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Stress Flips a Chromatin Switch to Wake Up Latent Virus

Daphne C. Avgousti[1,2] and Matthew D. Weitzman[1,2,*]
[1]Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA
[2]Division of Cancer Pathobiology, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA
*Correspondence: weitzmanm@email.chop.edu
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Stress-induced reactivation of latent herpesviruses requires disabling of repression, but the mechanism for converting silenced chromatin into an active state is unknown. In this issue of *Cell Host & Microbe*, Cliffe et al. (2015) suggest a methyl/phospho switch on histone H3 overcomes repression to facilitate reactivation of latent herpes simplex virus type 1 (HSV-1).

After initial infection, the HSV-1 genome remains dormant in neurons for the lifetime of the host in a state known as latency. From this dormant state, the viral genome can become periodically reactivated in response to stress and other stimuli, producing progeny in an active lytic infection. During latency, the viral promoters carry repressive chromatin marks (Bloom et al., 2010), which must be overcome to facilitate expression of viral genes and reestablish the lytic state. The transition from repressed chromatin to active expression of viral genes is not well understood. For reactivation to occur, viral transcription must be initiated in the absence of viral proteins, suggesting that host cell proteins are key to enabling viral gene expression.

Our appreciation of viral latency and reactivation comes from a combination of infection systems, including in vivo animal models (mice, guinea pigs, and rabbits) and cultured primary neurons infected in vitro (Wilson and Mohr, 2012). Spontaneous reactivation and virus shedding is difficult to capture; therefore, investigating reactivation often involves studying explanted ganglia. In mice, spontaneous shedding occurs very infrequently, although it can be induced by systemic stress such as heat shock and other methods. In rabbits and guinea pigs, reactivation is more frequent; however, the discrepancies in viral strains make these models less ideal. The primary sites for latent HSV-1 infection are the sensory neurons within the trigeminal ganglia (Wilson and Mohr, 2012). Primary neuronal in vitro models have the attractive feature of being amenable to pharmacological manipulation of cellular signaling pathways. However, these models are not able to account for stress signals from cells that would be nearby within the ganglia, such as CD8+ T cells. These neighboring cells may contribute both to immune repression and unwittingly to reactivation. Although rodent systems may not accurately represent latency and reactivation in human infection, much has been learned about the mechanism of reactivation from these models.

The molecular mechanism of reactivation within the cell is dependent on many factors. Inducing gene expression from silenced promoters during reactivation from latency may be mechanistically distinct from activating gene expression during lytic infection. During initial lytic HSV-1 infection, the incoming viral genomes are devoid of histones. In addition, they are accompanied by VP16, a viral transactivator that can bind to Oct1, HCF-1, and other chromatin modifiers to generate permissive chromatin at viral promoters. Once latency has been established, the histones associated with lytic regulatory genes carry marks of repressive chromatin, such as methylated histone H3 lysine 9 (H3K9me2/3) and lysine 27 (H3K27me3) (Cliffe et al., 2013). Reactivation can be triggered by neuronal stress induced by various means such as neuronal growth factor (NGF) deprivation, inhibition of PI3K that signals downstream of NGF, or heat shock. Using one of the rodent in vitro culture systems of sympathetic neurons from prenatal rat superior cervical ganglia, it was suggested that there are two waves of lytic gene transcription during reactivation induced by pharmacological agents such as PI3K kinase inhibitors (Kim et al., 2012; Wilson and Mohr, 2012). The first wave, termed Phase I, occurs approximately 15–20 hr post-induction and leads to concurrent transcription of immediate-early genes including VP16, early, and late gene transcription. VP16 is not thought to be essential for the transition to Phase II in which viral DNA replication also flourishes, although VP16 is required for full reactivation during Phase II (Wilson and Mohr, 2012).

Repressive chromatin marks have to be disabled for reactivation but the virus does not have the luxury of viral transactivators present to facilitate transcription. In addition, there may be several viral episomes at various stages of reactivation in a given neuron, suggesting that a threshold of viral gene expression needs to be reached to switch from continued repression to reactivation (Figure 1). In cellular chromatin, an ingenious strategy to overcome heterochromatin has been uncovered during an important step in the entry into M phase of the cell cycle. H3K9me3 is thought to be repressive because it serves as a binding site for heterochromatin protein 1 (HP1), which is recruited to regulate gene expression and heterochromatin formation. In 2005, Fischle et al. showed that phosphorylation on the adjacent serine residue, S10, was sufficient to eject HP1 without requiring demethylation of H3K9 (Fischle et al., 2005). This dual mark serves as an intermediate step between a repressive state and an active one, and the transition has been termed a methyl/phospho switch (Fischle et al., 2003). The kinase Aurora B was shown to be required for this phosphorylation of H3S10 during mitosis, although other kinases may also serve to modify this residue (Hirota et al., 2005).

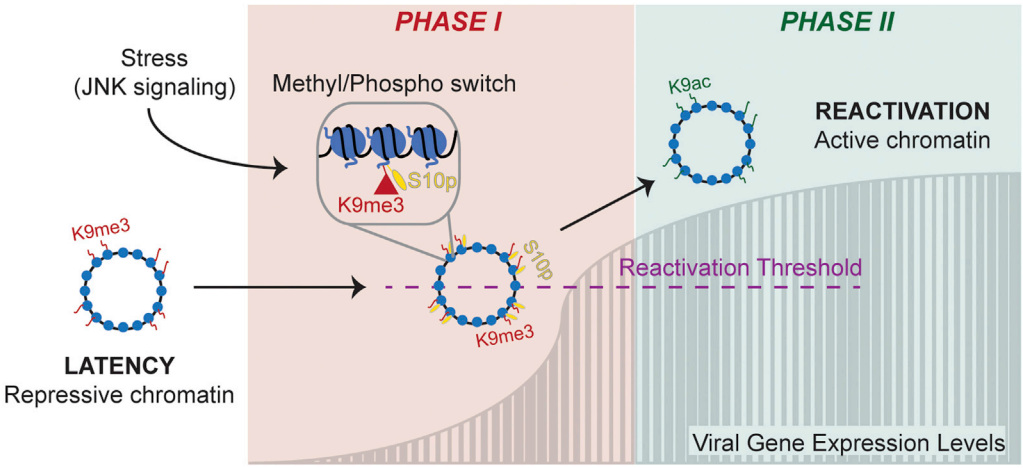

Figure 1. Schematic Illustrating the Correlation between Heightened Stress and Increased HSV Gene Expression and Reactivation
Initially, the episomal viral genome is coated with repressive chromatin marks such as H3K9me3 at promoters. As stress increases, the JNK signaling pathway is activated and phosphorylation on H3S10 allows transcription of viral genes without the removal of H3K9me3, passing the threshold for reactivation. With an increase in transcription of viral genes such as VP16, the reactivation enters Phase II with the re-writing of active marks such as H3K9ac on the viral genomes, viral replication, and progeny production.

Cliffe et al. (2015) developed a model of HSV-1 reactivation using mouse sympathetic neurons. The authors investigated stress-induced reactivation and demonstrated that induction of viral gene expression during Phase I of reactivation requires signaling a pathway involving c-Jun N-terminal kinase (JNK). They suggest that JNK activation is broadly essential for HSV-1 reactivation, since an inhibitor of JNK activity blocked HSV-1 reactivation in both sympathetic and sensory neurons triggered by multiple stimuli, as well as by axotomy of trigeminal ganglia from latently infected mice. Neurons maintain high levels of JNK kinases, which are regulated by accessory proteins to achieve physiological or stress-induced functions. Specifically, the dual leucine kinase (DLK) and JNK scaffold protein (JIP-3) are important for activation of the stress response by phosphorylating c-Jun in neurons (Tedeschi and Bradke, 2013). These activators were also required for activation of HSV-1 gene expression in the latent neuronal model but had no impact during lytic infection. Surprisingly, they found that demethylases LSD1 and JMJD3 were not required to remove the repressive methylation marks H3K9me3 and H3K27me3 for reactivation. This observation raises the conundrum: how does JNK signaling permit increased viral gene expression during the initial Phase I of reactivation without removing the presumed repressive heterochromatin marks? The solution comes from the methyl/phospho switch previously described in the context of cellular chromatin (Fischle et al., 2005; Gehani et al., 2010; Hirota et al., 2005). In an elegant set of experiments, Cliffe et al. (2015) show that JNK signaling leads to phosphorylation of H3S10, which presumably prevents binding of HP1 and negates the neighboring repressive mark without specifically removing it. Exploitation of the methyl/phospho switch by a virus further validates this mechanism as a strategy to convert from a repressed to an active state without the need for demethylases. In this model JNK provides the connection between stress signaling and chromatin modifications. Although the kinase was found enriched on viral lytic promoters, the direct involvement of kinase activity in mediating the histone switch requires additional investigation. This study further defines the difference between an active lytic state and the initiation of reactivation and provides insight into how chromatin is exploited by HSV-1 through signaling pathways.

This study also highlights how tractable cultured neuron models provide insight into the cellular switches that awaken latent genomes. JNK signaling may represent a common focal point for the different forms of neuronal stress that trigger HSV-1 reactivation, and this can be clearly demonstrated in cellular models. However, the relevance of in vitro findings with pure neuronal cultures to in vivo reactivation of natural latent HSV remains unclear. Deciphering how organismal stress or UV can lead to reactivation of latent HSV-1 in vivo is much more challenging. Virus has been isolated from a number of different neuronal cell types of the peripheral nervous system, but whether all of these are competent and responsible for reactivation is unknown. Even within a single neuron, there may be multiple viral episomes, and it is not known how consistent the chromatin marks are between them and to what extent differences impact reactivation potential. The establishment of latency may also be affected by the combination of marks deposited on histones of viral genomes during initial infection, and these may not be fully recapitulated with in vitro models. However, it is clear that viral systems of tightly regulated gene expression patterns provide attractive models to explore the potential of combinatorial histone modifications in gene regulation.

In this study, Cliffe et al. (2015) relied upon validated antibody recognition to detect the dually modified histone. Ultimately, the role of combinations of histone marks may only truly be revealed when techniques are developed to isolate proteins on viral genomes, combined with middle-down mass spectrometry to verify multiple modifications on the same histone. The methyl/phospho switch uncovered here highlights the importance of context and neighboring marks when

assessing the phenotypic effect on gene expression. This mechanism is reminiscent of the environment-dependent lysogenic/lytic switch of bacteriophage lambda, suggesting that mammalian viruses employ similar strategies to gauge the host cell environment. It will be interesting to see which other chromatin mechanisms are employed by viruses as they exploit cellular strategies to overcome host defenses and reactivate when the stress is right.

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Too Much Sugar Puts a Parasite in Jeopardy

Stanislas Tomavo[1,*]
[1]Laboratory of Cellular and Molecular Biology of *Toxoplasma*, Institut Pasteur de Lille, Université de Lille, 1 rue du Professeur Calmette, 59 000 Lille, France
*Correspondence: stan.tomavo@pasteur-lille.fr
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Toxoplasma gondii **and other coccidian parasites accumulate starch-like amylopectin stores whose functional significance is unclear. In this issue of *Cell Host & Microbe*, Uboldi et al. (2015) present a pioneering investigation into a signaling cascade with a pivotal role in amylopectin metabolism and transmission of encysted parasites during chronic infection.**

The Apicomplexa is an ancient phylum of some 5,000 diverse eukaryotic species. These intracellular parasites have gained notoriety because they cause acute disease and death in humans, as well as impacting the livestock industry worldwide. The phylum Apicomplexa includes *Plasmodium* species, which are responsible for malaria, and also *Cryptosporidium*, causing severe diarrhea in immunocompromised individuals; *Eimeria*, the agent of coccidiosis in poultry; and *Toxoplasma gondii*, one of the most ubiquitous intracellular parasites. While extremely common, infections by *T. gondii* generally remain subclinical because of a combination of a robust immune control and parasite-mediated subversion of the host enabling long-term survival. The infectious stages consist of the acute (tachyzoite), latent (bradyzoite), and spore-forming oocyst (sporozoite) stages. Infections initiated either by the ingestion of environmental oocysts or by tissue cysts are associated with the rapid replication and spread of tachyzoite forms. The control of this acute phase by the immune system eliminates the tachyzoites, while the few remaining parasites differentiate into the relatively quiescent and slowly growing bradyzoites, forming latent cysts that can persist mainly in the muscle and the central nervous system. The ensuing chronic infection is essentially asymptomatic, although cyst formation in the retina is a significant cause of blindness in some regions of South America (Glasner et al., 1992). Furthermore, in the absence of immune function, most notably observed in active HIV-AIDS, tissue cyst reactivation occurs, leading to life-threatening neurological disease and death if not treated. The chronic infection of *T. gondii* can also increase the risk of schizophrenia, bipolar disorder, and obsessive disorder (Arias et al., 2012; Hurley and Taber, 2012). The complex lifestyle of *T. gondii* relies on stage conversion and differentiation of tachyzoite to bradyzoite and the oocyst-forming sporozoites. One of the evolutionary hallmarks of the coccidian parasites is the presence of starch-like amylopectin stores (Harris et al., 2004; Figure 1). In contrast to plants, which contain starch in the form of branched amylopectin and amylose in their chloroplasts, the amylopectin of *T. gondii* is exclusively composed of a linear polymer of α(1-4)-linked glucose backbone modified with α(1-6)-branched glucose residues (Guérardel et al., 2005). It has been proposed that the loss of amylopectin from *Eimeria*