The Ticking CLOCK of HSV-2 Pathology

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Herpes simplex virus type 2 (HSV-2) is the causative agent of genital herpes. Matsuzawa et al have demonstrated that, in a mouse model, HSV-2 pathology is influenced by the time infection occurs. Increased expression of the HSV-2 receptor Nectin-1 under the control of CLOCK coincided with an increase in viral titer suggesting that HSV-2 infection is regulated by the host circadian clock

Human simplex virus type 2 (HSV-2) is predominantly a sexually transmitted infection and causes genital herpes through contact with open sores, fluids, or genital surfaces. Genital herpes can be asymptomatic, or alternatively present as one or more painful blisters or ulcers. Other symptoms may also include fever, muscular aches, and swollen lymph nodes. After an initial HSV-2 infection, symptoms may reoccur with approximately 5% of HSV-2 carriers having outbreaks once every 2 to 12 months over the duration of their lifetime. Approximately 417 million people are infected with the virus globally with rising infection rates reported in adolescents resulting in a huge medical and public health problem (WHO, 2017). In rare cases, neonatal herpes can be contracted by exposure to the virus in the genital tract during delivery resulting in neurological damage or death. In addition, the incidence of coinfection with HIV is increased in HSV-2-infected individuals by threefold, and people infected with both viruses are more likely to infect others with HIV-1 (WHO, 2017).

Infection with HSV-2 is incurable and lasts for the life time of the patient. Antiviral drugs such as acyclovir or valacyclovir are effective against HSV-2 infections, reducing the severity and duration of the symptoms, but they do not prevent recurrences unless used daily. In addition, these antiviral drugs have little impact on the spread of HSV-2 infection with 10 to 20 million per year continuing to acquire new infections. Research is underway to produce more effective methods to control HSV-2 infections such as vaccines of topical microbicides, but to date none are readily available for public use (Johnston et al., 2011; Nikolic and Piguet, 2010).

Matsuzawa et al. (2017) demonstrate that the expression of the HSV-2 receptor Nectin-1 in epithelial skin cells fluctuates with the host cell circadian (molecular) clock. Variable expression over time of Nectin-1 results in an enhanced uptake of HSV-2 during the "active phase" in a murine model, which further results in increased viral titer, cytokine expression, and HSV-2 pathology.

Studies have to date linked numerous molecular processes within cells to the coordinated oscillations of the circadian clock (24-hour cycle), and this connection has become an ever increasing field of interest in disease regulation. The body's internal clock is located in the hypothalamic suprachiasmatic nuclei. From here, neural and humoral signals regulate output pathways that control internal temperature, hormone regulation, metabolism, and cell cycle. On a molecular level, the circadian clock controls an independent transcription translation feedback loop including a negative feedback loop involving the genes Clock, Bmal1, Period1 *(Per1), Per2*, and cryptochrome1 *(Cry1)* and *Cry2*. The 24-hour cycle starts with CLOCK/NPAS2 binding to BMAL1 and activating the transcription of *Per* and *Cry* that heterodimerize and feedback to CLOCK: BMAL1 complex resulting in a block to further transcription (see **Figure 1**). In turn, the PER-CRY complex is degraded and CLOCK: BMAL1 can activate a new cycle. Hundreds of genes, approximately 10-20% of all genes, show circadian rhythm in their expression. Although these genes display a rhythmic cycle, they are not directly involved in the circadian mechanics and are therefore considered to be clock-controlled genes. These can encode a variety of proteins, including cytokines, chemokines, or receptors. Circadian cycles connected to and influencing molecular clocks can therefore contribute to variation of expression over a 24-hour cycle and potentially result in variation to disease susceptibility within the host (reviewed in Takahashi et al 2008).

Research on the relationship between pathogens and the host's circadian clock reveals new insights in pathogen-host interactions. Plasmodia have been previously reported to synchronize their replication cycle with the host's circadian rhythm to ensure a successful infection (O'Donnell et al 2011). Similarly, viruses may have also evolved to take advantage of the host's circadian clock, for example, hepatitis C virus has been shown to modulate clock gene expression with *Per2* being found to inhibit viral replication (Benegiamo et al 2013). Influenza A virus infection appears to modulate clock gene expression resulting in increased lung inflammation of the host (Sundar et al 2015). In addition, it has been reported

that viral replication of wild-type HSV-1 in mice was 10-fold higher during the resting phase as opposed to their active phase. It was also shown that *Bmal1* $^{-/-}$ mice, deficient in several circadian rhythms, had equal levels of viral replication independent of the time of infection, suggesting a relationship between viral infection and the circadian clock. The involvement of the host immune response was ruled out by showing that viral replication was still influenced by time in a cell model devoid of an active immune response. Viral replication was found to be linked to *Bmal1* expression levels, and the viral protein ICP0 of HSV-1 was found to enhance clock gene *Bmal1*, suggesting that other viruses may also have evolved to specifically target the circadian machinery to their advantage (Edgar et al 2016).

Matsuzawa et al now demonstrate that HSV-2 pathology, viral titer, and innate immune responses varied depending on the time at which the mice were infected, with mice showing more severe infection at the active phase (ZT18/night) in comparison to the rest phase (ZT6/day). Similar observations were made in RAG-2-deficient mice, suggesting that acquired immunity did not influence the differential infection levels observed, which agrees with previous studies. The authors went on to demonstrate that expression of the HSV-2 receptor Nectin1 (*Pvrl1*) is upregulated during the active phase in correlation to increased viral pathology and titer observed. Expression levels of *Pvrl1* were found to fluctuate over time, and the authors demonstrated that Nectin-1 expression was under the direct regulation of the *Clock* gene and therefore the host circadian clock (see **Figure 1**). Furthermore, and adding some clinical relevance to their findings, they also show that a larger dose of acyclovir is necessary to treat HSV-2 acquired in the active phase compared with the lower dose required to control infection acquired during the rest phase (Matsuzawa et al 2017).

In light of these studies, it would be of interest to see if changes in circadian gene expression and resulting effects on the host cells can also influence viral transmission to naıve hosts. For example, could fluctuating expression levels of viral genes influence the rate of transmission between hosts in a time dependent manner? However, as with all complex pathways, it should also be kept in mind that *Clock* genes control multiple host cellular pathways influencing the immune response and cell metabolism to name a few. In addition, research in this field will no doubt be of importance for novel therapeutic strategies looking into how the circadian clock may influence the administration of drugs, such as time of treatment. Finally, identification of circadian genes involved in viral propagation could be used as targets for antiviral therapies and help develop other novel strategies for disease prevention.

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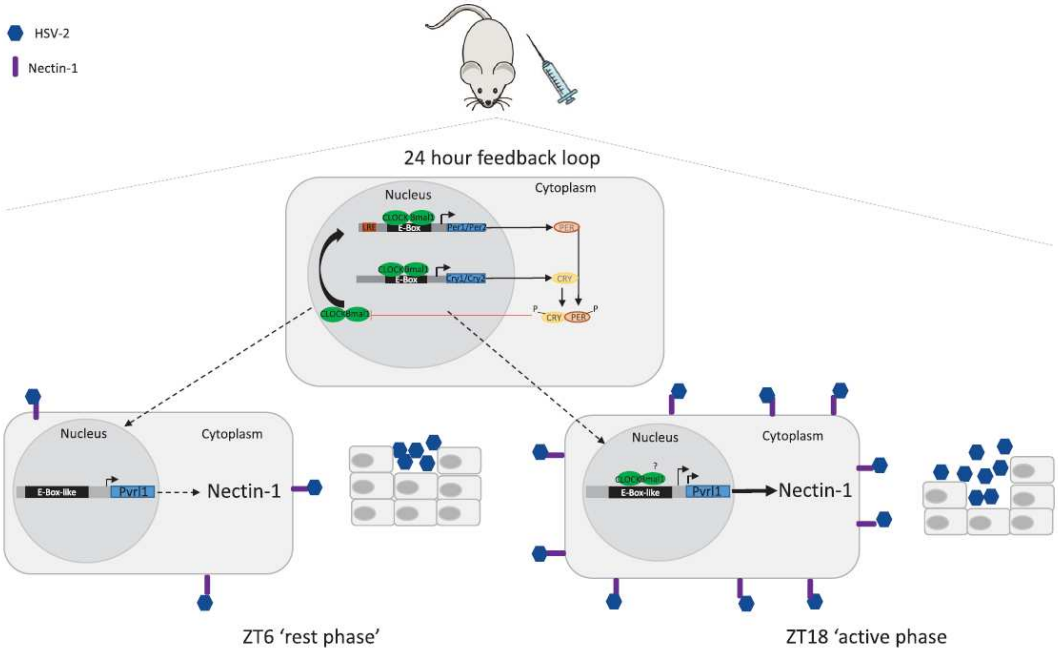
Figure 1.



Pvrl1 expression is under the control of the circadian CLOCK in murine keratinocytes resulting in increased HSV-2 pathology at the active phase.
The host circadian clock controls a transcription-translation feedback loop involving the genes CLOCK/NPAS2, Bmal1, Period1 (Per1), Per2 and Cryptochrome1 (Cry1) and Cry2. CLOCK: BMAL1 activate transcription of the Per1/2 and Cry1/2 genes. The PER and CRY proteins go on to heterodimerise resulting in translocation to the nucleus where they inhibit their own transcription by interacting with CLOCK:BMAL1 over a 24 hour period. Wild-type mice were intradermally infected with HSV-2 at ZT6 'rest phase' (ZT6) or 'active phase' (ZT18). CLOCK was found to bind to an E-box-like region present in the promoter region of the gene Prvl1 causing an increase in expression levels of the HSV-2 receptor Nectin-1 in the active phase which correlated to an observed increase in pathology and viral titre in comparison to mice infected at rest phase (ZT6).