ANNUAL REVIEWS Further
Click here to view this article's online features:
- Download figures as PPT slides
- Navigate linked references
- Download citations
- Explore related articles
- Search keywords

Epstein-Barr Virus: The Path from Latent to Productive Infection

Ya-Fang Chiu[1,2,3,4] and Bill Sugden[1]

[1]McArdle Laboratory for Cancer Research, University of Wisconsin–Madison, Madison, Wisconsin 53705; email: sugden@oncology.wisc.edu

[2]Morgridge Institute for Research, Madison, Wisconsin 53715

[3]Research Center for Emerging Viral Infections and Department of Microbiology and Immunology, Chang Gung University, Taoyuan 33302, Taiwan

[4]Department of Medical Laboratory, Chang Gung Memorial Hospital, Taoyuan 33305, Taiwan

Annu. Rev. Virol. 2016. 3:359–72

First published online as a Review in Advance on August 15, 2016

The *Annual Review of Virology* is online at virology.annualreviews.org

This article's doi: 10.1146/annurev-virology-110615-042358

Copyright © 2016 by Annual Reviews. All rights reserved

Keywords

Epstein-Barr virus, latent phase, lytic phase, transcriptional regulation

Abstract

The intrinsic properties of different viruses have driven their study. For example, the capacity for efficient productive infection of cultured cells by herpes simplex virus 1 has made it a paradigm for this mode of infection for herpesviruses in general. Epstein-Barr virus, another herpesvirus, has two properties that have driven its study: It causes human cancers, and it exhibits a tractable transition from its latent to its productive cycle in cell culture. Here, we review our understanding of the path Epstein-Barr virus follows to move from a latent infection to and through its productive cycle. We use information from human infections to provide a framework for describing studies in cell culture and, where possible, the molecular resolutions from these studies. We also pose questions whose answers we think are pivotal to understanding this path, and we provide answers where we can.

EPSTEIN-BARR VIRUS AS A HUMAN PATHOGEN

Most diseases caused by Epstein-Barr virus (EBV) reflect some form of its latent infection. By latency we mean that viral genomes are maintained in infected cells and some viral genes are likely expressed, but that viral progeny are not produced and the host cell survives. EBV's latent infections cause a gamut of diseases from self-limiting infectious mononucleosis to lethal cancers, including Burkitt's lymphoma and nasopharyngeal carcinoma (1). Here, causality has been demonstrated both by prospective epidemiological surveys and by using anti-EBV cytotoxic T cells to successfully treat some EBV-positive tumors (1). In these examples, the infected cells proliferate, express different viral genes, and, if they escape immune recognition, survive. In most of the 7,000,000,000 people currently infected with EBV, the virus resides in memory B cells (2) in an extreme state of latency, which we refer to as dormancy because the infected cells do not proliferate and the virus expresses few or no genes at a detectable level. These infected cells may well be ignored by the immune system.

We know of one disease associated with EBV's productive cycle, oral hairy leukoplakia (OHL) (3). This disease occurs most often in HIV-infected people and is associated with the immune suppression characteristic of their disease. Epithelial cells in the upper layers of the tongue are infected and support a productive infection in which viral progeny can be detected by electron microscopy (4, 5). Whether the basal cells are also infected in OHL is controversial. If so, this disease may reflect a reservoir of latently infected cells, which upon differentiation yield progeny cells in which EBV's productive cycle can occur, as has been found for human papillomaviruses (6). Sensitive assays using polymerase chain reaction and in situ hybridization detected EBV DNA in basal cells of 11 of 11 cases of OHL (7, 8), indicating that this benign disease may consist of both phases of EBV's life cycle, with its productive phase being pathognomonic.

EPSTEIN-BARR VIRUS IN CELL CULTURE

EBV is unique in its ability to infect resting human B cells and to induce and maintain their proliferation in cell culture. In experimental settings, more than 90% of freshly isolated peripheral B cells can be infected and begin to proliferate. Of these infected cells, 1–10% will go on to proliferate for tens of generations, and some small number often survive senescence to become immortalized. These cells efficiently secrete immunoglobulin, yielding $1–3 \times 10^7$ molecules per cell per hour (9). These cells, however, do not efficiently support the transition to EBV's productive cycle. They do not support it at all for the first 10 or so cell generations following initial infection, and later usually less than 1 in 10^4 cells per generation spontaneously supports the productive cycle (10).

Infection of primary epithelial cells by EBV in cell culture contrasts strikingly with that of B cells. Infection of epithelial cells is often inefficient, can rapidly lead to a productive infection, and does not provide these cells with an obvious selective advantage in vitro (11). Dissociated primary epithelial cells have been infected with EBV that was first bound to primary B cells, which were then cocultivated with the epithelial cells (12). The resulting epithelial infections did not lead to the long-term maintenance of EBV in the cells, and less than 1 in 1,000 of these infected cells expressed viral capsid antigens (13). EBV infections in organotypic epithelial raft cultures are strikingly different because they can be productive (14). These raft cultures are characterized by most of the infected cells expressing viral capsid antigens within four days of infection and eventually yielding significant titers of infectious particles. These results are therefore consistent with the idea that EBV infects tongue epithelial cells in OHL patients, directly yielding productive infections, a finding without parallel for infections of cells in monolayer cultures.

EBV can also infect cell lines, that is, cells that have survived senescence and are operationally immortalized. These cell lines include derivatives of EBV-positive Burkitt's lymphoma that have lost their endogenous virus, and EBV-negative gastric carcinoma cells. The B cell lines can be reinfected, and the epithelial cell lines can be infected by cocultivation (15, 16). In such cases, the newly introduced viral DNA can be maintained only under selection by engineering it to encode resistance to an antibiotic, such as hygromycin B or G418. Several epithelial cell lines initially positive for EBV, many derived from nasopharyngeal carcinomas, have lost EBV and have then been reinfected (17). Even though some of these lines appear to retain EBV, their provenance is uncertain because they appear to be derived from HeLa cervical carcinoma cells or to be somatic fusions with HeLa cells (18). None of these cell lines initially yields productive infections, despite the fact that in some cases the infected cells can eventually be induced to support productive EBV infections by various means. This failure has two consequences: First, we have no practical means of plaquing EBV in cell culture, meaning we cannot use standard virological approaches to examine EBV genetically. Second, the path from initial infection to productive infection is complex and usually indirect, including an initial latent infection. We need to understand this failure; in other words, What in the biology of EBV might necessitate its entering latency before supporting a productive cycle?

BEGINNING THE PATH BY EXITING LATENCY

Given our definition of latency as a virus-host state in which the infected cell survives and viral progeny are not produced, an initial question essential to address is, What triggers EBV's exit from latency? To answer this question, we first need to describe latency at the molecular level. EBV's genome is maintained as a plasmid during latency (**Figure 1**). It encodes an origin of DNA synthesis and an independent maintenance element, which together are termed *oriP*, and one protein, EBNA-1, which binds these sequences to mediate DNA synthesis and partitioning of the viral genomes (19). Enzymatic machinery encoded by the host cell provides all the other factors that are needed for EBV's genome replication. A variety of different viral genes can be expressed during latency. The regulation of their expression has been studied extensively for decades. We know now that this varied expression reflects the host cell's transcriptional machinery, the degree of methylation of the viral genomes, and which EBV genes are expressed (20–22). In general, the viral genes expressed in latently infected B cells both foster the proliferation of cells and block their dying by apoptosis (23). What these genes do in latently infected epithelial cells is not so clear. Once a latent infection is established in either B cells or epithelial cells, the viral genes needed to induce the productive cycle are not expressed. Thus, latency is maintained by the absence of certain viral proteins. The trigger to exit latency is the sufficient expression of at least two viral transcription factors, Zta and Rta (the gene products expressed from *BZLF1* and *BRLF1*, respectively), which when expressed can induce entry into EBV's productive cycle (**Figures 1** and **2**). The regulation of expression of Zta and Rta has also been examined extensively (24, 25). Treating different cells, for example, with tetradecanoyl phorbol acetate (TPA), sodium butyrate, or anti-immunoglobulin can induce the expression of one or both of these viral transcription factors, leading to the productive cycle in a fraction of the treated cells. TPA is an agonist of protein kinase C; sodium butyrate inhibits histone deacetylases; and anti-immunoglobulin can, by binding surface immunoglobulin (i.e., the B cell receptor), induce the signaling mediated by it. Each of these treatments in the appropriate cell can lead to the transcription of *BZLF1*, *BRLF1*, or both. The viral promoters of these two genes are complex but have been well dissected (26). Both Zta and Rta are required for EBV's productive cycle (27); however, the expression of one is often sufficient to induce the productive cycle because each can activate the other (28, 29).

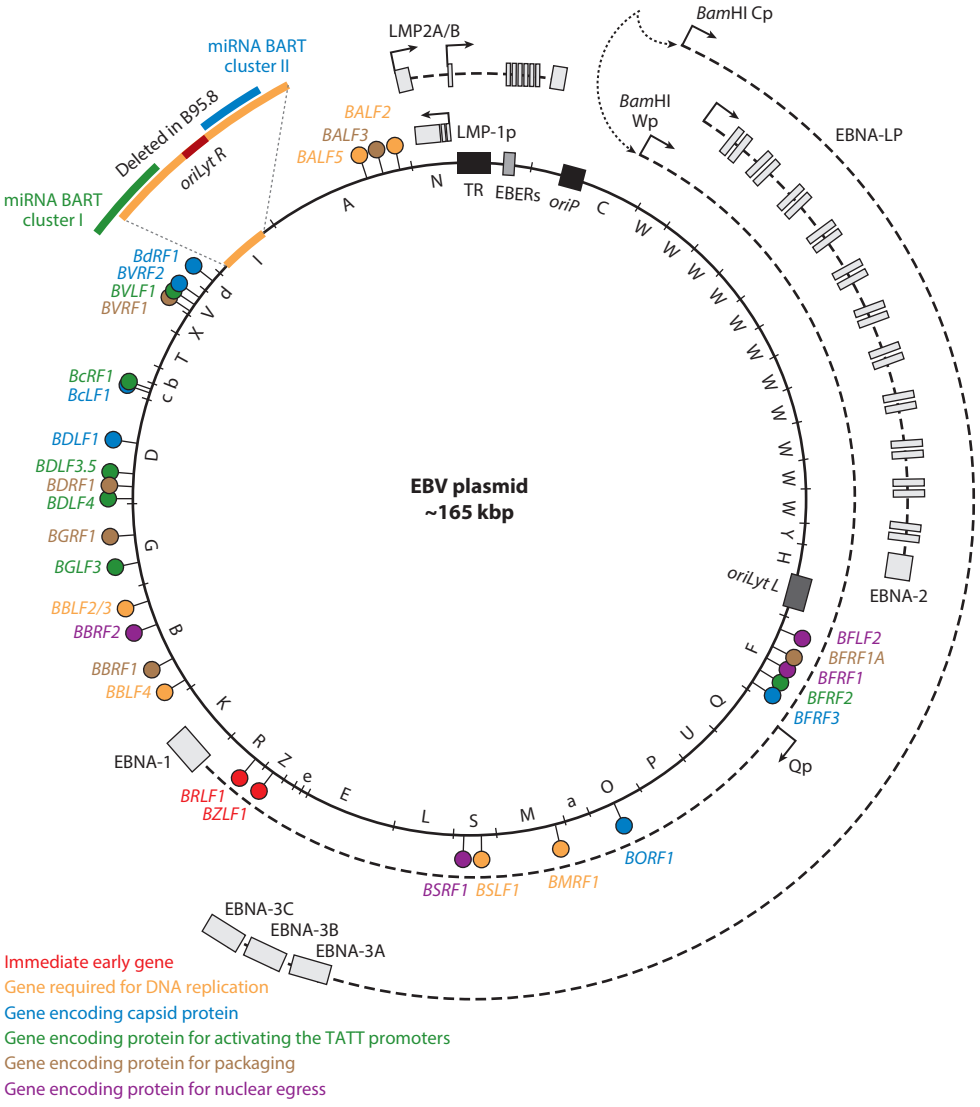


Figure 1

A map of the Epstein-Barr virus (EBV) genome. The letters on the inside of the annulus indicate the *Bam*HI fragments of EBV DNA used to assemble and sequence the viral genome (62). The colored lollipops on the outside of the annulus identify genes mapping to all or a portion of the adjacent *Bam*HI fragments, with each color indicating a functional category for the gene product. The outer partial annuli indicate the primary transcripts of genes expressed during the latent phase of the viral life cycle, with the gray boxes marking their exons. The names of the proteins encoded by these latent genes are noted alongside the gray boxes. The promoters for the latent transcripts are denoted with a lowercase p, such as the *Bam*HI Cp promoter. The black boxes denote *cis*-acting elements, and their names are indicated alongside the boxes. The EBERs are two small noncoding RNAs. The positions of two clusters of miRNAs are shown in green and blue. A third cluster (not shown) is located in the *Bam*H1 H fragment near *oriLyt* L.

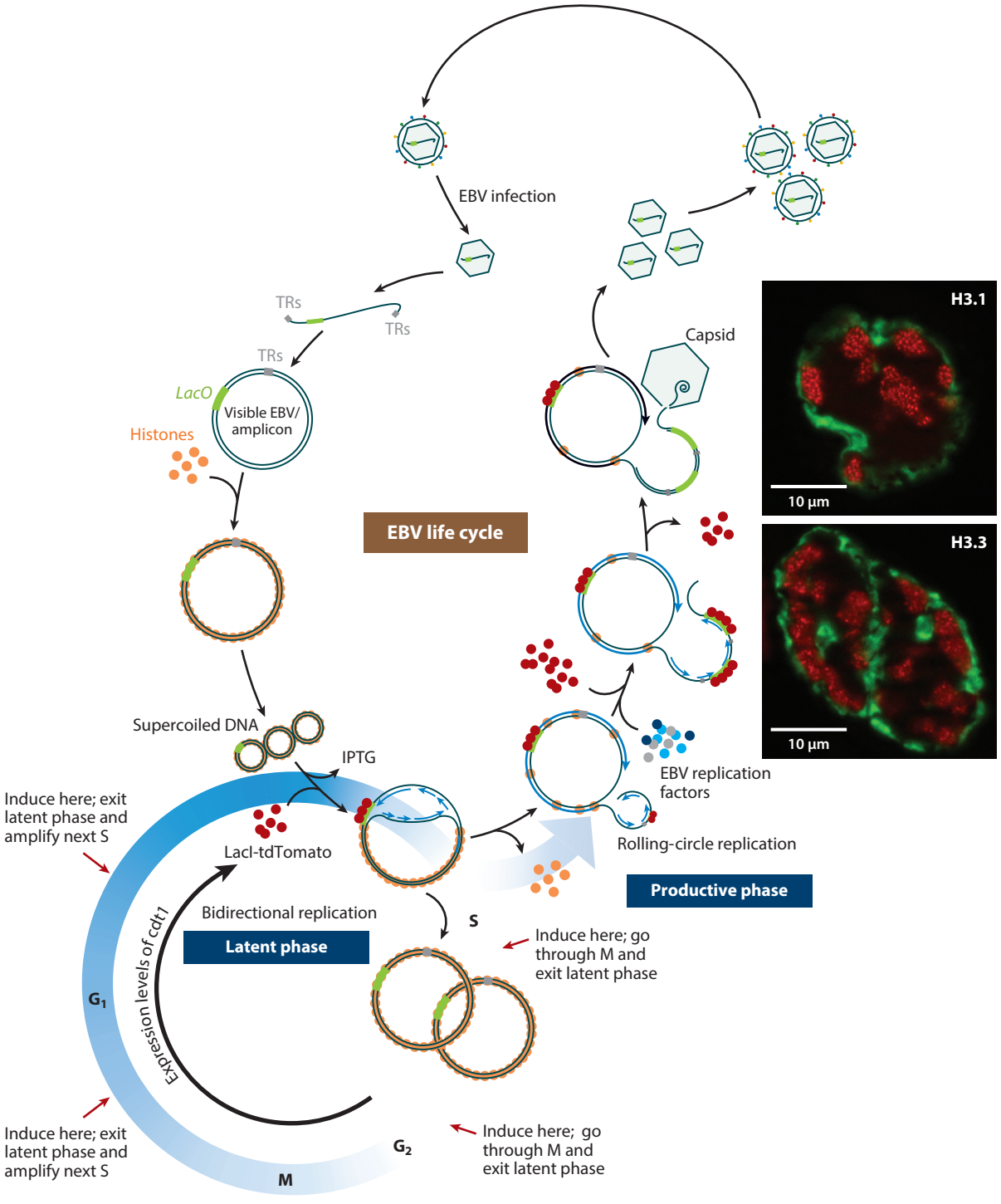
EARLY TRANSCRIPTIONAL EVENTS: ON THE PATH TO THE PRODUCTIVE CYCLE

The first events in EBV's productive cycle, following the accumulation of Zta and Rta, reflect these proteins' transcriptional activities. Zta alone in a variety of cell types in culture can induce EBV's productive cycle. Zta is a viral relative of the cellular transcription factor AP-1, and both can bind to similar DNA sequence motifs. Zta, however, preferentially binds some sites that are methylated (30, 31). This preferential binding to methylated DNA can explain EBV's initial inability to enter its productive cycle upon infecting primary B cells or any other cells tested in monolayer culture. Zta can bind some sites in the viral genome essential for EBV's productive cycle only when those sites are methylated. EBV DNA in virions is not methylated and gradually becomes methylated by cellular enzymes as the infected cells proliferate (32). For example, the promoter for the essential gene *BBLF4*, which encodes the ATPase subunit of EBV's helicase-primase complex, can be bound by Zta only when its binding sites are methylated (31). This knowledge leads to the following conundrum: If Zta cannot bind the viral genes necessary for EBV's productive cycle following primary infections, how can EBV enter its lytic cycle immediately on infecting tongue epithelial cells in raft cultures? Rta can bind unmethylated DNA to activate transcription; its introduction into an epithelial cell line maintaining latent EBV, in which the viral genome was largely unmethylated, induced the productive cycle. In parallel experiments, Zta did not induce the lytic cycle (33). Apparently EBV needs to express Rta immediately to induce its productive cycle on infecting epithelial cells, and to bypass the need for Zta to bind those sites, it can only bind when methylated. This bypass remains conjectural and needs testing (**Figure 3**).

There are two consequences of the early transcription mediated by Zta and Rta that are necessary for progression into EBV's productive cycle. First, viral genes that mediate virus-specific DNA synthesis are expressed, leading to the amplification of viral DNA. Second, viral genes that mediate the transcription of viral structural genes are expressed, leading to the accumulation of viral late proteins and subsequent capsid assembly.

ONE MILESTONE: DNA AMPLIFICATION

EBV encodes two origins of DNA synthesis, *oriLyt* L and *oriLyt* R, which are dedicated to its productive cycle (34) and bind Zta to function (**Figure 1**) (35). At least six viral gene products in addition to Zta are required for viral DNA synthesis during the productive cycle. The viral replication proteins that are recruited to the *oriLyt*s include a DNA polymerase (BALF5), a single-stranded DNA–binding protein (BALF2), a DNA polymerase processivity factor (BMRF1), and a primase-helicase complex (consisting of BSLF1, BBLF4, and BBLF2/3) (**Figure 1**) (36). The *oriLyt*s also are bound by cellular proteins, including Sp1 and ZBP-89, which are necessary for their efficient function (37). The initial product of this amplification is a covalently closed, circular DNA molecule with decreased negative superhelicity, consistent with its being bound by fewer nucleosomes (38). As amplification proceeds, it is thought that the product of viral DNA synthesis becomes a rolling circle as has been found for herpes simplex virus type 1 (HSV-1) (39). The amplified DNA concatemers are eventually packaged into assembled capsids and cleaved at specific sites at the termini of virion DNA to ensure a headful of DNA is packaged (**Figure 2**) (40). This simplified description of viral DNA amplification leads to the question, How does EBV subvert host functions to change from being a licensed replicon to managing its own unlicensed DNA amplification? Glimpses of an answer to this question have come from visualizing these events in live cells (see below).


EBV infection
TRs
TRs
TRs
LacO
Visible EBV/
amplicon
Histones
EBV life cycle
Capsid
H3.1
10 µm
H3.3
10 µm
Supercoiled DNA
IPTG
LacI-tdTomato
EBV replication
factors
Rolling-circle replication
Productive phase
Induce here; exit
latent phase and
amplify next S
Bidirectional replication
Latent phase
S
Induce here; go
through M and
exit latent phase
Expression levels of cdt1
G1
G2
M
Induce here; exit
latent phase and
amplify next S
Induce here; go
through M and
exit latent phase

A SECOND MILESTONE: LATE GENE TRANSCRIPTION

EBV encodes multiple transcription factors, including Zta and Rta, that facilitate transcription of viral genes during both latency and its productive cycle (41, 42). It also encodes its own version of a TATA-binding protein, BcRF1, which recognizes the TATT sequence found in the promoters of many late viral genes (**Figure 1**) (43–46). This TATT-binding protein is expressed when latently infected cells are induced to enter the productive cycle, for example, through the introduction of a vector that can express Zta. In parallel experiments, if the resident EBV lacks the *BcRF1* gene, the viral DNA can still undergo amplification, but late genes cannot be expressed (45). EBV's TATT-binding protein is not the only viral contribution required for late gene transcription. A combination of experiments using viral mutants and expression vectors with luciferase reporters has identified five additional genes—*BGLF3*, *BDLF4*, *BVLF1*, *BDLF3.5*, and *BFRF2*—as necessary for the transcription of late genes (**Figure 1**) (45, 46). These genes, along with *BcRF1*, share homology to genes found in other gammaherpesviruses, including murine herpesvirus 68 and Kaposi's sarcoma–associated herpesvirus. All three gammaherpesviruses appear to share the same mechanism to transcribe specifically genes encoding viral structural proteins. Alphaherpesviruses do not. Why? Different groups have disagreed on whether this mechanism depends on unlicensed viral DNA amplification. This disagreement leads to intriguing alternatives: Does viral DNA amplification simply provide more templates for transcription of the late genes, or are these templates functionally distinct from those used during latency or in the early phase of the productive cycle? As described below, experiments with live-cell imaging favor the second of these alternatives, which has been confirmed genetically (**Figure 3**) (45).

A THIRD MILESTONE: PARTICLE ASSEMBLY

Many EBV structural proteins share sequence homologies with those of other human herpesviruses (47). These homologies have helped to identify functional homologs in herpesviruses, such as HSV-1, whose virion assembly is well studied. EBV's minor capsid proteins BDLF1 and BORF1 have been identified by electron microscopy (48). These two proteins form a triplex. When expressed in insect cells, the major capsid protein (BcLF1), another minor capsid protein (BFRF3), a scaffold protein (BdRF1), and a protease (BVRF2), along with the BDLF1-BORF1 triplex, form an icosahedron that resembles a mature nucleocapsid (**Figure 1**) (49). These structural studies are a tantalizing beginning to answering the many questions arising from studies with other herpesviruses that have more amenable productive cycles. For example, Where do EBV nucleocapsids assemble? Where and how do they become filled with viral DNA? Where do they acquire their

Figure 2

Schematic counterclockwise depiction of the different facets of the life cycle of Epstein-Barr virus (EBV), focusing on its double-stranded DNA genome. At the top is a virion with its duplex, linear DNA ending in terminal repeats (TRs). The TRs are the site at which the DNA is circularized on infecting cells (40, 63). Recombinants of EBV with ~250 copies of *LacO* allow visualization of the virus when *LacO* is bound by a LacI-tdTomato fusion (60). Induction of EBV's productive cycle in cells carrying these recombinants, termed visible EBV, allows visual examination of viral DNA as it is amplified during the productive cycle. The timing of this amplification depends on when in the cell cycle the induction occurs and can be tracked by observing levels of a fusion between eGFP and a portion of Cdt1, which confers a cell cycle dependence on that fusion (60). The initial products of viral DNA replication following induction of the productive cycle are termed theta or Cairns structures and have a deficit of nucleosomes (38); these structures are likely to eventually yield rolling-circle intermediates. The synthesis of viral DNA occurs in amplification factories, which are devoid of histones. Images of these amplification factories are at the right; the amplified viral DNA is shown in red and the histones (H3.1 and H3.3) at the margins of the nuclei in green (detected by fusion to eGFP). The linear concatemers of viral DNA are packaged by a headful mechanism (50) into capsids by cleavages within the TRs (64).

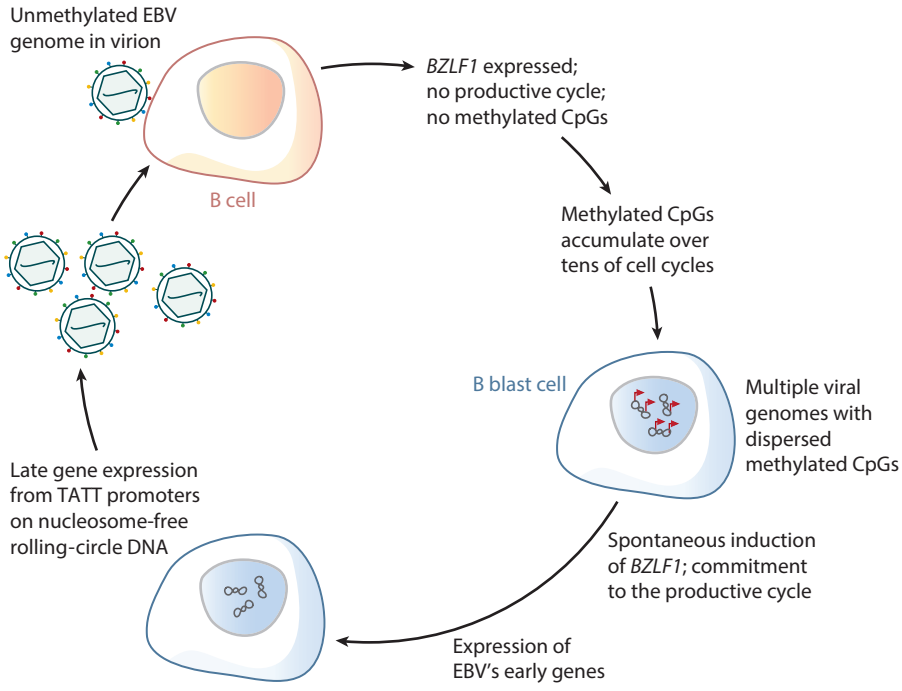


Figure 3

Schematic clockwise depiction of different facets of the life cycle of Epstein-Barr virus (EBV), focusing on the transcription of its DNA template in B cells. Viral DNA in virions is unmethylated (32); the virus, when infecting primary resting B lymphocytes, supports the transcription of genes such as *BZLF1*. The protein encoded by *BZLF1*, Zta, fails to transcribe viral genes involved in EBV's productive cycle because the promoters for these genes are not methylated (30, 31, 65). As the infected cells proliferate, viral DNA is progressively methylated (32), and the spontaneous induction of *BZLF1* allows a commitment to entering the productive cycle. The various triggers of this spontaneous induction are complex and are still being unraveled (24–26). Zta can bind promoters that have become methylated and mediate their transcription, allowing entry into EBV's productive cycle. Among these genes are those that mediate the amplification of viral DNA and transcription of the viral structural genes. EBV encodes its own version of a TATA-binding protein, which recognizes the TATT sequence upstream of multiple late genes (43, 45) and along with the products of five additional viral genes directs transcription of these late genes. This TATT-dependent machinery uses amplified viral DNA not bound by histones as a template for transcribing EBV's late genes (45).

tegument proteins? Where and how do they bud from the nucleus? Into what cytoplasmic compartment do they enter? And how do they exit from the cell? We can propose reasonable answers to some of these questions using insights gleaned from studies of other herpesviruses. We can also hope to answer some of these questions by performing live-cell imaging experiments.

PLAUSIBLE EXTRAPOLATIONS FOR LATE GENE FUNCTIONS FROM OTHER HERPESVIRUSES

Extensive comparisons of the sequences of the late genes of the herpesviruses have been made (47) and are informative because of the high levels of conservation exhibited among many of these viruses. We consider three sets of these genes whose functions are shared among all herpesviruses tested. Two sets of conserved late genes are involved in the packaging and cleavage of viral DNA as it enters the preformed capsid. For EBV, the products of the genes *BBRF1* and *BVRF1* are

predicted to form a portal and a covering for that portal through which virion DNA is fed into the capsid. Once a headful of DNA is inserted (50), it is cleaved by a terminase complex whose constituents are predicted to be encoded by the genes *BGRF1*, *BDRF1*, *BALF3*, and *BFRF1A* (**Figure 1**) (47). The capsid with its headful of DNA binds multiple tegument proteins, which are contained within the final viral envelope. Among these, the product of the gene *BBRF2* is predicted to support capsid exit from the nucleus, and the product of the gene *BSRF1* is predicted to foster exit from the cytoplasm (**Figure 1**) (47). In addition, the functions of some late gene products have been predicted from their conserved alignment among herpesvirus genomes. For example, two EBV late genes, *BFRF1* and *BFLF2*, are predicted on the basis of their positional homology to HSV-1 genes to promote efficient primary envelopment and egress of nucleocapsids, respectively (**Figure 1**) (51, 52). Compelling experimental findings confirm these predictions (51, 53). As with other herpesviruses, these steps in EBV's productive cycle likely involve first budding from the nucleus and acquiring an envelope from the inner nuclear membrane, and then fusing with the outer nuclear membrane to enter the cytoplasm. In the cytosol, the nucleocapsid, along with tegument proteins, acquires two layers of membrane while it passes through a cytoplasmic organelle, which is likely to be the Golgi or the *trans* Golgi network, allowing newly synthesized glycoproteins embedded in the Golgi or *trans* Golgi network to be incorporated into the virion. Finally, EBV is released from cells by fusing the second membrane with the plasma membrane (54).

ACQUISITION OF ENVELOPE GLYCOPROTEINS

Although the functions of many of EBV's late gene products can be predicted but have not yet been confirmed experimentally, the roles of its envelope glycoproteins during infections have been well studied. This research has illuminated how the virion's acquisition of one glycoprotein, gp42, during EBV's productive cycle depends on the cell in which this cycle occurs. gp42 is required for EBV to infect B cells (55), where it binds HLA class II as a coreceptor (56, 57), but it is not required for the virus to infect epithelial cells (55). When EBV particles mature in B lymphoid cells that express HLA class II molecules, the gp42 molecules apparently associate with the HLA class II molecules as they mature, leading to the degradation of much of the gp42 in these cells (58, 59). Epithelial cells in general do not express HLA class II molecules and thus do not support this mechanism of decreasing the levels of gp42 on EBV's envelope. EBV produced in B cells therefore has a higher infectivity-to-particle ratio for epithelial cells than for B cells; EBV produced in epithelial cells has the opposite preferential tropism (58, 59).

SEEING THE PATH: LIVE-CELL IMAGING OF EPSTEIN-BARR VIRUS DNA FROM LATENT TO PRODUCTIVE INFECTION

Insertion of approximately 250 binding sites for the Lac repressor (LacI) into EBV DNA renders it visible by fluorescence microscopy when the DNA also expresses LacI fused to the fluorescent protein tdTomato red. The genome of this virus, termed visible EBV, can be observed as punctate red fluorescent signals over multiple cell cycles (60). The cells can be engineered to express Zta conditionally so that, when they are treated with the inducer tamoxifen, the majority of them eventually exit from latency and pass through a complete productive cycle. Visualizing single cells that support this complete path over 60 h has allowed mapping of several of its features, some of which were unexpected (60).

The experiments have been conducted with clones of cells carrying visible EBV or an amplicon derived from it (60). In the latter case, the cells were also infected with an intact EBV. Even though the cells in these clones presumably all expressed Zta, no more than 60% of them eventually

supported a full productive cycle. In fact, in no case have we observed all cells in a clone supporting EBV's productive cycle. This failure indicates that entry into the productive cycle has some still unknown requirements (60). Second, entry into the productive cycle as evidenced by unlicensed amplification of viral DNA was not synchronous but depended on the phase of the cell cycle in which Zta was functionally induced. This insight came from expressing a portion of the cellular protein Cdt1 fused with eGFP in the cells with visible EBV. This fusion protein is expressed as a function of the cell cycle, becoming visible late in mitosis and peaking at the beginning of S phase. In cells induced early in G_1, EBV entered its productive cycle in S phase. In cells induced in S phase, EBV waited for the next cell cycle and entered its productive cycle in the ensuing S phase (**Figure 2**) (60). Thus, even though Zta is expressed in a cell, it can drive the cell to support EBV's lytic cycle only if the cell's milieu is conducive to lytic induction. How EBV senses where it is in a host's cell cycle and what its specific needs are to commit to its productive cycle are intriguing unknowns we still need to address. These findings also demonstrate that the asynchrony in inducing EBV's productive cycle will complicate interpretation of all but single-cell experiments.

In live cells, EBV was seen to amplify in defined sites consistent with the clustering of some replication proteins immunologically detected in static assays (60, 61). Visualizing these amplification factories has been satisfyingly informative. These factories were observed to grow in time as the productive cycle advanced. They increased at similar times within the same nucleus and at different times between nuclei. These factories were clearly delineated by the absence of histones, which were detected by fusing them with eGFP. Chromosomal DNA bound by the eGFP-tagged histones tended to be at the margin of the nuclei as the factories grew. When the factories were resolved as fixed samples in the absence of out-of-focus light, many individual signals were detected in each factory, which may reflect individual replicating molecules, and all were devoid of detectable histone-eGFP signals (**Figure 2**) (60).

EBV encodes its own clamp protein, BMRF1, for amplification of viral DNA during its productive cycle. Accordingly, the host cell's PCNA did not colocalize with the sites at which EBV DNA was being amplified (60). PCNA has multiple roles during DNA synthesis, including recruiting histone chaperones to sites of DNA synthesis. Its absence from the sites at which EBV DNA is amplified, coupled with EBV's inhibition of the synthesis of multiple histone chaperones, helps to explain the absence of histones from amplified EBV DNA and from encapsidated DNA (60). We propose that the absence of histones, and therefore the absence of nucleosomes, from amplified EBV DNA makes these templates functionally distinct and underlies the need for EBV and other beta- and gammaherpesviruses to encode their own machinery dedicated to late gene transcription (60). Histone modifications on nucleosomes in normal cellular chromatin, for example, are essential signals for dictating sites for the initiation of transcription. They are clearly lacking from the EBV DNA amplified during its productive cycle. It has now been shown that derivatives of EBV deleted for the *oriLyt*s cannot be induced to support late gene expression (45). In addition, RNAs encoding late genes accumulate at the sites where EBV DNA is being amplified, which lack detectable histones (**Figures 2** and **3**) (45). These results indicate that EBV has evolved its own rules for the transcriptional regulation of its late genes while linking that transcription to the amplification of its genomes.

REFINING THE PATH

Although the path from latency to the productive cycle for EBV is infrequently begun and rarely completed, it is essential for the virus. Several of its features warrant additional scrutiny. EBV resides dormant in memory B cells in most infected people. What signals are required for these

cells to evolve to support EBV's productive cycle? Why are all forms of latently infected cells so inefficient in their spontaneous transition to a productive infection? What are the mechanisms by which EBV commandeers cellular functions to enter, execute, and complete its productive cycle? Addressing these questions will illuminate our understanding of this human pathogen. The protracted duration of this transition for EBV makes its continued study appealing and tractable.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Dr. Eric Johannsen and Dr. Shih-Tung Liu for their insights and suggestions. This work was funded by the National Cancer Institute, the National Institutes of Health (grants P01 CA022443, R01 CA133027, and R01 CA070723), the Ministry of Science and Technology (grant MOST-105-2320-B-182-033), and the Morgridge Institute for Research. B.S. is an American Cancer Society Research Professor.

LITERATURE CITED

1. Sugden B. 2014. Epstein-Barr virus: the path from association to causality for a ubiquitous human pathogen. *PLOS Biol.* 12:e1001939
2. Babcock GJ, Decker LL, Volk M, Thorley-Lawson DA. 1998. EBV persistence in memory B cells in vivo. *Immunity* 9:395–404
3. Greenspan JS, Greenspan D, Lennette ET, Abrams DI, Conant MA, et al. 1985. Replication of Epstein-Barr virus within the epithelial cells of oral hairy leukoplakia, an AIDS-associated lesion. *N. Engl. J. Med.* 313:1564–71
4. Epstein JB, Fatahzadeh M, Matisic J, Anderson G. 1995. Exfoliative cytology and electron microscopy in the diagnosis of hairy leukoplakia. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* 79:564–69
5. Fernandez JF, Benito MA, Lizaldez EB, Montanes MA. 1990. Oral hairy leukoplakia: a histopathologic study of 32 cases. *Am. J. Dermatopathol.* 12:571–78
6. Bedell MA, Hudson JB, Golub TR, Turyk ME, Hosken M, et al. 1991. Amplification of human papillomavirus genomes in vitro is dependent on epithelial differentiation. *J. Virol.* 65:2254–60
7. Becker J, Leser U, Marschall M, Langford A, Jilg W, et al. 1991. Expression of proteins encoded by Epstein-Barr virus trans-activator genes depends on the differentiation of epithelial cells in oral hairy leukoplakia. *PNAS* 88:8332–36
8. Berean K, Epstein JB. 1996. Correspondence re: Brandwein M, Nuovo G, Ramer M, Orlowski W, Miller L: Epstein-Barr virus reactivation in hairy leukoplakia. *Mod Pathol* 9:298, 1996. *Mod. Pathol.* 9:869–70
9. Lee DY, Sugden B. 2008. The LMP1 oncogene of EBV activates PERK and the unfolded protein response to drive its own synthesis. *Blood* 111:2280–89
10. Sugden B. 1984. Expression of virus-associated functions in cells transformed in vitro by Epstein-Barr virus: Epstein-Barr virus cell surface antigen and virus-release from transformed cells. In *Immune Deficiency and Cancer: Epstein-Barr Virus and Lymphoproliferative Malignancies*, ed. TD Purtilo, pp. 165–77. New York: Springer
11. Tsao SW, Tsang CM, Pang PS, Zhang G, Chen H, Lo KW. 2012. The biology of EBV infection in human epithelial cells. *Semin. Cancer Biol.* 22:137–43
12. Shannon-Lowe CD, Neuhierl B, Baldwin G, Rickinson AB, Delecluse HJ. 2006. Resting B cells as a transfer vehicle for Epstein-Barr virus infection of epithelial cells. *PNAS* 103:7065–70

13. Shannon-Lowe C, Adland E, Bell AI, Delecluse HJ, Rickinson AB, Rowe M. 2009. Features distinguishing Epstein-Barr virus infections of epithelial cells and B cells: viral genome expression, genome maintenance, and genome amplification. *J. Virol.* 83:7749–60
14. Temple RM, Zhu J, Budgeon L, Christensen ND, Meyers C, Sample CE. 2014. Efficient replication of Epstein-Barr virus in stratified epithelium in vitro. *PNAS* 111:16544–49
15. Shimizu N, Tanabe-Tochikura A, Kuroiwa Y, Takada K. 1994. Isolation of Epstein-Barr virus (EBV)-negative cell clones from the EBV-positive Burkitt's lymphoma (BL) line Akata: Malignant phenotypes of BL cells are dependent on EBV. *J. Virol.* 68:6069–73
16. Imai S, Nishikawa J, Takada K. 1998. Cell-to-cell contact as an efficient mode of Epstein-Barr virus infection of diverse human epithelial cells. *J. Virol.* 72:4371–78
17. Glaser R, Zhang HY, Yao KT, Zhu HC, Wang FX, et al. 1989. Two epithelial tumor cell lines (HNE-1 and HONE-1) latently infected with Epstein-Barr virus that were derived from nasopharyngeal carcinomas. *PNAS* 86:9524–28
18. Strong MJ, Baddoo M, Nanbo A, Xu M, Puetter A, Lin Z. 2014. Comprehensive high-throughput RNA sequencing analysis reveals contamination of multiple nasopharyngeal carcinoma cell lines with HeLa cell genomes. *J. Virol.* 88:10696–704
19. Hammerschmidt W, Sugden B. 2013. Replication of Epstein-Barr viral DNA. *Cold Spring Harb. Perspect. Biol.* 5:a013029
20. Tempera I, Lieberman PM. 2014. Epigenetic regulation of EBV persistence and oncogenesis. *Semin. Cancer Biol.* 26:22–29
21. Kang MS, Kieff E. 2015. Epstein-Barr virus latent genes. *Exp. Mol. Med.* 47:e131
22. Price AM, Luftig MA. 2014. Dynamic Epstein-Barr virus gene expression on the path to B-cell transformation. *Adv. Virus Res.* 88:279–313
23. Vereide DT, Sugden B. 2011. Lymphomas differ in their dependence on Epstein-Barr virus. *Blood* 117:1977–85
24. Miller G, El-Guindy A, Countryman J, Ye J, Gradoville L. 2007. Lytic cycle switches of oncogenic human gammaherpesviruses. *Adv. Cancer Res.* 97:81–109
25. Murata T, Tsurumi T. 2014. Switching of EBV cycles between latent and lytic states. *Rev. Med. Virol.* 24:142–53
26. Kenney SC, Mertz JE. 2014. Regulation of the latent-lytic switch in Epstein-Barr virus. *Semin. Cancer Biol.* 26:60–68
27. Feederle R, Kost M, Baumann M, Janz A, Drouet E, et al. 2000. The Epstein-Barr virus lytic program is controlled by the co-operative functions of two transactivators. *EMBO J.* 19:3080–89
28. Adamson AL, Darr D, Holley-Guthrie E, Johnson RA, Mauser A, et al. 2000. Epstein-Barr virus immediate-early proteins BZLF1 and BRLF1 activate the ATF2 transcription factor by increasing the levels of phosphorylated p38 and c-Jun N-terminal kinases. *J. Virol.* 74:1224–33
29. Chang LK, Chuang JY, Nakao M, Liu ST. 2010. MCAF1 and synergistic activation of the transcription of Epstein-Barr virus lytic genes by Rta and Zta. *Nucleic Acids Res.* 38:4687–700
30. Bhende PM, Seaman WT, Delecluse HJ, Kenney SC. 2004. The EBV lytic switch protein, Z, preferentially binds to and activates the methylated viral genome. *Nat. Genet.* 36:1099–104
31. Bergbauer M, Kalla M, Schmeinck A, Gobel C, Rothbauer U, et al. 2010. CpG-methylation regulates a class of Epstein-Barr virus promoters. *PLOS Pathog.* 6:e1001114
32. Kintner C, Sugden B. 1981. Conservation and progressive methylation of Epstein-Barr viral DNA sequences in transformed cells. *J. Virol.* 38:305–16
33. Wille CK, Nawandar DM, Panfil AR, Ko MM, Hagemeier SR, Kenney SC. 2013. Viral genome methylation differentially affects the ability of BZLF1 versus BRLF1 to activate Epstein-Barr virus lytic gene expression and viral replication. *J. Virol.* 87:935–50
34. Hammerschmidt W, Sugden B. 1988. Identification and characterization of *oriLyt*, a lytic origin of DNA replication of Epstein-Barr virus. *Cell* 55:427–33
35. Schepers A, Pich D, Hammerschmidt W. 1993. A transcription factor with homology to the AP-1 family links RNA transcription and DNA replication in the lytic cycle of Epstein-Barr virus. *EMBO J.* 12:3921–29
36. Fixman ED, Hayward GS, Hayward SD. 1992. *trans*-Acting requirements for replication of Epstein-Barr virus ori-Lyt. *J. Virol.* 66:5030–39

37. Baumann M, Feederle R, Kremmer E, Hammerschmidt W. 1999. Cellular transcription factors recruit viral replication proteins to activate the Epstein-Barr virus origin of lytic DNA replication, *oriLyt*. *EMBO J*. 18:6095–105
38. Pfuller R, Hammerschmidt W. 1996. Plasmid-like replicative intermediates of the Epstein-Barr virus lytic origin of DNA replication. *J. Virol.* 70:3423–31
39. Falkenberg M, Lehman IR, Elias P. 2000. Leading and lagging strand DNA synthesis in vitro by a reconstituted herpes simplex virus type 1 replisome. *PNAS* 97:3896–900
40. Zimmermann J, Hammerschmidt W. 1995. Structure and role of the terminal repeats of Epstein-Barr virus in processing and packaging of virion DNA. *J. Virol.* 69:3147–55
41. Zhou H, Schmidt SC, Jiang S, Willox B, Bernhardt K, et al. 2015. Epstein-Barr virus oncoprotein super-enhancers control B cell growth. *Cell Host Microbe* 17:205–16
42. O'Grady T, Cao S, Strong MJ, Concha M, Wang X, et al. 2014. Global bidirectional transcription of the Epstein-Barr virus genome during reactivation. *J. Virol.* 88:1604–16
43. Wyrwicz LS, Rychlewski L. 2007. Identification of herpes TATT-binding protein. *Antivir. Res.* 75:167–72
44. Gruffat H, Kadjouf F, Mariame B, Manet E. 2012. The Epstein-Barr virus BcRF1 gene product is a TBP-like protein with an essential role in late gene expression. *J. Virol.* 86:6023–32
45. Djavadian R, Chiu YF, Johannsen E. 2016. An Epstein-Barr virus-encoded protein complex requires an origin of lytic replication in *cis* to mediate late gene transcription. *PLOS Pathog.* 12:e1005718
46. Aubry V, Mure F, Mariame B, Deschamps T, Wyrwicz LS, et al. 2014. Epstein-Barr virus late gene transcription depends on the assembly of a virus-specific preinitiation complex. *J. Virol.* 88:12825–38
47. Mocarski ES Jr. 2007. Comparative analysis of herpesvirus-common proteins. In *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis*, ed. A Arvin, G Campadelli-Fiume, E Mocarski, PS Moore, et al. Cambridge, UK: Cambridge Univ. Press
48. Wang WH, Chang LK, Liu ST. 2011. Molecular interactions of Epstein-Barr virus capsid proteins. *J. Virol.* 85:1615–24
49. Henson BW, Perkins EM, Cothran JE, Desai P. 2009. Self-assembly of Epstein-Barr virus capsids. *J. Virol.* 83:3877–90
50. Bloss TA, Sugden B. 1994. Optimal lengths for DNAs encapsidated by Epstein-Barr virus. *J. Virol.* 68:8217–22
51. Farina A, Feederle R, Raffa S, Gonnella R, Santarelli R, et al. 2005. BFRF1 of Epstein-Barr virus is essential for efficient primary viral envelopment and egress. *J. Virol.* 79:3703–12
52. Granato M, Feederle R, Farina A, Gonnella R, Santarelli R, et al. 2008. Deletion of Epstein-Barr virus BFLF2 leads to impaired viral DNA packaging and primary egress as well as to the production of defective viral particles. *J. Virol.* 82:4042–51
53. Lee CP, Liu PT, Kung HN, Su MT, Chua HH, et al. 2012. The ESCRT machinery is recruited by the viral BFRF1 protein to the nucleus-associated membrane for the maturation of Epstein-Barr virus. *PLOS Pathog.* 8:e1002904
54. Johnson DC, Baines JD. 2011. Herpesviruses remodel host membranes for virus egress. *Nat. Rev. Microbiol.* 9:382–94
55. Wang X, Kenyon WJ, Li Q, Mullberg J, Hutt-Fletcher LM. 1998. Epstein-Barr virus uses different complexes of glycoproteins gH and gL to infect B lymphocytes and epithelial cells. *J. Virol.* 72:5552–58
56. Li Q, Spriggs MK, Kovats S, Turk SM, Comeau MR, et al. 1997. Epstein-Barr virus uses HLA class II as a cofactor for infection of B lymphocytes. *J. Virol.* 71:4657–62
57. Haan KM, Kwok WW, Longnecker R, Speck P. 2000. Epstein-Barr virus entry utilizing HLA-DP or HLA-DQ as a coreceptor. *J. Virol.* 74:2451–54
58. Borza CM, Morgan AJ, Turk SM, Hutt-Fletcher LM. 2004. Use of gHgL for attachment of Epstein-Barr virus to epithelial cells compromises infection. *J. Virol.* 78:5007–14
59. Borza CM, Hutt-Fletcher LM. 2002. Alternate replication in B cells and epithelial cells switches tropism of Epstein-Barr virus. *Nat. Med.* 8:594–99
60. Chiu YF, Sugden AU, Sugden B. 2013. Epstein-Barr viral productive amplification reprograms nuclear architecture, DNA replication, and histone deposition. *Cell Host Microbe* 14:607–18
61. Sugimoto A, Sato Y, Kanda T, Murata T, Narita Y, et al. 2013. Different distributions of Epstein-Barr virus early and late gene transcripts within viral replication compartments. *J. Virol.* 87:6693–99

62. Baer R, Bankier AT, Biggin MD, Deininger PL, Farrell PJ, et al. 1984. DNA sequence and expression of the B95-8 Epstein-Barr virus genome. *Nature* 310:207–11
63. Kintner CR, Sugden B. 1979. The structure of the termini of the DNA of Epstein-Barr virus. *Cell* 17:661–71
64. Hammerschmidt W, Sugden B. 1989. Genetic analysis of immortalizing functions of Epstein-Barr virus in human B lymphocytes. *Nature* 340:393–97
65. Wen W, Iwakiri D, Yamamoto K, Maruo S, Kanda T, Takada K. 2007. Epstein-Barr virus BZLF1 gene, a switch from latency to lytic infection, is expressed as an immediate-early gene after primary infection of B lymphocytes. *J. Virol.* 81:1037–42