Ives and Bertke, 2017; Wilcox and Johnson, 1987; Wilcox et al., 1990). Infection of these primary neuronal cultures in the presence of acyclovir or phosphonoacteic acid (PAA) results in a quiescent infection that resembles latency. Importantly, to accurately define a quiescent infection in these model systems, it is imperative to show that replicating virus remains undetectable following the removal of viral DNA replication inhibitors. When a quiescent infection is properly established, these systems exhibit all of the known molecular hallmarks of latency, including accumulation of the LAT intron, expression of latency-associated miRNAs, absence of replicating virus and undetectable levels of viral proteins (Camarena et al., 2010; Cliffe et al., 2015; Jurak et al., 2014; Wilcox and Johnson, 1987). Furthermore, these models maintain the capacity to undergo reactivation triggered by a variety of stimuli, including NGF-deprivation, dexamethasone, inhibition of protein synthesis or high intracellular levels of cAMP (Camarena et al., 2010; Cliffe et al., 2015; Linderman et al., 2017; Colgin et al., 2001; Kobayashi et al., 2012; Wilcox and Johnson, 1987). Thus, these systems provide a powerful tool to study the molecular features of latency and reactivation, such as the role of cell stress pathways or chromatin modulation, in primary neuronal populations. Some caveats to these model systems is the absence of support cells that may also impact the nature of the latent infection and/or reactivation. The use of DNA replication inhibitors to promote latency is instead utilized to compensate for missing immune components. Whether DNA replication inhibitors impact the nature of latency or reactivation mechanisms is not known. However, it is worth noting that symptomatic primary HSV-1 is often treated with anti-viral compounds (James and Whitley, 2010). Moving forward, it will be important to determine if the mode infection, age of neurons, presence of immune mediators or addition of DNA replication inhibitors alters the nature of latency or impacts events that occur during reactivation, as this will have relevance to both the model systems used and human disease.

4. Latent viral chromatin structure: silent but poised?

Following the establishment of latent infection, viral lytic gene expression is silenced, and the lytic gene promoters are associated with repressive heterochromatin (Knipe and Cliffe, 2008). Key experiments performed in the 1980's indicated that latent genomes in the brain stems of infected mice have a nucleosomal structure (Deshmane and Fraser, 1989). Later studies confirmed that the latent viral genome associates with cellular histones in the trigeminal ganglia of mice (Cliffe et al., 2012; Kubat et al., 2004b; Wang et al., 2005). Coinciding with the silencing of lytic transcripts, the viral lytic gene promoters become enriched with characteristic heterochromatic histone modifications, namely histone H3 di- and tri-methylated at lysine 9 (H3K9me2/3) and H3K27me3 (Cliffe et al., 2012, 2009; Kwiatkowski et al., 2009; Nicoll et al., 2016; Wang et al., 2005). While it appears that factors intrinsic to neurons play a key role in the transcriptional silencing of the virus (Cliffe et al., 2012), viral gene products expressed during latent infection can also modulate the chromatin structure (Cliffe et al., 2009; Kwiatkowski et al., 2009; Raja et al., 2016; Wang et al., 2005). This modulation likely promotes long-term latency, while priming the