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Virus reactivation: a panoramic view in human infections

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Abstract

Viruses are obligate intracellular parasites, relying to a major extent on the host cell for replication. An active replication of the viral genome results in a lytic infection characterized by the release of new progeny virus particles, often upon the lysis of the host cell. Another mode of virus infection is the latent phase, where the virus is 'quiescent' (a state in which the virus is not replicating). A combination of these stages, where virus replication involves stages of both silent and productive infection without rapidly killing or even producing excessive damage to the host cells, falls under the umbrella of a persistent infection. Reactivation is the process by which a latent virus switches to a lytic phase of replication. Reactivation may be provoked by a combination of external and/or internal cellular stimuli. Understanding this mechanism is essential in developing future therapeutic agents against viral infection and subsequent disease. This article examines the published literature and current knowledge regarding the viral and cellular proteins that may play a role in viral reactivation. The focus of the article is on those viruses known to cause latent infections, which include herpes simplex virus, varicella zoster virus, Epstein-Barr virus, human cytomegalovirus, human herpesvirus 6, human herpesvirus 7, Kaposi's sarcoma-associated herpesvirus, JC virus, BK virus, parvovirus and adenovirus.

Keywords

adenoviridae; cellular factors; herpesviridae; latency; lytic; parvoviridae; polyomaviridae; reactivation

Virus replication is a complicated process. It involves different steps from the time a virus binds to the target cells until new progeny virions are made and released to the outside. A replication phase that yields progeny virions is referred to as a productive or lytic cycle of infection. Lytic infection involves the replication of a viral genome. This genome is

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packaged into a viral coat and released from the cell. This process of viral release from the cells results in lysis of cells, and hence, it is termed the 'lytic phase'. There are a few relevant human viruses that have another phase of replication, usually referred to as the 'latent phase' – in other words, the virus lays dormant in this latent phase of replication. Latent infections have the ability to be reactivated into a lytic form. The ability to move back and forth from latent to lytic infections helps the virus spread from infected individuals to uninfected individuals. Apart from these two phases, a few viruses also have yet another method of replication, termed 'persistent infection'. Persistent viruses (e.g., hepatitis B virus) are those that are not eliminated following primary infection and remain in specific cells of the infected individuals. An infected individual experiences a persistent infection in which the virus is capable of replicating slowly, silently or at low levels without causing excessive damage to the host cell. This article focuses primarily on the latent-to-lytic switch that leads to virus reactivation.

Reactivation is the mechanism whereby a latent virus that has infected a host cell switches to a lytic stage, undergoing productive viral replication and allowing the virus to spread. Viral reactivation is associated with several stress factors [1], including viral infection (with other viruses), nerve trauma, physiologic and physical changes (e.g., fever, menstruation and exposure to sunlight) and immunosuppression (as in cytomegalovirus [CMV] disease). However, increasing evidence suggest that reactivation frequently occurs in the absence of such stimuli. This premise asserts that viruses are continuously shed, but that reactivation only occurs when local immunity is compromised by stimuli such as fever, menstruation and exposure to sunlight [2]. Generally, a lytic cycle of virus infection *in vitro* can be induced in cells harboring a latent virus genome by treatment with 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) [3]. Although we have an idea of the potential stimuli that may trigger virus reactivation from latency, the critical molecular factor (SWITCH) that triggers virus reactivation is still not clear.

There are two sides to the molecular switch triggering virus reactivation: first, virus-encoded immediate-early (IE) genes, and; second, cellular components. Virus-encoded IE genes are those that initiate virus reactivation as monitored by the expression of virus-encoded transcripts. These genes, when artificially overexpressed in latently infected cells, can potentially trigger a cascade of events that would result in the expression of a series of lytic cycle genes. On the other hand, there is the role of cellular factors, which, of late, have been found to play a crucial role in virus reactivation. This article reviews the available literature on the roles for the virus-encoded machinery and the cellular events in the reactivation process.

Viral latency

Many viruses have a propensity to cause latent infections. The majority of these viruses are from the family of *Herpesviridae*: herpes simplex virus (HSV)-1, HSV-2, varicella zoster virus (VZV), Epstein-Barr virus (EBV), CMV, human herpesvirus (HHV)-6, HHV-7 and Kaposi's sarcoma-associated herpesvirus (KSHV/HHV)-8. Both JC and BK virus (which belong to the family *Polyomaviridae*), adenovirus (family: *Adenoviridae*) and parvovirus and adeno-associated virus (AAV; family: *Parvoviridae*), also have a latent phase of replication. The focus of this article will be on these viruses (Table 1).

HIV rarely causes a latent infection, with a frequency of 10^6 – 10^7 cells per infected individual. This occurs owing to transcriptional silencing [4]. After initial infection, HIV integrates into the chromosomes, more specifically into heterochromatin. Due to this mechanism, HIV can reproducibly create a latent infection. As HIV is a retrovirus and present as RNA, reverse transcription needs to occur if the RNA is to integrate with the host

DNA. The RNA is transcribed into a cDNA copy, and this is then integrated into the chromosome of the host cell via specific sequences and sites located on the viral and cellular genome [5]. The latent infection of CD4 cells with HIV causes great difficulty in eliminating the virus completely, as the virus is undetectable to the immune system, partly because HIV kills the very same CD4⁺ cells that would otherwise orchestrate the immune response. ART can stabilize the infection, but stopping this treatment and activating the cells can lead to a systemic infection due to production of the virus [6].

Along the same lines, integration of virus DNA into the host chromosome may indirectly support virus latency in other DNA viruses. Specifically, polyomaviruses and some herpesviruses, to a lesser extent, can have their genomes integrated into host chromosomes. Integration of the recently identified Merkel cell polyomavirus DNA leads to truncation of the large T antigen, thus preventing virus replication in Merkel cell carcinoma (MCC) cells [7,8]. However, the truncated large T antigen maintains a protein-binding domain that is capable of binding the retinoblastoma protein, which may aid in tumor progression [8]. Herpesvirus DNA integration is a rare occurrence, with EBV and HHV-6 being the most notable examples, as they are currently the only HHVs to have successfully demonstrated integration of their entire genomes [9]. Although several latency factors can be transcribed, evidence supporting reactivation from integrated herpesvirus genomes has not been described [9,10].

Virus reactivation

The genome of a virus that causes latent infection of cells must be transcribed and translated into viral proteins. This occurs when the virus is reactivated from a latent stage to a lytic stage. Certain viral genes that are specific to each virus initiate this reactivation process.

In general, lytic infection of a virus is characterized by expression of a variety of virus-encoded genes whose roles are directed in one way or the other to make progeny virions. During a lytic phase of infection, viral genes are temporally expressed. Based on the time of their expression with respect to the initial onset of reactivation, the genes are referred to as IE genes, early (E) genes and late (L) genes. The IE, E, and L genes encode for proteins that usually regulate gene transcription, viral replication and structural proteins that lead to virion formation, respectively. Accordingly, IE genes are said to play the most crucial role in the reactivation process. Each virus has a set of regulatory genes that are activated when going into its lytic state. Although some viral gene products have been identified, the exact mechanisms behind transcriptional activation that determine a virus' reactivation from latency are still unknown [11]. To make this process clearer, there have been various approaches to examining how a virus reactivates, some focusing on the regulatory cascade of gene expression in productive infections.

Herpes simplex virus-1 establishes latency in sensory ganglia as a nonintegrated, nucleosome-associated episome in the nucleus of infected cells. During latency, transcription of viral genome is limited to the latency-associated transcript (*LAT*) [12]. This gene is primarily involved in not only establishing and maintaining latency of the virus but may also aid in protecting the latent HSV-1 reservoir from cytopathic superinfections [13]. Interestingly, numerous studies have demonstrated *LAT* to inhibit apoptosis (programmed cell death) in the trigeminal ganglia of infected animals and transiently transfected cells [14–17]. Thus, *LAT* also has a role in virus reactivation by prolonging or inhibiting neuronal death during reactivation [18]. Experiments on HSV-1 have indicated that the viral transactivator proteins ICP0, ICP4 and VP16 are critical for virus reactivation from a latent state [19,20]. This was based on *trans* expression studies *in vitro* and *ex vivo* as well as latency studies involving HSV-1 recombinants deleted or mutated in the viral

transactivators. There have been conflicting reports that ICP0 is important in viral production but is not necessary for the initiation of reactivation [21]. This report points towards ICP0 being involved with the virus production after the lytic stage has been activated. Recent studies have also demonstrated that VP16 is essential for efficient stress-induced reactivation from QIF-PC12 cells, whereas ICP0 is not [22]. A multitude of different studies investigating HSV reactivation show that the mechanisms are extremely intricate and challenging to understand. Such a complex process is synonymous with HSV-2 where ICP0 is sufficient to reactivate the latent virus in an *in vitro* system [23].

The VZV is also known as HHV-3. It displays a primary lytic infection that causes chicken pox and can reactivate from its latent state to produce an incapacitating disease in adults called shingles/zoster [24]. The incidence of zoster in the USA is approximately 5–6.5 per 1000 individuals per annum at 60 years of age, increasing to 8–11 per 1000 at 70 years of age [25]. Unlike varicella, which occurs primarily during the spring, there is no seasonal preference for zoster. Immunodeficiency may be a vital predisposing factor for the development of zoster. It is a concern in patients with a natural decline in VZV-specific cell-mediated immunity with age, and also for those with more serious immune deficits such as those seen in cancer patients and transplant recipients, and more so in AIDS patients [26]. Although VZV was the first viral herpesvirus to be sequenced, not much information is available regarding viral reactivation, partly because of the fact that it is an exclusively human pathogen. Transcription of ORF63 is the signature of VZV latent infection [27].

Epstein–Barr virus (HHV-4) displays latent and lytic cycles mainly in B lymphocytes and epithelial cells [28]. EBV is an oncogenic γ -herpes virus that persistently infects over 95% of the human population [29]. EBV Zta protein is the crucial transactivator of a variety of viral and host genes that are essential for the reactivation of EBV from latency [30]. EBV-encoded Zta's role in virus reactivation was recently demonstrated using a severe combined immunodeficiency mouse model [31]. Zta-knockout EBV cannot enter a complete lytic cycle in severe combined immunodeficiency mice, showing the key role for Zta in initiating virus reactivation.

Another member of the herpesvirus family, the human cytomegalovirus (HCMV/HHV-5), persists as a subclinical, lifelong infection in the human host owing to its ability to stay dormant. *In vivo*, cells of the myeloid lineage harbor HCMV in a latent phase [32]. The differentiation of myeloid cells may hold the crucial link for HCMV reactivation. For example, differentiation of myeloid progenitor cells specifically to dendritic cells not only reactivated viral lytic gene expression but also led to the production of infectious virus particles [33]. The mechanisms by which CMV replication and latency are regulated remain unclear. IE genes are required for viral replication. The major IE (*MIE*) gene products, which are autoregulatory transactivators that trigger the expression of downstream viral genes in transient assays, are found to be key players governing the productive cycle and are often found to be repressed in nonpermissive or latent infections [34]. Reactivation from the latent state must be mediated first by inducible host cell factors acting at the transcriptional level on the *MIE* enhancer. The CMV *MIE* gene products, IE1 and IE2 proteins, are presumed to be involved directly in regulating subsequent gene expression during the viral lytic cascade, as well as acting as potential triggers of the switch between latent and lytic infection [35].

Human herpesvirus 6 and HHV-7 establish latent infections predominantly in macrophages and T lymphocytes [36,37]. Both of these viruses are shed in the saliva of healthy people. The reactivated virus is associated with asymptomatic infection; however, it may cause severe disease conditions in transplant recipients [38]. More recently, this has been demonstrated by investigating the HHV-6 *IE2* gene (equivalent to the *MIE* within HCMV),

which is important in viral growth and transcriptional regulation [39]. Interestingly, HHV-6 has been shown to activate the lytic replication of KSHV, which may suggest that the virus pathways and open reading frames are closely related [40]. HHV-7 is closely related to HHV-6 [41]. As with VZV, not much is known about the crucial viral genes that trigger virus reactivation in HHV-6 and HHV-7.

The last addition to the list of human herpes-viruses is KSHV, which is also commonly referred to as HHV-8. This belongs to the γ 2-herpesvirus family (genus: *Rhadinovirus*) [42]. It is broadly known that KSHV Rta activates KSHV E lytic genes, including virus-encoded IL-6 and polyadenylated nuclear RNA, and a L gene, small viral capsid antigen [43]. It is considered to be the switch that triggers virus reactivation. Interestingly, KSHV Rta is the functional equivalent of EBV Zta [44]. KSHV reactivation can be triggered *in vitro* by treating cells with TPA or infecting cells with HCMV [45,46].

The exact triggers for the virus reactivation process in the cases of adenovirus, JC, BK and parvovirus in terms of virus-encoded proteins are poorly understood phenomena. It is hypothesized that major gene rearrangements, and possibly nucleotide sequence alterations in transcription binding sites, could serve as the 'switch' between latent and lytic infections [47]. AAV (another parvovirus) replication is dependent on one or more adenovirus E genes. HSV E genes are not necessary for AAV replication, yet some may be able to directly participate in AAV DNA replication [48].

Role of cellular proteins in virus reactivation

As it is, different viruses use different mechanisms in terms of viral encoded proteins to trigger their replication. These proteins that initiate virus replication are held in check by certain other proteins that promote virus latency – usually not more than a couple of transcripts. On the other hand, cellular factors are said to play a crucial role in initiating signals critical for the switch from latent to lytic infection. In this section, we have attempted to understand the set of crucial cellular signatures that have the potential to set up virus reactivation.

Herpes simplex virus reactivation from latency is initiated by external stimuli like stress and immunosuppression that stimulate viral gene expression [18]. Using microarrays, it was determined that the expression of certain early response genes was elevated in latently infected trigeminal ganglion, supporting virus reactivation triggered by stress [49]. The early response gene mRNAs that were increased during virus reactivation were heat-shock proteins (HSP40 and HSP60), basic transcription factors (TFs; BTF T62), DNA repair enzyme, MAPK, stress-induced protein kinase, oxidative stress-induced protein, manganese superoxide dismutase precursor-2 and cyclooxygenase 2. The cell-division cycle can be defined as the series of events that occur between one cell division and the next, and is a key factor in mediating HSV reactivation. Cyclin D2 gene expression was increased upon treating latently infected mice with immunosuppressant drugs that induced HSV reactivation [50]. Cyclin D consists of three subtypes of cyclins: cyclin D1, cyclin D2 and cyclin D3. Cyclin D2 contributes to not only sequestering the cell-cycle inhibitor p27 but also to switching from the G1 phase of the cell cycle through to S phase [51]. Cyclins, in general, bind a family of proteins referred to as cyclin-dependent kinases (CDKs) to phosphorylate and thus activate them [52]. CDKs regulate cell-cycle progression. HSV replication is dependent on the expression of cellular CDKs [53]. Inhibiting CDKs (specifically CDK-1, -2 and -5) significantly lowers virus replication [54]. CDKs are absent in quiescent neurons. Along with the loss of NGF-mediated latency, the expression of CDK-1 and -2 is elevated, likely supporting HSV reactivation [55,56]. Recent studies have also implicated a role for cyclin D3 in HSV replication [57]. HSV-1-encoded ICP0 is a regulatory protein that recruits

cyclin D3 to the nucleus. The authors hypothesize that this facilitates the availability of activated CDK-4 in the replication compartment, which is a necessity for the onset and maintenance of viral DNA synthesis.

Multiple signaling pathways seem to have a role in VZV reactivation. JNK/SAPK and p38/MAPK pathways appear to be essential for VZV replication. Rahaus and colleagues have shown that inhibition of ERK1/2-associated signaling cascade resulted in a decline in viral progeny, thus suggesting that this pathway also plays an important role in viral replication [58]. The mechanism of activation of the above signaling cascades resulting in a lytic mode of replication remains unclear.

Both Zta and Rta are key lytic transactivators that autostimulate their own expression, reciprocally induce one another and cooperatively direct the downstream expression cascade of EBV lytic genes [59]. How these factors, in combination with cellular TFs, cause expression of lytic genes remains undetermined. Studies have demonstrated that Zta and Rta are involved in the activation of p38, ERK and JNK signal transduction pathways, which may play a role in EBV reactivation [60–62]. *In vitro*, the lytic cycle can be induced by a variety of stimuli including changes in intracellular calcium concentration, treatment with phorbol ester, sodium butyrate, antihuman immunoglobulin, or TGF. *In vivo*, the mechanism by which the lytic cycle of EBV activation occurs is unknown.

A current theory about CMV infection is that undifferentiated monocytes harbor the virus in its latent phase, thereby allowing for extensive spread throughout the body [63]. Lytic infection is commonly observed in monocytes only after differentiation. In other words, cell-cycle progression does have a role to play in CMV reactivation as well. Reactivation of CMV is dependent on the CMV IE gene enhancer/promoter that regulates IE gene products and initiates CMV replication. Both NF- κ B and *c-Jun* play a crucial role in activating the CMV IE promoter and hence viral reactivation [64,65]. In the case of transplanted patients, allogeneic responses induce IE gene expression, which in turn results in the expression of TNF and subsequent activation of TFs, NF- κ B and AP-1 [66]. Cellular factors that are known to modify chromatin structure are said to play roles in cell differentiation-dependent CMV reactivation [67]. It is now well established that the regulation of many cellular genes involves interference at the level of their chromatin structure: active promoters are associated with acetylated histones whereas under-acetylated/methylated histones repress transcription of genes [68]. Chromatinization of IE gene promoters is implicated in CMV reactivation as in the case of EBV and HSV [28,34,69].

The phase of the cell cycle has a key role to play in HHV-6 infection. HHV-6 infection of target cells induces cell-cycle arrest by modulating expression of E2F TFs [70]. TF p53 induces cell-cycle arrest or apoptosis in target cells [71]. Recent studies have shown the ability of p53 to lower HHV-6 replication without actually influencing cell-cycle progression or apoptosis [72]. Limited research has been conducted to understand the role of cellular factors aiding in HHV-7 reactivation given the fact that its association with disease conditions is rare [73]. However, it has been documented that HHV-7 has the potential to reactivate HHV-6 latent infections [74,75].

Recently, it was shown that TPA-induced reactivation was optimal in KSHV-infected human B cells that were in the S phase of the cell cycle compared with the other phases [45,76]. In addition, using microarrays, we demonstrated that the cells in S phase of the cell cycle have their machinery tuned to provide signals that promote cell survivability and active DNA replication, and increased lipid metabolism, while blocking cell-cycle progression to M phase. All of these facets play a major role in terminating KSHV latency [45]. It is widely believed that a combination of well-orchestrated interactions between

KSHV and the cellular environment leads to reactivation of latency. Recently, separate studies from our laboratory and another described a role for the MAPK in inducing KSHV reactivation [77,78]. These studies demonstrated that either inhibiting or overexpressing the B-Raf-associated MEK-ERK1/2 signaling resulted in lowering or enhancing the TPA-induced KSHV reactivation process, respectively. These findings have been further confirmed by a systematic and elaborate study published recently [79,80]. Furthermore, two other separate studies have described that inhibiting NF- κ B signaling can promote KSHV reactivation under selective conditions [81,82]. However, the exact biomolecular switch that triggers KSHV reactivation remains elusive. KSHV-encoded Rta is the switch carried by the virus to initiate virus reactivation [83]. Multiple pathways involving a variety of TFs, including autoactivation by Rta, have been shown to either enhance or inhibit *ORF50* gene transcription [78,84–89]. Such a repertoire of different and, to some extent, redundant activating factors or pathways makes for an efficient mechanism to control one of the key features of virus replication – specifically, reactivation during different stages of pathogenesis as well as under different cellular environments. All the factors that have been described thus far to regulate the expression of Rta can be classified as cellular proteins, nuclear-associated virus-encoded proteins or chemicals. Recently, we have described the ability of the KSHV-encoded membrane-bound glycoprotein B (gB; an envelope-associated protein) to promote latency by regulating expression of a TF, early growth response-1 (Egr-1) [90].

Adenovirus can cause disease in immuno-compromised patients, owing to the reactivation of the virus [91]. Infections may also arise after transplantation, suggesting that adenovirus is reactivated endogenously [91]. Adenovirus maintains latency through E3 immunoregulatory genes that contain NF- κ B binding sites, and are activated during inflammation due to the TNF- α -induced signal transduction pathway [92]. Adenovirus *E1A* gene products are transactivators that can interact with cellular TFs, transcriptional cofactors and cell-cycle-regulatory proteins crucial for reactivation [93]. Also, proteins encoded by *E3* and *E4* genes seem to have a critical role in the lysis of infected cells [94,95].

JC and BK virus reactivation is regulated by the nuclear factor of T cells via its ability to interact with a variety of signaling molecules such as c-Jun, c-fos and NF- κ B p65 subunits [96–98]. Incidentally, HIV Tat protein has been demonstrated to activate BK virus from latency by activating NF- κ B p65 activity [99].

Parvovirus B19 maintains a latent infection by regulating pathways that include AP-1 and SP1, NF- κ B pathway, TNF- α and p53 through the virus-encoded NS1 protein [100]. However, the exact mechanism by which cellular events initiate parvovirus reactivation is yet to be elucidated.

A possible unifying theme for the molecular trigger from within the cells that initiates virus reactivation

We believe that cellular signaling plays a very critical role in inducing virus reactivation based on the following reasons: first, viruses are non-living things; and second, stress almost always seems to be a major factor leading to the stimulation of all the virus reactivation processes. Different viruses utilize different IE gene products to drive their replication in response to cellular signaling. The single most common cellular signal that seems to be driving the reactivation of viruses is MAPK in conjunction with the NF- κ B pathway, to a lesser extent. Stress, along with various other growth factors (GFs)/inflammatory cytokines, has the propensity to trigger these cascades (Figure 1). There exists a complicated set of interactions between these cellular events, leading up to activating a set of different TFs, ultimately resulting in cell proliferation/differentiation; in other words, cell-cycle

progression. As one might recall, virus reactivation is commonly observed in cells that are actively dividing. Thus, the stress-induced MAPK/NF- κ B signaling provides apt conditions for virus reactivation by inducing cell-cycle progression. In our earlier studies, we demonstrated that cells in the S phase of the cell cycle provide conditions that promote active DNA replication that is crucial for virus replication [45].

Since many viruses target signaling pathways that regulate responses such as mitosis, apoptosis, motility, proliferation and differentiation, they have a means to manipulate cellular function, including reactivation and lytic replication [101]. With valid consideration of MAPK/NF- κ B as a unifying theme in all virus reactivation, testing inhibitors and blocking this signaling could be one possible route to reactivation inhibition (Table 2). For example, it has been determined that upon inhibition of the p38 MAPK pathway, bacterium-mediated induction of lytic KSHV infection is greatly reduced [102]. More specifically, the effect of dehydroxymethylepoxyquinomicin (DHMEQ), a relatively new NF- κ B inhibitor, was examined on primary effusion lymphoma cells, a refractory malignancy that can be caused by both KSHV and EBV infections. NF- κ B is constitutively activated in these cells. Interestingly, it was shown that DHMEQ transiently destroys NF- κ B activation, irreversibly triggering the apoptosis cascade without reactivating KSHV lytic genes, hence not inducing virus replication. Just as DHMEQ may be a promising candidate for molecular target therapy of the primary effusion lymphoma, further developments of MAPK/NF- κ B inhibitors may prove to be effective therapies for other viruses and complications resulting from those viruses [103].

Conclusion: latency, a ‘double-edged sword’

The majority of the world’s population becomes infected with multiple herpesviruses during childhood, and after clearance of acute infection, viral latency is established in the host and persists for life [104,105]. On one hand, latency is deemed a beneficial symbiotic relationship, but to this notion’s detriment, it is also noted that viral persistence in latent phase is the greatest obstacle for effective antiviral therapy [105,106].

Reactivation from viral latency is associated with an array of human pathologies. Although normally controlled in immunocompetent adults, β -herpesvirus, CMV, can cause severe disease such as hepatitis following reactivation in immunosuppressed hosts [104]. VZV, for example, can reactivate from its latent state to cause shingles/zoster in the elderly [24]. Seemingly, in such cases, maintenance of viral latency would be a godsend. In a 2009 letter, Barton *et al.* state that a virus that increases the odds that its host will survive to a reproductive age, even if the period of enhanced fitness lasts only months, confers a lifelong benefit and meets the definition of a beneficial symbiont [107]. Also, the perks from latency may provide more than protection from pathogens. For instance, data support the ‘hygiene’ hypothesis, in which prior infection protects against development of allergy [107].

On the flipside, there is an opposing view based on the mechanism of immune evasion and lifelong persistence, in which latency is deemed exclusively pathogenic [107]. It has been suggested that viral miRNAs are a component of the immune evasion repertoire controlling viral latency in the case of herpesviruses, and it is speculated that they are a necessity in the virus lifecycle. As controllers of virus latency, viral miRNAs play an essential role in immune evasion by inhibiting immune surveillance and extending the life of the infected host cell. This is regarded as a hindrance to vaccine development [108].

To date, virus vaccines are completely ineffective during latency; only upon reactivation to lytic phase are current vaccines beneficial treatments. For instance, the key protein target for all the successful α -herpesvirus antivirals available is the virus-encoded DNA-polymerase.

For selectivity, other compounds depend on their phosphorylation by the herpesvirus thymidine kinase. However, during latency, the virus does not typically express genes coding for virus proteins, including both DNA-polymerase and thymidine kinase. Likewise, there is no obvious solution of how to destroy herpesvirus DNA concealed in latently infected cells, as latent viruses are unaffected by any of the conventional nucleoside analogs or drugs that rely on viral protein targets. Although vaccination against herpesviruses has been difficult, advancements have been made; in fact, HSV was among the very first infections to be treated successfully using antiviral compounds, proving that a viral disease could be successfully treated in this way [106].

Whether virus latency is considered to be advantageous or disadvantageous, both views aspire to achieve the common goal of improving human health and alleviating human suffering from virus-associated health complications through either maintenance of latency and/or the development of vaccines. In comparison to many other viruses, development of herpesvirus antivirals is at the forefront of current research. The majority of parvovirus cases do not require specific therapy, adenovirus is extensively used as a vector in gene therapy, and although BK and JC polyomaviruses are common [109], they are highly asymptomatic and only pathogenic when reactivated in immunosuppressed individuals [110–112].

Future perspective

With regard to future therapies, current inhibitors for pathways such as MAPK/NF- κ B are unspecific, as they inhibit activity in both host and infected cells. We propose that a strategy for effective antiviral therapies would be to design specifically regulated inhibitors that do not lower signaling below functional physiological levels. By doing so, we can ensure that the activity of such signaling molecules is not reduced below threshold levels in uninfected or tumor cells. In summary, research providing further understanding of the molecular biology of viruses as well as the specifics of the molecular switch that triggers virus reactivation is warranted in an effort to demystify latency.

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Executive summary

- Virus replication is a complicated process. Viruses may undergo three different phases of replication: lytic, latent and persistent.
- Not all viruses establish latency upon infection.
- Viruses belonging to the *Herpesviridae*, *Polyomaviridae*, *Parvoviridae* and *Adenoviridae* families commonly seem to establish latent infections in target cells.
- Reactivation is a process by which virus latency is terminated, leading to a lytic phase of replication.
- The exact mechanism that triggers this switch from latent-to-lytic replication is not known.
- Both viral and cellular factors play a critical role in virus reactivation.
- Activation of MAPK, and NF- κ B signaling to a lesser extent, seems to be a common theme within cells supporting virus reactivation. Furthermore, specific targeting of these pathways may prove to be advantageous in developing future therapies to treat such virus-associated disease conditions.
- Whether virus reactivation is considered to be advantageous or disadvantageous, knowledge of the trigger(s) that initiates such an event is viewed as getting one step closer to improving human health and alleviating human suffering from virus-associated health complications.

Figure 1. Major signaling pathways that are involved in virus reactivation

Alternative signaling pathways that involve Ras/Raf/NF-κB as key players in reactivation of different viruses are depicted. The Raf/MEK/ERK pathway of signaling interacts with other signal transduction pathways, leading to the activation of a variety of transcription factors that are critical for the transcription of specific cellular genes and in inducing viral reactivation. Signaling molecules that are boxed in blue are critical upstream components in the divergent MAPK and NF-κB signaling pathways. These molecules can be activated by one or more of the ligands listed above.

Table 1

Viruses that have the potential to cause both latent and lytic cycles of replication.

Virus	Associated disease conditions	Ref.
Herpesviridae		
HSV-1	Cold sores, herpetic whitlow, encephalitis, herpetic keratitis and herpes pharyngitis	[113,114]
HSV-2	Genital herpes	[115]
VZV	Chicken pox and shingles	[116]
EBV	Mononucleosis, Burkitt's lymphoma, naso-pharyngeal carcinoma, hairy oral leukoplakia and non-Hodgkin's lymphoma	[117,118]
CMV	Congenital diseases, heterophile-negative mononucleosis syndrome, hypertension and atherosclerosis	[119,120]
HHV-6	Roseola	[121]
HHV-7	Roseola	[122]
KSHV (HHV-8)	Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman disease	[123,124]
Papillomaviridae		
JC virus	Progressive multifocal leukoencephalopathy	[97]
BK virus	BK virus nephropathy	[125]
Parvoviridae		
Parvovirus b19	Fifth disease and 'gloves-and-socks' syndrome	[126]
AAV	No disease is associated with this virus as of yet	[127]
Adenoviridae		
Adenovirus	Pharyngitis, keratoconjunctivitis, gastroenteritis, hemorrhagic cystitis and meningoencephalitis	[128,129]

AAV: Adeno-associated virus; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; HHV: Human herpesvirus; HSV: Herpes simplex virus; KSHV: Kaposi's sarcoma-associated herpesvirus; VZV: Varicella zoster virus.

Table 2

Examples of inhibitors of signal transduction pathways currently in clinical trials that have the potential to serve as antiviral agents.

Drug	Company (location)	Mode of action	Stage of clinical testing	Role in virus replication?	Ref.
MAPK inhibitors					
PLX4032	Plexxikon (co-) Roche (WI, USA)	Inhibits BRAF	Phase II and III	N/A	[130]
E6201	Eisai Inc. (MA, USA)	Inhibits MEK-1 and MEK kinase-1	Phase I	N/A	[131]
ARRY-142886	AstraZeneca (DE, USA) and Array BioPharma Inc. (CO, USA)	Inhibits basal and epidermal growth factor-induced ERK1/2	Phase II	Yes	[132,133]
PD-0325901	Pfizer (NY, USA)	Oral MAPK/ERK kinase inhibitor	Phase II	Yes	[133,134]
SB-681323	GlaxoSmithKline (Middlesex, USA)	Inhibits p38 MAPK	Phase II	N/A	[135]
VX 702	Vertex Pharmaceuticals (MA, USA)	p38 MAPK inhibitor	Phase II	N/A	[136]
AS601245	Enzo Life Sciences (PA, USA)	ATP-binding site inhibitor of JNK	In rat and gerbil models	Yes	[137,138]
NF-κB inhibitors					
Bortezomib	Millennium Pharmaceuticals (MA, USA)	Protease inhibitor that indirectly blocks NF-κB	Phase I and II	Yes	[139-143]
Curcumin	Sabinsa (NJ, USA)	NF-κB and tumor inhibitor	Phase II	Yes	[144,145]

N/A: Not applicable.