Noise Cancellation: Viral Fine Tuning of the Cellular Environment for Its Own Genome Replication

Yoshitaka Sato¹²³, Tatsuya Tsurumi¹⁴*¹

¹ Division of Virology, Aichi Cancer Center Research Institute, Nagoya, Japan, ² Department of Virology, Nagoya University Graduate School of Medicine, Nagoya, Japan, ³ Department of Cell Biology, G-COE, Kobe University School of Medicine, Kobe, Japan, ⁴ Department of Oncology, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan

Abstract: Productive replication of DNA viruses elicits host cell DNA damage responses, which cause both beneficial and detrimental effects on viral replication. In response to the viral productive replication, host cells attempt to attenuate the S-phase cyclin-dependent kinase (CDK) activities to inhibit viral replication. However, accumulating evidence regarding interactions between viral factors and cellular signaling molecules indicate that viruses utilize them and selectively block the downstream signaling pathways that lead to attenuation of the high S-phase CDK activities required for viral replication. In this review, we describe the sophisticated strategy of Epstein-Barr virus to cancel such “noisy” host defense signals in order to hijack the cellular environment.

Introduction

Cellular DNA damage responses initiate with activation and rapid recruitment of repair proteins to DNA damage sites [1,2]. Until the damage is repaired, cells are prevented from transitioning to the next stage of the cell cycle. The tumor suppressor p53 is phosphorylated by DNA damage–responsive kinases, resulting in stabilization of p53 and an increase in its protein level [3,4]. This leads to activation of target gene transcription including p53 itself, which subsequently causes cell cycle arrest or apoptosis [3,4]. The replicated viral genomes of DNA viruses, including adenoviruses, the polyomavirus, and herpesviruses, are recognized by cellular DNA damage sensors, triggering activation of DNA damage responses [3,6,7,8,9]. Several lines of evidence revealed viral approaches to create an optimal environment for viral replication by manipulating the host defense systems. In this review, we describe the elegant strategies used by Epstein-Barr virus (EBV) to cancel “noisy” cellular signaling in order to manipulate the cellular environment for its own genome replication.

Life Cycle of the Epstein-Barr Virus

EBV, a human lymphotropic herpesvirus, infects more than 90% of world’s population and is now known to contribute to a variety of human disorders, including infectious mononucleosis, nasopharyngeal carcinoma, Burkitt’s lymphoma, and lymphoproliferative diseases occurring in immune-compromised individuals [10]. The lifecycle of EBV is quite distinctive, featuring two alternative infection cycles: “latent” and “lytic.” Primary EBV infection targets resting B lymphocytes, inducing their continuous proliferation. In the resultant B lymphoblastoid cell lines that express a limited number of EBV gene products, the viral genomes are maintained as circular plasmids forming nucleosomal structures with histones [11], and there is no production of virus particles, this being called “latent” infection. In the latent state, viral DNA is replicated only once during S phase, just as host chromosomal DNA [11]. Only a small percentage of infected cells switch their states from the latent stage into the “lytic” cycle to produce progeny viruses. EBV DNA replication occurs at discrete sites in nuclei called “replication compartments,” where all of the viral replication proteins are assembled [12]. During lytic replication, the circular genome becomes a ready template for amplification by the viral replication machinery, generating thousands of copies per cell. This reactivation is correlated with the emergence of human cancers [13,14]. The switch from latent to lytic replication is triggered by expression of the EBV BZLF1 gene product (also called Zta or ZEBRA) [15]. The BZLF1 protein is a lytic replication origin binding protein and acts to transactivate various viral promoters [16], leading to an ordered cascade of viral gene expression: activation of early genes followed by viral genome replication and late gene expression. Using the EBV system, the alteration in cellular conditions, from latent to virus-productive infection without overlapping signals triggered by virus entry, can be monitored [17,18].
uncontrolled cellular proliferation without inducing apoptosis in latent EBV-infected cells.

**The Important Role of p53 in the Early Stages of EBV Lytic Replication**

We propose that, during the course of lytic replication, BZLF1 protein plays two distinct roles in the regulation of p53-mediated transactivation, which depend on the progression of lytic replication: the early stage and the middle to late stages (described below) (Figure 1B). Previous studies demonstrated that the EBV immediate-early protein BZLF1, which was either conditionally expressed [22,23] or overexpressed by a recombinant adenovirus [24,25], could induce G1 arrest in some cell lines. The BZLF1 protein causes the accumulation of both mRNA and protein of CDK inhibitor p21Cip1/Waf1 [23], a well-known p53-target gene product. BZLF1 protein accelerates the rate of p53-DNA complex formation through physical interaction with p53 [26]. In the early phases of lytic replication, p53 is hypophosphorylated and therefore exhibits weak DNA binding ability to its recognition sequences [9]. The BZLF1 protein helps the hypophosphorylated p53 to bind to its recognition sequences, leading to the enhancement of p53-dependent transcription [26]. Levels of p53 and p21Cip1/Waf1 are transiently elevated in the early stages of lytic replication, and then decline with the progression of lytic infection [26], probably reflecting the effects of BZLF1 expression.

Recently, we and other groups have shown that p53 is involved in reactivation of EBV [26,27]. Tsai and his colleagues have reported that induction of viral lytic proteins by a chemical inducer, sodium butylate, does not occur in p53-negative H1299A and Saos2A cells [27], although the ability of BZLF1 or BRLF1 protein to transactivate its downstream genes is not notably affected by the lack of p53 [28,29]. This implies that p53 might instead be required for a switch from the latent to the lytic cycle. Indeed, we found that overexpression of p53 in the early stages of lytic replication enhances subsequent viral genome replication [26].

In the case of human cytomegalovirus (HCMV), the level of p53 is elevated upon viral infection, but its downstream transcriptional targets remain inactivated [30,31]. It has been reported that cells infected with HCMV in the absence of p53 produce fewer infectious viral particles and cause delays in viral protein production and trafficking [30]. The HCMV genome has 21 potential p53-responsive sites [32]. HCMV gene expression is thought to be influenced by p53 molecules bound to HCMV genome at immediate-early and early stages of the infection, which

---

**Figure 1. Stage-specific regulation of p53 during EBV infection.** (A) The ubiquitination of p53 is regulated by both MDM2 E3 ligase and USP7 deubiquitinase in uninfected cells. During EBV latent infection, EBV latent EBNA1 protein inhibits USP7 and thereby drives the ubiquitination of p53. Phosphorylated p53 is ubiquitinated by BZLF1 protein-associated E3 ligase independently of MDM2 during lytic infection. (B) During the latent phase of EBV infection, p53 is quantitatively regulated by MDM2 ubiquitin ligase via the ubiquitin-proteasome pathway [36], serving as a guardian of genome stability. Expression of BZLF1 protein induces virus-productive (lytic) replication through the ordered cascades of viral gene expression, and concomitantly host DNA damage responses [9], leading to p53 phosphorylation and release of p53 from the MDM2-dependent regulation [36]. In the early stages of lytic infection, the inactive (hypophosphorylated) form of p53 cooperates with viral factors including BZLF1 protein to stimulate virus replication [26,27]. In the middle and late stages of infection, active (hyperphosphorylated) p53 is ubiquitinated by BZLF1 protein–associated ECS ubiquitin ligase complexes and is degraded in a proteasome-dependent manner to inhibit apoptosis [37].

doi:10.1371/journal.ppat.1001158.g001

---

**A**

Uninfected cells

EBV-infected cells (Latent)

EBV-infected cells (Lytic)

**B**

| Latent phase | Lytic phase |
|--------------|-------------|
| Early        | Middle & Late |

| BZLF1 expression | p53 | Protein abundance | Phosphorylation | Regulation | Specific function |
|------------------|-----|------------------|----------------|------------|-----------------|
| MD2-dependent    | MD2-dependent |
| Guarding the genomic integrity | Cooperation to reactivation | Activated p53 is degraded to avoid induction of apoptosis | low | high |
could explain the mechanism for reduced and delayed production of virions in p53-negative cells. Similarly, potential p53 recognition sequences are present on the EBV genome [T. Murata et al., unpublished results]. Indeed, we have found that p53 is associated with EBV replication compartments [9]. Thus, in the early stages of EBV lytic infection, p53 could be recruited to the EBV genomic regions through its direct binding to the recognition sequences. The BZLF1-mediated enhancement of p53-DNA binding may therefore contribute to the expression of viral genes (Figure 1B).

Newly Synthesized Viral DNA Elicits Host DNA Damage Responses

Herpesviruses such as HSV, HCMV, and EBV modulate the cell cycle to promote a transition through G1-S phase and achieve the cellular environment with high S-phase CDK activities, called the S-phase-like condition, for virus-productive replication (reviewed in [18]). During the EBV lytic replication, the levels of cyclin E and cyclin A continue to be elevated, and cyclin E- and cyclin A-associated CDK activities actually increase [9]. Moreover, this elevation of S-phase CDK activities drives accumulation of the hyperphosphorylated form of retinoblastoma protein (Rb) and an increase in the level of E2F-1 transcription factor [9]. The observation that chemical CDK inhibitors, such as purvalanol A and roscovitine, block viral lytic replication through prevention of viral immediate-early and early gene expression [33] suggests that a cellular environment featuring high CDK activities is required for efficient viral replication. It is conceivable that expression of proteins involved in DNA metabolism may be promoted under S-phase conditions, when energy generation and other resources support viral replication [18]. However, cellular DNA synthesis is almost entirely blocked during the lytic phase of EBV DNA replication, despite S-phase-like cellular conditions with high CDK activities [17]. The EBV-encoding protein kinase (PK) BGLF4 phosphorylates MCM complex to inhibit its replicative helicase activity (Figure 2) [34]. Although the precise mechanism remains unclear, it might be one of the reasons for inhibition of chromosomal DNA replication and for the blockade of the cell-cycle progression from S to G2 phase.

The host cell DNA damage-sensing machinery recognizes the newly synthesized viral DNA in the lytic phase as “abnormal” DNA, activating ATM-dependent DNA damage signaling [9] (Figure 2). ATM phosphorylates histone H2AX (H2AX), which initiates the DNA damage response. The EBV BGLF4 PK might further amplify this response through phosphorylation of H2AX [35]. ATM phosphorylates p53 at Ser-15, which liberates p53 from MDM2-mediated degradation. The downstream kinases of ATM, Chk1, and Chk2 also phosphorylate p53 at various sites. Therefore, elicitation of DNA damage responses in general activates the transcriptional functions of p53.

Ubiquitin-Mediated Degradation of p53 in the Middle and Late Stages of Lytic Infection

Paradoxically, reactivation of EBV induces cellular DNA damage responses that causes phosphorylation of p53, which could lead to accumulation of p53 and subsequent activation of p53 downstream signaling (Figure 1), at the same time it establishes the S-phase-like cellular environment. At the middle to late stages of the lytic replication, the p53 target gene products are indeed maintained at low levels [9,17,26,36]. An explanation for this comes from the observation that p53 is degraded via the ubiquitin-proteasome pathway in the middle and late stages of lytic infection, allowing EBV to exploit cellular environments with high CDK activities for efficient viral replication (Figure 2).

A series of recent studies have shown that induction of the EBV lytic program leads to degradation of p53 via a ubiquitin-proteasome pathway independently of MDM2 [36]. The BZLF1 protein functions as an adapt complex in the regulation of diverse cellular processes [52,53] provides us with new insights into their significance as potential targets of viruses manipulating the host cellular system. Post-translational modifications, especially phosphorylation and ubiquitination, play a crucial role in cell-cycle progression.

Regulation of CDK Inhibitors during Lytic Replication

The large body of evidence implicating Cullin-based E3 ubiquitin ligase in the regulation of diverse cellular processes [52,53] provides us with new insights into their significance as potential targets of viruses manipulating the host cellular system. Post-translational modifications, especially phosphorylation and ubiquitination, play a crucial role in cell-cycle progression.
Phosphorylation controls the activity of proteins involved in G1-S and G2-M transitions. Ubiquitination and its mediated proteolysis are commonly facilitated to maintain threshold levels of cell-cycle regulators. Two distinct classes of E3 ubiquitin ligase regulate cell-cycle progression [52], possessing an adaptor protein to determine substrate specificity [54,55,56]. E3 ligase activity of the anaphase-promoting complex is required for the G2-M transition [57]. The SCF (Skp1-Cul1-F-box protein) family of E3 ligase promotes ubiquitination of phosphorylated substrates and typically targets the mediators of G1-S transition [58]. For instance, ubiquitination-mediated degradation of p27Kip1 is regulated by the SCF complex only when p27Kip1 is phosphorylated at Thr-187 by the cyclin E-CDK2 complex, which induces S phase conditions [59,60,61].

The EBV lytic program promotes specific cell cycle-associated activity involved in progression from G1 to S phase, since virus-productive replication occurs under S-phase-like circumstances [18]. Similar to p53, CDK inhibitors are also regulated during lytic replication, contributing to establishment of an S-phase-like cellular environment with high-CDK activities [9,33]. γ-Herpesviruses possess their own strategies to degrade p27Kip1. For example, Kaposi’s sarcoma-associated herpesvirus (KSHV)-encoding cyclin (v-cyclin), a latent viral protein, forms a complex with CDK6 to phosphorylate Thr-187 on p27Kip1, leading to down-regulation at the protein level [62,63]. Also, the viral cyclin encoded by murine herpesvirus 68 preferentially associates with CDK2 to phosphorylate Thr-187 on p27Kip1 [64]. While EBV does not encode any v-cyclin homologue in its genome, our recent study revealed that the EBV protein kinase BGLF4 can phosphorylate the Thr-187 residue of p27Kip1, resulting in its ubiquitination and degradation in an SCFSkp2 ubiquitin ligase-dependent manner [65] (Figure 2).

Manipulating the ubiquitin system by EBV involves two aspects of its regulation: attachment of ubiquitin to a substrate and removal from its substrate. As an EBV-encoding deubiquitinating enzyme, BPLF1 deubiquitinates and reduces activity of EBV
ribonucleotide reductase [66]. In this case, deubiquitination influences the function of the protein rather than targeting it for proteasomal degradation. A recent paper documented that BPLF1 also act as a de neddylase [67]. Neddylation, which is a conjugation of ubiquitin-like modifier NEDD8 to its substrate, is an important mechanism for regulating Cullin-based E3 ubiquitin ligases [68]. The EBV BPLF1 binds to Cullins and attenuates the activity of the Cullin-RING ligases, resulting in accumulation of the licensing factor Cdt1 and induction of DNA re-replication. Inhibition of BPLF1 during the lytic infection prevents viral replication in the cells that carries a recombinant EBV [67]. These findings support the idea that manipulating ubiquitin system by virus promotes viral productive replication. Furthermore, two lytic proteins (BSLF1 and BXLFI) are found as deubiquitinases by a bioinformatic search on the EBV genome [69], although their functions in viral replication remain obscure. Further investigations are needed to determine the exact role of deubiquitination in the context of EBV lytic infection.

The level of another CDK inhibitor protein p21Cip1/Waf1, of course, becomes low during lytic replication [36]. Although the detailed mechanisms remain unknown, one reason is that p53 is actively degraded during lytic infection and another is that the SCFSkp2 ubiquitin ligase directs p21Cip1/Waf1 for degradation through S-phase CDK-mediated phosphorylation [70]. Recent study showed that KSHV-encoding microRNA, miR-K1 represses expression of p21Cip1/Waf in latent infection [71]. As an additional mechanism, an EBV-encoding microRNA that has yet to be discovered might regulate p21Cip1/Waf1 for maintaining S-phase-like conditions.

On the other hand, maintaining low levels of CDK inhibitors results in accumulation of the hyperphosphorylated Rb protein due to high S-phase CDK activities and causes accumulation of active E2F-1 as lytic replication progresses [9]. E2F-1 in turn activates the transcription of many proteins involved in cellular DNA synthesis and cell-cycle progression [72], and probably transcription of the EBV DNA polymerase gene as well [73]. The available data suggest that E2F activity is required for lytic viral DNA replication. Alternatively, the EBV immediate-early transactivator BZLF1 and BRLF1 proteins are reported to increase the level of E2F-1 [74,75]. Furthermore, since activated ATM or Chk2 phosphorylates and activates E2F-1 in response to DNA damage [76,77], the DNA damage response induced by EBV lytic replication could activate E2F-1. To achieve effective viral lytic replication, EBV therefore possesses a variety of strategies to maintain the S-phase-like cellular environment.

**Beneficial Aspects of DNA Damage Signaling on EBV DNA Replication**

During EBV lytic replication, phosphorylated ATM and Mre11/Rad50/Nbs1 (MRN) complexes are targeted to replication compartments in nuclei. Simultaneously, homologous recombinational repair (HRR) factors such as replication protein A (RPA), Rad51, and Rad52, as well as MRN complex, are recruited and loaded onto the newly synthesized viral genome in replication compartments [40]. The 32 kDa subunit of RPA is extensively phosphorylated at sites in accordance with these events [40]. Hyperphosphorylation of RPA52 causes a change in RPA conformation, resulting in a switch from catalysis of DNA replication to participation in DNA repair. RNAi knockdown of RPA32 and Rad51 prevents viral DNA synthesis, suggesting that homologous recombination and/or repair of the viral DNA genome might occur, coupled with viral DNA replication to facilitate viral genome synthesis (Figure 2). Thus, the host DNA damage response induced by productive viral replication is essential for efficient EBV lytic genomic replication.

**Conclusions**

Replication of DNA viruses in host cells triggers a variety of cellular signaling cascades, including the DNA damage response. Recent studies indicate that such cellular responses to viral genomic replication paradoxically play a crucial role in EBV lytic replication by establishing cellular conditions appropriate for efficient viral replication. To achieve these conditions, EBV manipulates host ubiquitin-proteasome systems, and thereby cancels host antivirus signals. During lytic infection, the interaction between BZLF1 protein and ECS E3 ligase complexes leads to p53 degradation, and the SCF E3 complex recognizes and ubiquinates phosphorylated p27Kip1 through viral protein kinase. Therefore, by skipping the induction of checkpoint signaling and apoptosis, virus-producing cells stay in a persistent S-phase-like environment with high CDK activity.

**Accession numbers**

The Entrez Gene (http://www.ncbi.nlm.nih.gov/gene) accession numbers for genes and gene products discussed in this study are as follows: p53 (7157), p21Cip1/Waf1 (1026), p27Kip1 (1027), USP7 (7874), MDM2 (4193), E2F-1 (1869), ATM (472), Chk2 (11200), H2AX (3014), PARP (142), Skp2 (6502), Cdt1 (81620), ubiquitin (7314), NEDD8 (4738), Rb (5925), Cyclin E (898), Cyclin A (890), CDK2 (1017), CDK6 (1021), RPA32 (6118), Rad51 (5888), Rad52 (5893), KSHV v-cyclin (4961471), and EBV EBNA1 (3783709), BGLF4 (3783704) BPLF1 (3783726), BSLF1 (3783705), USP7 (7874), RAD51 (890), RPA32 and Rad51 prevents viral DNA synthesis, suggesting that homologous recombination and/or repair of the viral DNA genome might occur, coupled with viral DNA replication to facilitate viral genome synthesis (Figure 2). Thus, the host DNA damage response induced by productive viral replication is essential for efficient EBV lytic genomic replication.

**Acknowledgments**

We thank Dr. Y. Nishiyama (Nagoya University) and Dr. S. Seino (Kobe University) for encouragement, and Dr. T. Igaki (Kobe University) for sharing unpublished data.

**References**

1. Sancar A, Lindsey-Boltz LA, Urai-Kaczmak K, Liu S (2004) Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 73: 39–85.
2. Rose J, Jackson SP (2002) Interfaces between the detection, signaling, and repair of DNA damage. Science 297: 547–551.
3. Haffner R, Oren M (1995) Biochemical properties and biological effects of p53. Curr Opin Genet Dev 5: 84–90.
4. Ko LJ, Prives C (1996) p53: puzzle and paradigm. Genes Dev 10: 1054–1072.
5. Kudoh A, Fujita M, Zhang L, Shirata N, Daikoku T, et al. (2005) Epstein-Barr virus lytic replication elicits ATM checkpoint signal transduction while providing a dual signaling environment. J Virol 61: 1743–1746.
6. Dahl J, You J, Benjamin TL (2005) Induction and utilization of an ATM signaling pathway by polyomavirus. J Virol 79: 13007–13017.
7. Shirata N, Kudoh A, Daikoku T, Tatsumi Y, Fujita M, et al. (2003) Activation of ataxia telangiectasia-mutated DNA damage checkpoint signal transduction elicited by herpes simplex virus infection. J Biol Chem 278: 30336–30341.
8. Gaspar M, Shenk T (2006) Human cytomegalovirus inhibits a DNA damage response by modulating checkpoint proteins. Proc Natl Acad Sci U S A 103: 2821–2826.
9. Kudoh A, Fujita M, Zhang L, Shirata N, Daikoku T, et al. (2005) Epstein-Barr virus lytic replication elicits ATM checkpoint signal transduction while providing an S-phase-like cellular environment. J Biol Chem 280: 8156–8163.
10. Young LS, Rickinson AB (2004) Epstein-Barr virus: 40 years on. Nat Rev Cancer 4: 757–768.
11. Adams A (1987) Replication of latent Epstein-Barr virus genomes in Raji cells. J Virol 61: 1743–1746.
12. Daikoku T, Kudoh A, Fujita M, Sugaya Y, Isomura H, et al. (2005) Architecture of replication compartments formed during Epstein-Barr virus lytic replication. J Virol 79: 3499-3418.

13. Josh I, Nicola CJ, Schaeb G, de-The G, Clause B, et al. (1991) Detection of anti-Epstein-Barr Virus-transactivator (ZEBRA) antibodies in sera from patients with nasopharyngeal carcinoma. Int J Cancer 46: 647-649.

14. Feng WH, Cohen JJ, Fischer S, Li L, Smeller M, et al. (2004) Reactivation of latent Epstein-Barr virus by methotrexate: a potential contributor to methotrexate-induced arthralgias. J Natl Cancer Inst 96: 1691-1702.

15. Hammerschmidt W, Sugden B (1988) Identification and characterization of oriLyt, a lyric origin of DNA replication of Epstein-Barr virus cell. Cell 55: 427-433.

16. Flemington EK, Goldfeld AE, Speck SH (1991) Efficient transcription of the Epstein-Barr virus immediate-early BZLF1 and BRLF1 genes requires protein synthesis. J Virol 65: 7075-7077.

17. Kudoh A, Fujita M, Kiyono T, Kuzushima K, Sugaya Y, et al. (2003) Reactivation of lytic replication from B cells latently infected with Epstein-Barr virus occurs with high S-phase cyclin-dependent kinase activity while inhibiting nuclear DNA replication. J Virol 77: 8535-8540.

18. Tsurumi T, Fujita M, Kudoh A (2005) Latent and lytic Epstein-Barr virus replication strategies. Rev Med Virol 15: 3–13.

19. Li M, Chen D, Shih L, Luo J, Nikolaeff AV, et al. (2002) Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. Nature 414: 648-653.

20. Saridakis V, Sheng Y, Sarkari F, Holowaty MN, Shirke K, et al. (2005) Structure of the p53 binding domain of HAUSP/USP7 bound to Epstein-Barr nuclear antigen 1 implications for EBV-mediated germ cell cancer. Mol Cell 18: 25-36.

21. Sivachandran N, Sarkari F, Frappier L (2008) Epstein-Barr nuclear antigen 1 implications for EBV-mediated germ cell cancer. Mol Cell 18: 25-36.

22. Chang SS, Lo YC, Chua HH, Chiu HY, Tsai SC, et al. (2008) Critical role of cyclin-dependent kinase inhibitor in inhibiting nuclear DNA replication. J Virol 82: 7745-7751.

23. Cayrol C, Flemington EK (1996) The Epstein-Barr virus B-ZIP transcription factor Zta causes G0/G1 cell cycle arrest through induction of cyclin-dependent kinase inhibitors. EMBO J 15: 2748-2759.

24. Kudoh A, Fujita M, Kiyono T, Kuzushima K, Sugaya Y, et al. (2003) Architecture of replication compartments formed during Epstein-Barr virus lytic replication. J Virol 79: 3499-3418.

25. Kudoh A, Fujita M, Kiyono T, Kuzushima K, Sugaya Y, et al. (2003) Architecture of replication compartments formed during Epstein-Barr virus lytic replication. J Virol 79: 3499-3418.

26. Sato Y, Shirata N, Murata T, Nakasu S, Kudoh A, et al. (2010) Transient phosphorylated p53 by Viral Protein-ECS E3 Ligase Complex. PLoS Pathog 5: e1001158.

27. Saridakis V, Sheng Y, Sarkari F, Holowaty MN, Shirke K, et al. (2005) Structure of the p53 binding domain of HAUSP/USP7 bound to Epstein-Barr nuclear antigen 1 implications for EBV-mediated germ cell cancer. Mol Cell 18: 25-36.

28. Phase2b HCMV IE2-Trans activation of p533, reinitiates transcription from virus latency in cells infected with human cytomegalovirus. J Virol 72: 2033-2039.

29. Hsu CH, Chang MD, Tai KY, Yang YT, Wang PS, et al. (2004) HCMV IE2-mediated inhibition of HAT activity downregulates p53 function. EMBO J 23: 2269-2280.

30. Wilcock D, Lane DP (1991) Localization of p53, reinitiates transcription from virus latency in cells infected with human cytomegalovirus. J Virol 72: 2033-2039.

31. Sato Y, Shirata N, Murata T, Kudoh A, et al. (2009) Phosphorylation of p53 by Viral Protein-ECS E3 Ligase Complex. PLoS Pathog 5: e1001158.
Degradation through SCFSkp2 Ubiquitin Ligase Actions during Viral Lytic Replication. J Biol Chem 284: 18923–18931.

66. Whitehurst CB, Ning S, Beatz GL, Dufour F, Gershburg E, et al. (2009) The Epstein-Barr virus (EBV) deubiquitinating enzyme BPLF1 reduces EBV ribonucleotide reductase activity. J Virol 83: 4345–4353.

67. Gastaldello S, Hildebrand S, Faridani O, Callegari S, Palmkvist M, et al. (2010) A de neddylyase encoded by Epstein-Barr virus promotes viral DNA replication by regulating the activity of cullin-RING ligases. Nat Cell Biol 12: 551–561.

68. Rabut G, Peter M (2008) Function and regulation of protein neddylation. ‘Protein modifications: beyond the usual suspects’ review series. EMBO Rep 9: 969–976.

69. Sompallae R, Gastaldello S, Hildebrand S, Zinin N, Hassink G, et al. (2008) Epstein-barr virus encodes three bona fide ubiquitin-specific proteases. J Virol 82: 10477–10486.

70. Bornstein G, Bloom J, Sity-Sheval D, Nakayama K, Pagano M, et al. (2003) Role of the SCFSkp2 ubiquitin ligase in the degradation of p21Cip1 in S phase. J Biol Chem 278: 22752–22757.

71. Gottwein E, Cullen BR. (2010) A human herpesvirus microRNA inhibits p21 expression and attenuates p21-mediated cell cycle arrest. J Virol 84: 5229–5237.

72. Adams PD, Kaelin WG, Jr. (1995) Transcriptional control by E2F. Semin Cancer Biol 6: 99–108.

73. Liu C, Sista ND, Pagano JS (1996) Activation of the Epstein-Barr virus DNA polymerase promoter by the BRLF1 immediate-early protein is mediated through UNF and E2F. J Virol 70: 2545–2553.

74. Mauser A, Holley-Guthrie E, Zanation A, Yarborough W, Kaufmann W, et al. (2002) The Epstein-Barr virus immediate-early protein BZLF1 induces expression of E2F-1 and other proteins involved in cell cycle progression in primary keratinocytes and gastric carcinoma cells. J Virol 76: 12543–12552.

75. Swenson JJ, Mauser AE, Kaufmann WK, Kenney SC (1999) The Epstein-Barr virus protein BRLF1 activates S phase entry through E2F1 induction. J Virol 73: 6540–6550.

76. Lin WC, Lin FT, Nevins JR (2001) Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation. Genes Dev 15: 1833–1844.

77. Stevens C, Smith L, La Thangue NB (2005) Chk2 activates E2F-1 in response to DNA damage. Nat Cell Biol 3: 401–409.