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Transcriptional and post-transcriptional regulation of viral gene expression in the gamma-herpesvirus Kaposi's sarcoma-associated herpesvirus

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Abstract

Purpose of review—Kaposi's sarcoma-associated herpesvirus (KSHV), the etiological agent of the AIDS-associated tumor Kaposi's sarcoma, is a complex virus that expresses ~90 proteins in a regulated temporal cascade during its replication cycle. Although KSHV relies on cellular machinery for gene expression, it also uses specialized regulators to control nearly every step of the process. In this review we discuss the current understanding of KSHV gene regulation.

Recent findings—High-throughput sequencing and a new robust system to mutate KSHV have paved the way for comprehensive studies of KSHV gene expression, leading to the characterization of new viral factors that control late gene expression and post-transcriptional steps of gene regulation. They have also revealed key aspects of chromatin-based control of gene expression in the latent and lytic cycle.

Summary—The combination of mutant analysis and high-throughput sequencing will continue to expand our model of KSHV gene regulation and point to potential new targets for anti-KSHV drugs.

Keywords

KSHV; lytic genes; gene expression

Introduction

Kaposi's sarcoma-associated herpesvirus (KSHV) is the most recently discovered human herpesvirus [1]. In patients with compromised immune systems, particularly AIDS patients

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and transplant recipients, infection with KSHV can cause Kaposi's sarcoma (KS), two rare but aggressive lymphoproliferative diseases, primary effusion lymphoma (PEL) and a variant of Multicentric Castleman's disease, or KSHV inflammatory cytokine syndrome [2, 3]. KS was one of the AIDS-defining illnesses prior to diagnosis by HIV testing, and KSHV was first isolated from an AIDS patient in 1994 [1]. Like other herpesviruses, KSHV infection can be latent or lytic [4]. In KS lesions, most of the cells are latently infected, and latent infection promotes cell proliferation and survival [5]. A role for active lytic replication in disease was uncovered by the observation that treatment with anti-herpesviral drugs that block lytic replication causes tumor regression [6]. It is now thought that lytically reactivating cells create a tumorigenic microenvironment by secreting paracrine factors that promote tumor formation and growth [3, 5]. Indeed, at least some KS patients display evidence of lytic viral gene expression in tumors [7]. Also, retrospective studies show that antibodies against the lytic protein K8.1 are more abundant in KSHV-infected patients that go on to develop KS, compared to ones that do not, and that this difference is detectable as early as six years before the KS diagnosis [8]. Thus, the lytic cycle contributes not only to the spread of KSHV to new individuals, but also directly to KS pathogenesis.

The KSHV genome is over 140 kb long and encodes ~90 open reading frames, designated ORF4 to 75 if homologous to those in the related herpesvirus saimiri, or K1 to 15 if divergent, as well as several non-coding RNAs [9]. In latently infected cells, the genome of KSHV is circularized and tethered to the host genome by the latent protein LANA (latency associated nuclear antigen) [10]. The KSHV mRNAs are transcribed from this episome by the cellular RNA polymerase II complex (RNAPII) and are virtually identical to host transcripts, with a 5' cap and a 3' poly(A) tail that mediate recognition by the cellular translation machinery. A small subset of genes, including LANA, is expressed during latency, while all the genes are expressed during the lytic cycle, but with varying kinetics [11–15]. The mechanisms that shape gene expression and the viral and cellular proteins that regulate it are only partially characterized. In this review, we will summarize the current knowledge of regulation of KSHV genes, focusing particularly on lytic infection. A major advance in KSHV research in the past few years was the construction of a new bacterial artificial chromosome system to make mutations in KSHV, termed BAC16 [16]. Many of the recent studies cited in this review have made use of this system, which has allowed a much closer look at the role of KSHV proteins in gene regulation.

Transcriptome-wide profiling reveals waves of gene expression in KSHV-infected cells

Multiple studies have profiled the kinetics of KSHV gene expression using microarrays and RNAseq. These studies utilized cells isolated from PEL patients [11–14] or a recently developed epithelial cell culture system with tighter control on latency, the iSLK.219 cells [15, 17, 18], because PEL cells are thought to spontaneously reactivate at low frequency. Reactivation from latency can be triggered by overexpression of the immediate early protein RTA, which is sufficient to drive lytic cycle entry and is considered the master lytic regulator [19, 20]. In these studies, RTA overexpression was achieved by treatment with 12-O-Tetradecanoyl-phorbol-13-acetate (TPA) [11–13], which induces RTA expression through

the Raf/MEK/ERK pathway [21], or using a doxycycline-inducible RTA transgene in TREx-RTA-BCBL1 cells [14] and iSLK.219 cells [15]. The results of the transcriptomic studies generally agree, although there are small discrepancies on the timing of expression of specific genes. All studies detected multiple waves of gene expression, which likely correspond to well-characterized kinetic classes of herpesviral genes: i) latent genes, expressed during both lytic replication and latency; ii) immediate early genes, also called alpha genes in other herpesviruses; iii) delayed early genes or beta genes, which require expression of immediate early viral proteins for transcription; and iv) late genes or gamma genes, whose expression is dependent on viral DNA replication, and thus are expressed only later in the replication cycle. In particular, Lu *et al.* [11] examined KSHV gene expression in cells treated with an inhibitor of viral DNA replication, cidofovir, which allowed them to precisely identify late genes. Interestingly, they found that loss of DNA replication can still reduce, but not completely abolish, transcription of some early genes [11]. The studies also noted that the timing of lytic gene expression matches the role of viral proteins in the replication cycle. Immediate early genes include gene regulators required for delayed early gene expression, while delayed early genes include the viral DNA polymerase and associated proteins, as well as immune modulators. Most virion proteins, including capsid proteins and envelope glycoproteins, are expressed with late kinetics [11]. Arias *et al.* coupled transcriptome profiling of iSLK.219 cells with ribosome profiling (Ribo-seq) to determine translation rates [15]. The relative translation efficiency of mRNAs matches their relative expression levels, suggesting that the timing of gene expression is mostly controlled at the level of transcription [15]. In most of the studies, the levels of viral mRNAs consistently rise throughout the timecourse irrespective of their kinetic class, which is distinct from the pattern of gene expression observed in other herpesviruses, where each class of genes is turned on and off in waves [22]. It is unclear whether this difference reflects a real biological phenomenon or an artifact of the inefficient reactivation systems. In fact, in the doxycycline-inducible TREx-RTA-BCBL1 cells, the lytic cycle induction is more efficient and rapid, and many of the genes appear to get turned on and off in waves [14].

In terms of latent gene expression, the studies agree that LANA/ORF73, v-cyclin/ORF72, vFLIP/ORF71 and the KSHV microRNAs are transcribed during latency [11–15]. LANA, vFLIP, v-cyclin and the microRNAs promote the survival and proliferation of infected cells [23] and LANA tethers the viral episome to the host genome [10]. In contrast, the latent expression of several other signaling and immunomodulatory proteins (vIL-6/K2, Kaposins/K12, vFGARAT/ORF75, K10.5/LANA2/viral Interferon regulatory factor-like 3 (vIRF-3), K1 and K15) varies depending on cell type [11–15, 24, 25]. Interestingly, ribosome profiling suggests that some of the latent genes, including v-cyclin and vFLIP, are transcribed but not translated in latently infected iSLK.219 cells [15]. In contrast, v-cyclin and vFLIP proteins are present in latently infected BCBL1 cells [15]. This may be due to the fact that SLK cells only express a tricistronic LANA-v-cyclin-vFLIP mRNA, whereas PEL cells also express a bicistronic v-cyclin-vFLIP mRNA [15]. Only the bicistronic mRNA supports v-cyclin and vFLIP translation [26–28]. Other mRNAs (for example ORF75) are also present during latency, but do not bind the ribosome [15]. Since the ribosome profiling was only done in the iSLK.219 cells, it is unknown whether there is additional translation-based variability in latent protein expression in other cell types as well.

These experiments all profiled lytic gene expression during long-term latency and reactivation. While *de novo* viral infection, at least in tissue culture, results in establishment of latent rather than lytic infection, profiling of the gene expression pattern in *de novo* infections revealed that there is a burst of lytic gene expression immediately after infection, which then is suppressed [29–32]. Interestingly, both RTA and LANA are initially expressed; however, RTA expression is transient, peaking earlier than LANA before being repressed [29]. LANA recruitment of the cellular transcriptional repressors Nrf2 and KAP1 to the viral genome may contribute to the lytic burst and subsequent rapid repression [33, 34] (Figure 1). The lytic gene burst may contribute to establishment of latency and tumorigenesis through expression of immunomodulatory and anti-apoptotic proteins [29]. It may also be required for a brief burst of amplification of the viral genome, because the viral DNA polymerase co-factor ORF59 is one of the genes expressed during *de novo* infection and its mutation leads to reduction in the genome copy number once latency is established [30].

Chromatin remodeling in latency and lytic replication

Selective gene expression during latency is in part controlled through chromatinization of the KSHV genome, which has been the subject of several excellent recent reviews [35–38]. In the last ten years, the advent of genome-wide approaches to analyze chromatin modifications, chromatin immunoprecipitation coupled to microarrays (ChIP-on-Chip) or high-throughput sequencing (ChIP-Seq), has allowed multiple groups to profile the chromatin modifications of the KSHV genome during *de novo* infection, latency and reactivation [31, 39–41]. Upon *de novo* infection, the KSHV genome becomes circularized, methylated and chromatinized [35–38]. During latency, most genes are bound by histones that display modifications associated with repression, with the exception of the locus encoding the latency proteins, which is consistent with their continued expression [39–41]. These results explain why inhibiting histone deacetylases with sodium butyrate potently induces the lytic cycle, as acetylation of histones promotes open chromatin and transcription [4]. There is also evidence that several loci on the KSHV genome are primed for fast activation during reactivation. The promoter of RTA and other immediate early genes, for example, display markings of bivalent chromatin, i.e. chromatin that is poised for gene expression in the presence of the correct signals [39, 40]. In addition, RNAPII is pre-bound to the promoter of several early lytic genes and is kept in a paused state by interactions with negative elongation factor (NELF) [42]. LANA is responsible for repressive chromatin formation on the genome upon *de novo* infection through recruitment of polycomb repressive complexes [43], while lytic factors like ORF59 promote chromatin remodeling [44] (Figure 1). Besides acting as the viral DNA polymerase processivity factor, ORF59 blocks the arginine methylase PRMT5 from binding the viral genome and modifying histone 4 [44]. PRMT5 symmetrically methylates arginine 3 of histone 4 (H4R3me2s) [44], which in turn promotes the tri-methylation of histone 3 on lysine 27 (H3K27me3), a modification that represses transcription [45]. Thus, by blocking PRMT5, ORF59 promotes formation of active chromatin and lytic gene transcription. The highly abundant KSHV long non-coding RNA PAN is also thought to promote lytic gene expression through chromatin remodeling,

although it likely has multiple functions in maintaining the lytic cycle and gene expression, as reviewed recently in [46–48].

ChIP-Seq and other high-throughput based techniques such as chromatin conformation capture have also been used to define the role of higher-order DNA structures in the control of gene expression in KSHV. Three-dimensional chromatin looping is now widely appreciated to facilitate and control RNAPII access to promoters in mammalian genomes [49]. Several recent studies have found that cellular proteins involved in gene looping, such as the chromatin insulator protein CTCF and the cohesin complex, contribute to repressing lytic genes during latency and also facilitate reactivation [50–54]. In particular, long-range interactions between promoter regions near the latency genes and promoter regions near RTA and other immediate early genes are key for the coordinate regulation of latent and lytic gene expression [51]. The role of long-range interactions was most recently demonstrated by precise mutation of interaction sites proximal to the RTA promoter, which lead to reduced levels of distal genes and decreased ability of the virus to reactivate [54].

Control of early gene transcription

Overexpression or induction of the master lytic regulator RTA/ORF50 is sufficient to drive entry into the lytic cycle [20]. A recent study using 3D microscopy, RNA fluorescence in situ hybridization and immunofluorescence showed that there is a physical movement of the cellular RNAPII to discrete locations in the nucleus during early activation of viral gene transcription in TPA-reactivated cells [55]. The RNAPII relocalization creates viral gene expression factories that can be visualized by probing for nascent RTA transcripts [55]. These experiments also revealed that transcription may only occur from a subset of the genomes in the cell [55]. Experimental evidence over the years has demonstrated that RTA directly regulates transcription of many early genes, but that additional cellular and viral factors also play a role [56] (Figure 1). While ChIP-on-Chip revealed that RTA binds the promoters of as many as 40 viral genes [57, 58], Bu *et al.* found that only very few genes are independently induced by RTA when translation of other viral proteins is inhibited with hygromycin [59]. Several additional viral transcription factors that act on early genes have been described, particularly K-bZIP/K8, which has both positive and negative effects on viral gene expression [58] and vIRF4/K10, which regulates its own transcription and the transcription of vIRF1 [60] (Figure 1). It is likely that other viral factors may also have yet uncharacterized roles in early gene transcription. Among the cellular transcription factors, RBP-J κ , a downstream target of the Notch signaling pathway, is the best described co-factor of RTA (reviewed in [61]), but multiple additional cellular proteins also act in concert with RTA (reviewed in [56]). To date, the function of these cellular transcription factors has been well described only for specific promoters, but they may have more pervasive effects that were not investigated in the original studies. In particular, the transcriptional control of RTA itself has been a major focus of study [62]. For example, regulation of RTA by the hypoxia-related transcription factors HIF-1 α and HIF-2 α was investigated because hypoxic conditions trigger lytic reactivation [63]. More genome-wide studies are needed to understand the coordinated function of cellular and viral factors in early gene expression. Moreover, an additional level of regulation may be provided by post-translational modifications of RTA and other regulatory proteins. Sumoylation of RTA, which occurs

during lytic replication, affects RTA-dependent induction of transcripts, but this effect can be positive or negative depending on the gene [64]. Also, sumoylation of the histone demethylase JMJD2A is needed to promote chromatin remodeling and lytic gene expression [65]. In both cases, the SUMO ligase involved appears to be K-bZIP [66], showing that this protein has multiple functions in gene regulation [64, 65].

Control of late gene transcription

The most significant advance of the last five years in understanding KSHV gene expression is the identification of an alternative viral transcription complex specific to late gene promoters. At the start of transcription in mammalian cells, core promoter sequences proximal to the transcription start site, particularly the TATA box, are recognized by the general transcription factors, which include the TATA-binding protein (TBP) [67]. General transcription factors form the transcription pre-initiation complex (PIC) and recruit RNAPII [67]. However, late gene promoters contain TATT sequences instead of the canonical TATA boxes and therefore require an alternative viral PIC to be transcribed [68]. Homologous viral PICs were identified in beta- and gamma-herpesviruses, and consist of proteins that are required for replication and have no homologs in alpha-herpesviruses [68]. In KSHV, the viral PIC is composed of ORF18, ORF24, ORF30, ORF31, ORF34 and ORF66 [69–73] (Figure 1). One study suggested that ORF23 may also be part of the complex [69], but its role is in question because ORF23 activity is dispensable for replication in the murine gamma-herpesvirus MHV68 [74]. Although viral DNA replication is required for late gene transcription during infection, co-expression of the six proteins is sufficient to drive transcription of a reporter with a late gene promoter [70]. This is consistent with the observation that reporters with late gene promoters were expressed in cells undergoing lytic replication, i.e. cells in which the alternative PIC was present [75]. The six proteins are also all necessary for late gene expression, as omission of any of them from transfection experiments [70] or deletion of most of them from the genome using the BAC16 system [16] prevents late gene expression (ORF18, ORF30: [71]; ORF24: [72]; ORF31: [73]; ORF34: [69]; ORF66 not yet tested). Interestingly, ORF24 appears to provide two key functions of the complex: DNA binding and RNAPII recruitment. The N-terminal domain of ORF24 contains a conserved RLLLG motif that mediates RNAPII binding, and the central domain is structurally homologous to cellular TBP and binds DNA [72]. As expected based on the lack of TATA sequences, cellular TBP does not bind late promoters [72]. Instead, ORF24 substitutes for TBP in the recruitment of other cellular general transcription factors, while also directly recruiting RNAPII [72]. ORF24 only binds one other member of the viral PIC, ORF34, via amino acids 323–339 of ORF24 and the C-terminal domain of ORF34 [69, 70]. In turn, ORF34 may serve as a scaffold, as it interacts with all of the other proteins through a central domain [69, 70]. The exact function of the other four components of the viral PIC remains unclear. While this complex of proteins acts as an equivalent of the general transcription factors, it is unknown whether signal-regulated transcription factors also control late gene expression. Blattman *et al.* reported that in some cell types, NF κ B is required for the production of select components of the virions, including some late genes [76]. As a result, in the absence of NF κ B, replication appears normal and viral particles are produced, but the virions are not infectious [76].

Post-transcriptional control of viral gene expression

While transcriptional mechanisms control the temporal dynamics of gene expression, non-transcriptional mechanisms of gene regulation may be necessary to ensure abundant production of KSHV proteins. Although KSHV transcripts are very similar to host mRNAs, there are some differences that could reduce the accumulation of KSHV proteins. For example, some viral genes have extremely short 3' untranslated regions (UTRs), and most of them are encoded from intronless genes [15]. In principle these differences could impair nuclear export and translation of viral mRNAs, because RNA binding proteins deposited during splicing mediate interactions with nuclear export factors and promote translation. The viral protein ORF57 was originally proposed to counteract this problem and promote export of the intronless KSHV mRNAs through interactions with nuclear export proteins like Aly/REF [77, 78]. However, subsequent studies have suggested that its predominant function may be to stabilize and promote accumulation of the RNAs [79–81]. ORF57 is now considered a multifunctional protein with roles in many RNA metabolic processes, including RNA stability, export, translation and even transcription (reviewed recently in [82–84], Figure 1). One recent addition to this body of literature is a transcriptome-wide profile of ORF57 targets using an ORF57-null virus [85]. While the expression of many lytic genes of all kinetic classes is reduced in the absence of ORF57, this study revealed that eight transcripts in two gene clusters are particularly dependent on ORF57 [85]. The mechanism by which ORF57 selectively increases their expression remains unclear, as it depends on the sequence of the coding region rather than promoter sequences or polyadenylation signals [85]. Interestingly, Vogt *et al.* found a similar effect, reporting that ORF57 is required to sustain the levels of some viral mRNAs containing higher AT nucleotide content, which they propose is suboptimal compared to cellular nucleotide content [86]. In a separate study vFLIP was demonstrated to have suboptimal codon usage, which impaired vFLIP RNA accumulation [87]. In this case, the resulting low levels of vFLIP were optimal for lytic virus production [87]. This suggests that codon and sequence composition may be used by KSHV to further modulate its gene expression. Another recently described function of the ORF57 protein is binding the dsRNA-activated kinase PKR to prevent it from phosphorylating the translation initiation factor eIF2 α [88]. eIF2 α brings the initiator methionine tRNA to the 40S ribosome, and eIF2 α phosphorylation by kinases like PKR is a cellular strategy to shut down translation in response to stress and infection [89]. dsRNA may be produced during KSHV replication because in some loci both DNA strands are transcribed, and could trigger translational shutoff through PKR activation. Thus, in addition to promoting RNA accumulation, ORF57 may also potentiate viral protein production by reducing cell-driven translational shutoff (Figure 1).

Another viral protein, ORF10, was recently reported to regulate nuclear RNA export through interactions with the export factor Rae1 [90] (Figure 1). However, while ORF10 appears to promote the expression of late lytic mRNAs, this effect may be indirectly due to ORF10 inhibition of cellular mRNA export [90]. Thus, at present it remains unclear how KSHV intronless mRNAs are efficiently exported without the benefits of interactions with the cellular splicing machinery.

Besides eIF2 α , multiple other translation initiation factors are regulated through phosphorylation. Because initiation is a critical point of translation regulation, these phosphorylation events control translational output during stress and infection. KSHV regulates some of these phosphorylation events, presumably to boost viral protein translation. The activity of the cap binding protein eIF4E, which recruits the 40S subunit of the ribosome to the 5' capped end of mRNAs, increases after phosphorylation by the MAP kinase-interacting kinase Mnk1 [91]. In addition, eIF4E binding to the regulatory 4E binding proteins (4EBPs) prevents association with other initiation factors, and phosphorylation of 4EBPs by mTOR promotes translation by disrupting eIF4E-4EBP interactions [91]. Both 4EBP1 and eIF4E are phosphorylated by KSHV reactivation, promoting translation of RTA and thus lytic replication [92]. The levels of other viral proteins were not examined, but in principle their increased accumulation could also contribute to increased lytic replication. One of the viral proteins that may trigger these phosphorylation events is vGPCR, a signaling protein that activates mTOR signaling [93]. The activity of the initiation factor eIF4B, which promotes scanning of the 5' UTR by the 40S ribosome in complex with the initiator tRNA, is also potentiated through phosphorylation [94]. KSHV ORF45 activates ribosomal S6 kinase (RSK), which phosphorylates eIF4B and the ribosomal protein S6 to stimulates translation [95–98] (Figure 1). Using the BAC16 system, the Zhu lab mutated the F66 residue in ORF45, which mediates RSK binding, and showed that RSK activation by ORF45 promotes viral protein production, particularly late protein production [98]. Interestingly, the positive translational effect of ORF45 may be most dramatic for mRNAs containing highly structured 5' UTRs, both viral and cellular [99], which may be a strategy used by KSHV to translationally regulate a subset of its own mRNAs as well as also cellular mRNAs important for KSHV pathogenesis.

While most of the KSHV genes are translated in a canonical cap-dependent manner, KSHV expresses several polycistronic transcripts containing multiple ORFs [15], which is different from the predominantly monocistronic human mRNAs. These polycistronic transcripts occur as a result of the limited number of polyadenylation signals in the KSHV genome [15, 100, 101], which means that transcription starting at different promoters ends at the same position. Although this potentially offers additional levels of regulation, in most cases only the 5' proximal ORF is translated from each transcript, as expected based on the canonical cap-dependent model of translation [15]. There is one reported internal ribosome entry sites (IRES), which drives translation of vFLIP from the v-cyclin-vFLIP transcript [26–28]. Surprisingly, IRES-mediated translation of vFLIP still requires all tested initiation factors including the cap-binding protein eIF4E, which is unique among IRESs [102]. Another protein that is expressed from a bicistronic transcript is ORF36, which is the distal ORF in the ORF35-ORF36 transcript [103]. In this case, the presence of small upstream ORFs (uORFs) of 9–11 amino acids in the 5' UTR of the transcript regulates ORF36 expression [103]. uORFs are known to have roles in translation regulation during stress [104]. Translation of the uORF that overlaps with the start codon of ORF35 prevents ORF35 translation and instead promotes ribosome engagement of the ORF36 start codon, which is downstream of the uORF stop codon, through a non-canonical mechanism of translation reinitiation [103, 105]. Because of inherent variability in engagement of the start codons, ORF35 expression from the transcript is lower than ORF36 expression, but not absent [103,

105]. The recent ribosome profiling of the KSHV genome revealed that other KSHV mRNAs also have uORFs in their 5' UTRs that appear to be actively translated [15], but it is not known whether they also have regulatory functions on the expression of viral proteins or facilitate expression of multiple proteins from polycistronic transcripts.

m⁶A methylation of viral transcripts

N⁶-methyladenosine (m⁶A) is an RNA modification that is found at high frequency in mRNAs [106]. In the last few years, advances in techniques to isolate and sequence methylated sites on mRNAs have resulted in an explosion of studies on m⁶A [106]. This has led to the discovery of many roles of this modification in RNA metabolism with implications in health and disease [106]. Not surprisingly, some KSHV mRNAs also carry methylated adenosines [107–109]. Knock-down of an m⁶A “writer”, the methylase METTL3, reduces virus production [107, 109], while removal of an m⁶A “eraser”, the demethylase FTO, increases it slightly [107]. There are some discrepancies in the current studies as to which “reader” (i.e. m⁶A binding) proteins mediate the effect of m⁶A modifications, perhaps because different infected cell lines were used [108, 109]. m⁶A modifications appear to increase the half-life of multiple lytic mRNAs [108], and also promote the accumulation of RTA protein without changing its RNA levels [107, 109]. This may be due to a failure to properly splice the RTA transcript [107], which is consistent with a well-established role for m⁶A in regulating RNA splicing [106]. However, some of the m⁶A modifications may also restrict KSHV gene expression, as one study has shown increased virus production after knock-down of one of the m⁶A reader protein [108].

Conclusions

Recent advances in the tools available to study the biology of KSHV have pushed forward our understanding of gene expression throughout the viral replication cycle, and will continue to do so in the coming years. The identification of the viral factors that control late gene transcription [69–73] has filled an important gap in our knowledge of KSHV gene regulation, and was facilitated by a more streamlined system to make mutations in KSHV [16]. High-throughput based methods have led to more detailed transcript maps of KSHV genes [15], as well as maps of chromatin modifications [39–41] and RNA modifications [107–109], that are unveiling new aspects of gene regulation with potential implications for pathogenesis and treatment. The fact that several novel viral regulators of gene expression have recently been identified suggests that there are aspects of KSHV gene regulation that remain unexplored. Indeed, in a screen for potential gene regulators, we have found that several KSHV proteins not classically associated with gene expression affect the levels of reporter constructs (Butnaru, Rivas and Gaglia, unpublished). Moreover, both characterized and uncharacterized viral gene regulators have the potential to also control cellular gene expression, another area which has not been fully explored. Ultimately, the hope is that understanding the replication cycle of KSHV will point to new targets for drug development, as currently no drugs are available that directly treat this viral infection.

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References

Recently published papers of particular interest have been highlighted as:

• Of importance

•• Of major importance

1. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. 1994; Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science*. 266:1865–1869. [PubMed: 7997879]
2. Yarchoan R, Uldrick TS. 2018; HIV-Associated Cancers and Related Diseases. *N Engl J Med*. 378:1029–1041. [PubMed: 29539283]
3. Marignò G, Koch S, Schulz TF. 2017; Kaposi sarcoma herpesvirus pathogenesis. *Philos Trans R Soc Lond B Biol Sci*. doi: 10.1098/rstb.2016.0275
4. Miller G, Heston L, Grogan E, Gradoville L, Rigsby M, Sun R, Shedd D, Kushnaryov VM, Grossberg S, Chang Y. 1997; Selective switch between latency and lytic replication of Kaposi's sarcoma herpesvirus and Epstein-Barr virus in dually infected body cavity lymphoma cells. *J Virol*. 71:314–324. [PubMed: 8985352]
5. Ganem D. 2010; KSHV and the pathogenesis of Kaposi sarcoma: listening to human biology and medicine. *J Clin Invest*. 120:939–949. [PubMed: 20364091]
6. Martin DF, Kuppermann BD, Wolitz RA, Palestine AG, Li H, Robinson CA. 1999; Oral ganciclovir for patients with cytomegalovirus retinitis treated with a ganciclovir implant. Roche Ganciclovir Study Group. *N Engl J Med*. 340:1063–1070. [PubMed: 10194235]
7. Hosseini MC, Sweet KM, Xiong J, et al. 2014; Viral Profiling Identifies Multiple Subtypes of Kaposi's Sarcoma. *mBio*. 5:e01633–14. [PubMed: 25249280]
8. Wakeham, Katie; Thomas, Johnston W; Angela, Nalwoga; Emily L, Webb; Billy N, Mayanja; Wendell, Miley; Alison M, Elliott; Denise, Whitby; Robert, Newton. 2015; Trends in Kaposi's sarcoma-associated Herpesvirus antibodies prior to the development of HIV-associated Kaposi's sarcoma: A nested case-control study. *Int J Cancer*. 136:2822–2830. [PubMed: 25395177]
9. Russo JJ, Bohenzky RA, Chien M-C, et al. 1996; Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proc Natl Acad Sci*. 93:14862–14867. [PubMed: 8962146]
10. Cotter MA, Robertson ES. 1999; The latency-associated nuclear antigen tethers the Kaposi's sarcoma-associated herpesvirus genome to host chromosomes in body cavity-based lymphoma cells. *Virology*. 264:254–264. [PubMed: 10562490]
11. Lu M, Suen J, Frias C, Pfeiffer R, Tsai M-H, Chuang E, Zeichner SL. 2004; Dissection of the Kaposi's Sarcoma-Associated Herpesvirus Gene Expression Program by Using the Viral DNA Replication Inhibitor Cidofovir. *J Virol*. 78:13637–13652. [PubMed: 15564474]
12. Jenner RG, Albà MM, Boshoff C, Kellam P. 2001; Kaposi's Sarcoma-Associated Herpesvirus Latent and Lytic Gene Expression as Revealed by DNA Arrays. *J Virol*. 75:891–902. [PubMed: 11134302]
13. Paulose-Murphy M, Ha N-K, Xiang C, Chen Y, Gillim L, Yarchoan R, Meltzer P, Bittner M, Trent J, Zeichner S. 2001; Transcription Program of Human Herpesvirus 8 (Kaposi's Sarcoma-Associated Herpesvirus). *J Virol*. 75:4843–4853. [PubMed: 11312356]

14. Nakamura H, Lu M, Gwack Y, Souvlis J, Zeichner SL, Jung JU. 2003; Global changes in Kaposi's sarcoma-associated virus gene expression patterns following expression of a tetracycline-inducible Rta transactivator. *J Virol.* 77:4205–4220. [PubMed: 12634378]
15. Arias C, Weisburd B, Stern-Ginossar N, Mercier A, Madrid AS, Bellare P, Holdorf M, Weissman JS, Ganem D. 2014; KSHV 2.0: a comprehensive annotation of the Kaposi's sarcoma-associated herpesvirus genome using next-generation sequencing reveals novel genomic and functional features. *PLoS Pathog.* 10:e1003847. [PubMed: 24453964]
16. Brulois KF, Chang H, Lee AS-Y, et al. 2012; Construction and manipulation of a new Kaposi's sarcoma-associated herpesvirus bacterial artificial chromosome clone. *J Virol.* 86:9708–9720. [PubMed: 22740391]
17. Myoung J, Ganem D. 2011; Generation of a doxycycline-inducible KSHV producer cell line of endothelial origin: maintenance of tight latency with efficient reactivation upon induction. *J Virol Methods.* 174:12–21. [PubMed: 21419799]
18. Stürzl M, Gaus D, Dirks WG, Ganem D, Jochmann R. 2013; Kaposi's sarcoma-derived cell line SLK is not of endothelial origin, but is a contaminant from a known renal carcinoma cell line. *Int J Cancer.* 132:1954–1958. [PubMed: 22987579]
19. Lukac DM, Renne R, Kirshner JR, Ganem D. 1998; Reactivation of Kaposi's Sarcoma-Associated Herpesvirus Infection from Latency by Expression of the ORF 50 Transactivator, a Homolog of the EBV R Protein. *Virology.* 252:304–312. [PubMed: 9878608]
20. Sun R, Lin S-F, Gradoville L, Yuan Y, Zhu F, Miller G. 1998; A viral gene that activates lytic cycle expression of Kaposi's sarcoma-associated herpesvirus. *Proc Natl Acad Sci.* 95:10866–10871. [PubMed: 9724796]
21. Yu F, Harada JN, Brown HJ, et al. 2007; Systematic Identification of Cellular Signals Reactivating Kaposi Sarcoma-Associated Herpesvirus. *PLOS Pathog.* 3:e44. [PubMed: 17397260]
22. Honess RW, Roizman B. 1974; Regulation of Herpesvirus Macromolecular Synthesis I. Cascade Regulation of the Synthesis of Three Groups of Viral Proteins. *J Virol.* 14:8–19. [PubMed: 4365321]
23. Giffin L, Damania B. 2014; KSHV: pathways to tumorigenesis and persistent infection. *Adv Virus Res.* 88:111–159. [PubMed: 24373311]
24. Chandriani S, Ganem D. 2010; Array-Based Transcript Profiling and Limiting-Dilution Reverse Transcription-PCR Analysis Identify Additional Latent Genes in Kaposi's Sarcoma-Associated Herpesvirus. *J Virol.* 84:5565–5573. [PubMed: 20219929]
25. Rivas C, Thlick AE, Parravicini C, Moore PS, Chang Y. 2001; Kaposi's sarcoma-associated herpesvirus LANA2 is a B-cell-specific latent viral protein that inhibits p53. *J Virol.* 75:429–438. [PubMed: 11119611]
26. Low W, Harries M, Ye H, Du M-Q, Boshoff C, Collins M. 2001; Internal Ribosome Entry Site Regulates Translation of Kaposi's Sarcoma-Associated Herpesvirus FLICE Inhibitory Protein. *J Virol.* 75:2938–2945. [PubMed: 11222719]
27. Bielecki L, Talbot SJ. 2001; Kaposi's sarcoma-associated herpesvirus vCyclin open reading frame contains an internal ribosome entry site. *J Virol.* 75:1864–1869. [PubMed: 11160685]
28. Grundhoff A, Ganem D. 2001; Mechanisms governing expression of the v-FLIP gene of Kaposi's sarcoma-associated herpesvirus. *J Virol.* 75:1857–1863. [PubMed: 11160684]
29. Krishnan HH, Naranatt PP, Smith MS, Zeng L, Bloomer C, Chandran B. 2004; Concurrent Expression of Latent and a Limited Number of Lytic Genes with Immune Modulation and Antiapoptotic Function by Kaposi's Sarcoma-Associated Herpesvirus Early during Infection of Primary Endothelial and Fibroblast Cells and Subsequent Decline of Lytic Gene Expression. *J Virol.* 78:3601–3620. [PubMed: 15016882]
30. Purushothaman P, Thakker S, Verma SC. 2015; Transcriptome Analysis of Kaposi's Sarcoma-Associated Herpesvirus during De Novo Primary Infection of Human B and Endothelial Cells. *J Virol.* 89:3093–3111. [PubMed: 25552714]
31. Jha HC, Lu J, Verma SC, Banerjee S, Mehta D, Robertson ES. 2014; Kaposi's Sarcoma-Associated Herpesvirus Genome Programming during the Early Stages of Primary Infection of Peripheral Blood Mononuclear Cells. *mBio.* 5:e02261–14. [PubMed: 25516617]

32. Bruce AG, Barcy S, DiMaio T, Gan E, Garrigues HJ, Lagunoff M, Rose TM. 2017; Quantitative Analysis of the KSHV Transcriptome Following Primary Infection of Blood and Lymphatic Endothelial Cells. *Pathogens*. 6:11.
33. Gjysli O, Roy A, Dutta S, Veetil MV, Dutta D, Chandran B. 2015; Activated Nrf2 Interacts with Kaposi's Sarcoma-Associated Herpesvirus Latency Protein LANA-1 and Host Protein KAP1 To Mediate Global Lytic Gene Repression. *J Virol*. 89:7874–7892. [PubMed: 25995248]
34. Sun R, Liang D, Gao Y, Lan K. 2014; Kaposi's sarcoma-associated herpesvirus-encoded LANA interacts with host KAP1 to facilitate establishment of viral latency. *J Virol*. 88:7331–7344. [PubMed: 24741090]
35. Lieberman PM. 2013; Keeping it quiet: chromatin control of gammaherpesvirus latency. *Nat Rev Microbiol*. 11:863–875. [PubMed: 24192651]
36. Toth Z, Brulois K, Jung JU. 2013; The Chromatin Landscape of Kaposi's Sarcoma-Associated Herpesvirus. *Viruses*. 5:1346–1373. [PubMed: 23698402]
37. Pentland I, Parish JL. 2015; Targeting CTCF to Control Virus Gene Expression: A Common Theme amongst Diverse DNA Viruses. *Viruses*. 7:3574–3585. [PubMed: 26154016]
38. Uppal T, Jha HC, Verma SC, Robertson ES. 2015; Chromatinization of the KSHV Genome During the KSHV Life Cycle. *Cancers*. 7:112–142. [PubMed: 25594667]
39. Toth Z, Maglinte DT, Lee SH, et al. 2010; Epigenetic analysis of KSHV latent and lytic genomes. *PLoS Pathog*. 6:e1001013. [PubMed: 20661424]
40. Günther T, Grundhoff A. 2010; The epigenetic landscape of latent Kaposi sarcoma-associated herpesvirus genomes. *PLoS Pathog*. 6:e1000935. [PubMed: 20532208]
41. Toth Z, Brulois K, Lee H-R, Izumiya Y, Tepper C, Kung H-J, Jung JU. 2013; Biphasic euchromatin-to-heterochromatin transition on the KSHV genome following de novo infection. *PLoS Pathog*. 9:e1003813. [PubMed: 24367262]
42. Toth Z, Brulois KF, Wong L-Y, Lee H-R, Chung B, Jung JU. 2012; Negative Elongation Factor-Mediated Suppression of RNA Polymerase II Elongation of Kaposi's Sarcoma-Associated Herpesvirus Lytic Gene Expression. *J Virol*. 86:9696–9707. [PubMed: 22740393]
43. Toth Z, Papp B, Brulois K, Choi YJ, Gao S-J, Jung JU. 2016; LANA-Mediated Recruitment of Host Polycomb Repressive Complexes onto the KSHV Genome during De Novo Infection. *PLoS Pathog*. 12:e1005878. [PubMed: 27606464]
44. Strahan RC, McDowell-Sargent M, Uppal T, Purushothaman P, Verma SC. 2017; KSHV encoded ORF59 modulates histone arginine methylation of the viral genome to promote viral reactivation. *PLOS Pathog*. 13:e1006482. [PubMed: 28678843]
45. Tae S, Karkhanis V, Velasco K, Yaneva M, Erdjument-Bromage H, Tempst P, Sif S. 2011; Bromodomain protein 7 interacts with PRMT5 and PRC2, and is involved in transcriptional repression of their target genes. *Nucleic Acids Res*. 39:5424–5438. [PubMed: 21447565]
46. Rossetto CC, Pari GS. 2014; PAN's Labyrinth: Molecular Biology of Kaposi's Sarcoma-Associated Herpesvirus (KSHV) PAN RNA, a Multifunctional Long Noncoding RNA. *Viruses*. 6:4212–4226. [PubMed: 25375885]
47. Campbell M, Kung H-J, Izumiya Y. 2014; Long Non-Coding RNA and Epigenetic Gene Regulation of KSHV. *Viruses*. 6:4165–4177. [PubMed: 25375882]
48. Conrad NK. 2016; New insights into the expression and functions of the Kaposi's sarcoma-associated herpesvirus long noncoding PAN RNA. *Virus Res*. 212:53–63. [PubMed: 26103097]
49. Pombo A, Dillon N. 2015; Three-dimensional genome architecture: players and mechanisms. *Nat Rev Mol Cell Biol*. 16:245–257. [PubMed: 25757416]
50. Chen H-S, Wikramasinghe P, Showe L, Lieberman PM. 2012; Cohesins repress Kaposi's sarcoma-associated herpesvirus immediate early gene transcription during latency. *J Virol*. 86:9454–9464. [PubMed: 22740398]
51. Kang H, Wiedmer A, Yuan Y, Robertson E, Lieberman PM. 2011; Coordination of KSHV latent and lytic gene control by CTCF-cohesin mediated chromosome conformation. *PLoS Pathog*. 7:e1002140. [PubMed: 21876668]
52. Kang H, Cho H, Sung G-H, Lieberman PM. 2013; CTCF regulates Kaposi's sarcoma-associated herpesvirus latency transcription by nucleosome displacement and RNA polymerase programming. *J Virol*. 87:1789–1799. [PubMed: 23192870]

53. Li D-J, Verma D, Mosbrugger T, Swaminathan S. 2014; CTCF and Rad21 act as host cell restriction factors for Kaposi's sarcoma-associated herpesvirus (KSHV) lytic replication by modulating viral gene transcription. *PLoS Pathog.* 10:e1003880. [PubMed: 24415941]
54. Campbell M, Watanabe T, Nakano K, Davis RR, Lyu Y, Tepper CG, Durbin-Johnson B, Fujimuro M, Izumiya Y. 2018; KSHV episomes reveal dynamic chromatin loop formation with domain-specific gene regulation. *Nat Commun.* 9:49. [PubMed: 29302027]
55. Chen CP, Lyu Y, Chuang F, Nakano K, Izumiya C, Jin D, Campbell M, Izumiya Y. 2017; Kaposi's Sarcoma-Associated Herpesvirus Hijacks RNA Polymerase II To Create a Viral Transcriptional Factory. *J Virol.* 91:e02491–16. [PubMed: 28331082]
56. Deng H, Liang Y, Sun R. 2007; Regulation of KSHV lytic gene expression. *Curr Top Microbiol Immunol.* 312:157–183. [PubMed: 17089797]
57. Chen J, Ye F, Xie J, Kuhne K, Gao S-J. 2009; Genome-wide identification of binding sites for Kaposi's sarcoma-associated herpesvirus lytic switch protein, RTA. *Virology.* 386:290–302. [PubMed: 19233445]
58. Ellison TJ, Izumiya Y, Izumiya C, Luciw PA, Kung H-J. 2009; A comprehensive analysis of recruitment and transactivation potential of K-Rta and K-bZIP during reactivation of Kaposi's sarcoma-associated herpesvirus. *Virology.* 387:76–88. [PubMed: 19269659]
59. Bu W, Palmeri D, Krishnan R, Marin R, Aris VM, Soteropoulos P, Lukac DM. 2008; Identification of Direct Transcriptional Targets of the Kaposi's Sarcoma-Associated Herpesvirus Rta Lytic Switch Protein by Conditional Nuclear Localization. *J Virol.* 82:10709–10723. [PubMed: 18715905]
60. Xi X, Persson LM, O'Brien MW, Mohr I, Wilson AC. 2012; Cooperation between Viral Interferon Regulatory Factor 4 and RTA To Activate a Subset of Kaposi's Sarcoma-Associated Herpesvirus Lytic Promoters. *J Virol.* 86:1021–1033. [PubMed: 22090118]
61. DeCotiis JL, Lukac DM. 2017; KSHV and the Role of Notch Receptor Dysregulation in Disease Progression. *Pathog Basel Switz.* doi: 10.3390/pathogens6020034
62. Guito J, Lukac DM. 2012; KSHV Rta Promoter Specification and Viral Reactivation. *Front Microbiol.* doi: 10.3389/fmicb.2012.00030
63. Haque M, Davis DA, Wang V, Widmer I, Yarchoan R. 2003; Kaposi's Sarcoma-Associated Herpesvirus (Human Herpesvirus 8) Contains Hypoxia Response Elements: Relevance to Lytic Induction by Hypoxia. *J Virol.* 77:6761–6768. [PubMed: 12767996]
64. Wang J, Guo Y, Wang X, Zhao R, Wang Y. 2017; Modulation of global SUMOylation by Kaposi's sarcoma-associated herpesvirus and its effects on viral gene expression. *J Med Virol.* 89:2011–2019. [PubMed: 28639696]
65. Yang W-S, Campbell M, Chang P-C. 2017; SUMO modification of a heterochromatin histone demethylase JMJD2A enables viral gene transactivation and viral replication. *PLOS Pathog.* 13:e1006216. [PubMed: 28212444]
66. Chang P-C, Izumiya Y, Wu C-Y, Fitzgerald LD, Campbell M, Ellison TJ, Lam KS, Luciw PA, Kung H-J. 2010; Kaposi's Sarcoma-associated Herpesvirus (KSHV) Encodes a SUMO E3 ligase That Is SIM-dependent and SUMO-2/3-specific. *J Biol Chem.* 285:5266–5273. [PubMed: 20034935]
67. Thomas MC, Chiang C-M. 2006; The general transcription machinery and general cofactors. *Crit Rev Biochem Mol Biol.* 41:105–178. [PubMed: 16858867]
68. Gruffat H, Marchione R, Manet E. 2016; Herpesvirus Late Gene Expression: A Viral-Specific Pre-initiation Complex Is Key. *Front Microbiol.* 7:869. [PubMed: 27375590]
69. Nishimura M, Watanabe T, Yagi S, Yamanaka T, Fujimuro M. 2017; Kaposi's sarcoma-associated herpesvirus ORF34 is essential for late gene expression and virus production. *Sci Rep.* 7:329. [PubMed: 28336944]
70. Davis ZH, Hesser CR, Park J, Glaunsinger BA. 2016; Interaction between ORF24 and ORF34 in the Kaposi's Sarcoma-Associated Herpesvirus Late Gene Transcription Factor Complex Is Essential for Viral Late Gene Expression. *J Virol.* 90:599–604. [PubMed: 26468530]
71. Gong D, Wu NC, Xie Y, et al. 2014; Kaposi's Sarcoma-Associated Herpesvirus ORF18 and ORF30 Are Essential for Late Gene Expression during Lytic Replication. *J Virol.* 88:11369–11382. [PubMed: 25056896]

72. Davis ZH, Verschueren E, Jang GM, et al. 2015; Global mapping of herpesvirus-host protein complexes reveals a transcription strategy for late genes. *Mol Cell*. 57:349–360. [PubMed: 25544563]
73. Brulois K, Wong L-Y, Lee H-R, Sivadas P, Ensser A, Feng P, Gao S-J, Toth Z, Jung JU. 2015; Association of Kaposi's Sarcoma-Associated Herpesvirus ORF31 with ORF34 and ORF24 Is Critical for Late Gene Expression. *J Virol*. 89:6148–6154. [PubMed: 25810551]
74. Ohno S, Steer B, Sattler C, Adler H. 2012; ORF23 of murine gammaherpesvirus 68 is non-essential for in vitro and in vivo infection. *J Gen Virol*. 93:1076–1080. [PubMed: 22258865]
75. Chang J, Ganem D. 2000; On the control of late gene expression in Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8). *J Gen Virol*. 81:2039–2047. [PubMed: 10900043]
76. Blattman NN, Lagunoff M, Blattman JN, Corey L. 2014; Nuclear factor kappa B is required for the production of infectious human herpesvirus 8 virions. *Front Microbiol*. doi: 10.3389/fmicb.2014.00129
77. Malik P, Blackburn DJ, Clements JB. 2004; The Evolutionarily Conserved Kaposi's Sarcoma-associated Herpesvirus ORF57 Protein Interacts with REF Protein and Acts as an RNA Export Factor. *J Biol Chem*. 279:33001–33011. [PubMed: 15155762]
78. Boyne JR, Colgan KJ, Whitehouse A. 2008; Recruitment of the complete hTREX complex is required for Kaposi's sarcoma-associated herpesvirus intronless mRNA nuclear export and virus replication. *PLoS Pathog*. 4:e1000194. [PubMed: 18974867]
79. Nekorchuk M, Han Z, Hsieh T-T, Swaminathan S. 2007; Kaposi's sarcoma-associated herpesvirus ORF57 protein enhances mRNA accumulation independently of effects on nuclear RNA export. *J Virol*. 81:9990–9998. [PubMed: 17609285]
80. Pilkington GR, Majerciak V, Bear J, Uranishi H, Zheng Z-M, Felber BK. 2012; Kaposi's Sarcoma-Associated Herpesvirus ORF57 Is Not a Bona Fide Export Factor. *J Virol*. 86:13089–13094. [PubMed: 22993146]
81. Li D-J, Verma D, Swaminathan S. 2012; Binding of cellular export factor REF/Aly by Kaposi's sarcoma-associated herpesvirus (KSHV) ORF57 protein is not required for efficient KSHV lytic replication. *J Virol*. 86:9866–9874. [PubMed: 22761374]
82. Majerciak V, Zheng Z-M. 2015; KSHV ORF57, a protein of many faces. *Viruses*. 7:604–633. [PubMed: 25674768]
83. Vogt C, Böhne J. 2016; The KSHV RNA regulator ORF57: target specificity and its role in the viral life cycle. *Wiley Interdiscip Rev RNA*. 7:173–185. [PubMed: 26769399]
84. Schumann S, Jackson BR, Baquero-Perez B, Whitehouse A. 2013; Kaposi's Sarcoma-Associated Herpesvirus ORF57 Protein: Exploiting All Stages of Viral mRNA Processing. *Viruses*. 5:1901–1923. [PubMed: 23896747]
85. Verma D, Li D-J, Krueger B, Renne R, Swaminathan S. 2015; Identification of the physiological gene targets of the essential lytic replicative Kaposi's sarcoma-associated herpesvirus ORF57 protein. *J Virol*. 89:1688–1702. [PubMed: 25410858]
86. Vogt C, Hackmann C, Rabner A, Koste L, Santag S, Kati S, Mandel-Gutfreund Y, Schulz TF, Böhne J. 2015; ORF57 overcomes the detrimental sequence bias of Kaposi's sarcoma-associated herpesvirus lytic genes. *J Virol*. 89:5097–5109. [PubMed: 25694606]
87. Bellare P, Dufresne A, Ganem D. 2015; Inefficient Codon Usage Impairs mRNA Accumulation: the Case of the v-FLIP Gene of Kaposi's Sarcoma-Associated Herpesvirus. *J Virol*. 89:7097–7107. [PubMed: 25926644]
88. Sharma NR, Majerciak V, Kruhlak MJ, Zheng Z-M. 2017; KSHV inhibits stress granule formation by viral ORF57 blocking PKR activation. *PLoS Pathog*. 13:e1006677. [PubMed: 29084250]
89. Carpenter S, Ricci EP, Mercier BC, Moore MJ, Fitzgerald KA. 2014; Post-transcriptional regulation of gene expression in innate immunity. *Nat Rev Immunol*. 14:361–376. [PubMed: 24854588]
90. Gong D, Kim YH, Xiao Y, et al. 2016; A Herpesvirus Protein Selectively Inhibits Cellular mRNA Nuclear Export. *Cell Host Microbe*. 20:642–653. [PubMed: 27832591]
91. Siddiqui N, Sonenberg N. 2015; Signalling to eIF4E in cancer. *Biochem Soc Trans*. 43:763–772. [PubMed: 26517881]

92. Arias C, Walsh D, Harbell J, Wilson AC, Mohr I. 2009; Activation of host translational control pathways by a viral developmental switch. *PLoS Pathog.* 5:e1000334. [PubMed: 19300492]
93. Sodhi A, Chaisuparat R, Hu J, Ramsdell AK, Manning BD, Sausville EA, Sawai ET, Molinolo A, Gutkind JS, Montaner S. 2006; The TSC2/mTOR pathway drives endothelial cell transformation induced by the Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor. *Cancer Cell.* 10:133–143. [PubMed: 16904612]
94. Shahbazian D, Roux PP, Mieulet V, Cohen MS, Raught B, Taunton J, Hershey JW, Blenis J, Pende M, Sonenberg N. 2006; The mTOR/PI3K and MAPK pathways converge on eIF4B to control its phosphorylation and activity. *EMBO J.* 25:2781–2791. [PubMed: 16763566]
95. Kuang E, Fu B, Liang Q, Myoung J, Zhu F. 2011; Phosphorylation of eukaryotic translation initiation factor 4B (EIF4B) by open reading frame 45/p90 ribosomal S6 kinase (ORF45/RSK) signaling axis facilitates protein translation during Kaposi sarcoma-associated herpesvirus (KSHV) lytic replication. *J Biol Chem.* 286:41171–41182. [PubMed: 21994950]
96. Kuang E, Tang Q, Maul GG, Zhu F. 2008; Activation of p90 ribosomal S6 kinase by ORF45 of Kaposi's sarcoma-associated herpesvirus and its role in viral lytic replication. *J Virol.* 82:1838–1850. [PubMed: 18057234]
97. Kuang E, Wu F, Zhu F. 2009; Mechanism of sustained activation of ribosomal S6 kinase (RSK) and ERK by kaposi sarcoma-associated herpesvirus ORF45: multiprotein complexes retain active phosphorylated ERK AND RSK and protect them from dephosphorylation. *J Biol Chem.* 284:13958–13968. [PubMed: 19304659]
98. Fu B, Kuang E, Li W, Avey D, Li X, Turpin Z, Valdes A, Brulois K, Myoung J, Zhu F. 2015; Activation of p90 ribosomal S6 kinases by ORF45 of Kaposi's sarcoma-associated herpesvirus is critical for optimal production of infectious viruses. *J Virol.* 89:195–207. [PubMed: 25320298]
99. Avey D, Tepper S, Li W, Turpin Z, Zhu F. 2015; Phosphoproteomic Analysis of KSHV-Infected Cells Reveals Roles of ORF45-Activated RSK during Lytic Replication. *PLoS Pathog.* 11:e1004993. [PubMed: 26133373]
100. Majercki V, Ni T, Yang W, Meng B, Zhu J, Zheng Z-M. 2013; A Viral Genome Landscape of RNA Polyadenylation from KSHV Latent to Lytic Infection. *PLOS Pathog.* 9:e1003749. [PubMed: 24244170]
101. McClure LV, Kincaid RP, Burke JM, Grundhoff A, Sullivan CS. 2013; Comprehensive Mapping and Analysis of Kaposi's Sarcoma-Associated Herpesvirus 3' UTRs Identify Differential Posttranscriptional Control of Gene Expression in Lytic versus Latent Infection. *J Virol.* 87:12838–12849. [PubMed: 24067953]
102. Othman Z, Sulaiman MK, Willcocks MM, Ulryck N, Blackburn DJ, Sargueil B, Roberts LO, Locker N. 2014; Functional analysis of Kaposi's sarcoma-associated herpesvirus vFLIP expression reveals a new mode of IRES-mediated translation. *RNA.* 20:1803–1814. [PubMed: 25246653]
103. Kronstad LM, Brulois KF, Jung JU, Glaunsinger BA. 2013; Dual short upstream open reading frames control translation of a herpesviral polycistronic mRNA. *PLoS Pathog.* 9:e1003156. [PubMed: 23382684]
104. Young SK, Wek RC. 2016; Upstream Open Reading Frames Differentially Regulate Gene-specific Translation in the Integrated Stress Response. *J Biol Chem.* 291:16927–16935. [PubMed: 27358398]
105. Kronstad LM, Brulois KF, Jung JU, Glaunsinger BA. 2014; Reinitiation after Translation of Two Upstream Open Reading Frames (ORF) Governs Expression of the ORF35-37 Kaposi's Sarcoma-Associated Herpesvirus Polycistronic mRNA. *J Virol.* 88:6512–6518. [PubMed: 24623444]
106. Peer E, Rechavi G, Dominissini D. 2017; Epitranscriptomics: regulation of mRNA metabolism through modifications. *Curr Opin Chem Biol.* 41:93–98. [PubMed: 29125941]
107. Ye F, Chen ER, Nilsen TW. 2017; Kaposi's Sarcoma-Associated Herpesvirus Utilizes and Manipulates RNA N6-Adenosine Methylation To Promote Lytic Replication. *J Virol.* doi: 10.1128/JVI.00466-17

- 108•. Tan B, Liu H, Zhang S, et al. 2018; Viral and cellular N⁶-methyladenosine and N^{6,2'}-O²-dimethyladenosine epitranscriptomes in the KSHV life cycle. *Nat Microbiol.* 3:108–120. [PubMed: 29109479]
- 109•. Hesser C, Karijolic J, Dominissini D, He C, Glaunsinger BA. 2018; N⁶-methyladenosine modification and the YTHDF2 reader protein play cell type specific roles in lytic viral gene expression during Kaposi's sarcoma-associated herpesvirus infection. *PLoS Pathog.* 14:e1006995. [PubMed: 29659627]

Figure 1. KSHV gene regulation by virus-encoded factors

Viral mRNAs are transcribed, processed and translated by host cellular machinery, but multiple viral proteins (listed) contribute to the regulation of transcription, export, mRNA stability and translation to increase viral protein production and ensure the correct temporal pattern of gene expression.