Virus-Encoded microRNAs: An Overview and a Look to the Future

Rodney P. Kincaid, Christopher S. Sullivan*

The University of Texas at Austin, Molecular Genetics & Microbiology, Austin, Texas, United States of America

Abstract: MicroRNAs (miRNAs) are small RNAs that play important roles in the regulation of gene expression. First described as posttranscriptional gene regulators in eukaryotic hosts, virus-encoded miRNAs were later uncovered. It is now apparent that diverse virus families, most with DNA genomes, but at least some with RNA genomes, encode miRNAs. While deciphering the functions of viral miRNAs has lagged behind their discovery, recent functional studies are bringing into focus these roles. Some of the best characterized viral miRNA functions include subtle roles in prolonging the longevity of infected cells, evading the immune response, and regulating the switch to lytic infection. Notably, all of these functions are particularly important during persistent infections. Furthermore, an emerging view of viral miRNAs suggests two distinct groups exist. In the first group, viral miRNAs mimic host miRNAs and take advantage of conserved networks of host miRNA target sites. In the larger second group, viral miRNAs do not share common target sites conserved for host miRNAs, and it remains unclear what fraction of these targeted transcripts are beneficial to the virus. Recent insights from multiple virus families have revealed new ways of interacting with the host miRNA machinery including noncanonical miRNA biogenesis and new mechanisms of posttranscriptional cis gene regulation. Exciting challenges await the field, including determining the most relevant miRNA targets and parlaying our current understanding of viral miRNAs into new therapeutic strategies. To accomplish these goals and to better grasp miRNA function, new in vivo models that recapitulate persistent infections associated with viral pathogens are required.

Introduction

In recent years, non-protein-coding regulatory RNAs have been the subject of increasing interest in both procaryotic and eukaryotic fields. A new understanding of the mammalian genome is emerging where a majority (50%–85%) of the genome is transcribed with at least some noncoding RNA (ncRNA) transcripts being functionally relevant [1]. Although it is likely that new functions and classes remain to be described, diverse ncRNAs have already been implicated in regulating gene expression at multiple levels, including chromatin modification, transcription, and posttranscriptional mechanisms (reviewed in [2]).

RNA interference (RNAi), the process whereby small ncRNAs (<30 nts) serve to direct gene silencing via specific protein machinery, is evolutionarily conserved throughout most eukaryotes. Discovered in studies of the nematode C. elegans [3], with important contributions from the plant and Drosophila research communities, RNAi commonly functions to defend hosts against harmful nucleic acids such as endogenous transposons or exogenous viruses [reviewed in [4–6]]. While the antiviral role of RNAi is well-established in plants, insects, and nematodes, this does not seem to be the case in most (if not all) mammalian cell contexts. When compared to some plants and invertebrates, strong experimental evidence supporting an antiviral role for mammalian RNAi is lacking yet remains the subject of ongoing debate [7–9]. Nevertheless, at least some components of the RNAi machinery appear to protect mammalian cells against endogenous transposon activity [10–12].

Prokaryotes also possess a nucleic acid-based defense called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs). Like RNAi, CRISPRs can be thought of as a nucleic acid-based adaptive immune response providing protection against plasmids, transposons, or phage. Similar to RNAi, some bacterial CRISPR systems use double stranded RNA (dsRNA) and RNase III enzymes in the process of generating effectors that silence gene expression, typically through cleavage of targeted DNA [13]. Functional CRISPR machinery has been lost or gained numerous times in bacterial lineages. Similarly, RNAi has been lost in some eukaryotic lineages including the important model organism Saccharomyces cerevisiae, and some loss-of-functions in either RNAi or CRISPRs have been associated with the gain of beneficial foreign genetic elements [14–16]. It has been proposed that some bacteria evolved to adapt CRISPR machinery to regulate self protein-coding gene expression [17]. Similarly, at least once and possibly multiple times, eukaryotic lineages have evolved to use components of the RNAi machinery to regulate self protein-coding gene expression via a class of small RNAs called microRNAs (miRNAs) [18].

miRNAs are small, approximately 22 nt RNAs that typically silence gene expression by directing repressive protein complexes to the 3’ untranslated region (UTR) of target messenger RNA (mRNA) transcripts. The first miRNAs were discovered in C.
Drosophila

Drosha, along with its binding partner DGCR8 (Pasha in miRNAs) are liberated from the larger pri-miRNA via the loop hairpin structures. In mammals, the precursor miRNAs (pre-miRNAs) are transcribed by RNA polymerase II (pol II) (Figure 1). Pri-miRNAs contain at least one, but often several, precursor(s) of imperfectly complementary stem-loop hairpin structures. In mammals, the precursor miRNAs (pre-miRNAs) are liberated from the larger pri-miRNA via the RNAseIII-like endonuclease Drosa (also known as Drosha), comprise the Microprocessor complex that binds to the pri-miRNA, where multiple structural cuses position cleavage towards the base of the hairpin stem. The newly liberated ~60 nt hairpin pre-miRNA is then exported from the nucleus to the cytoplasm via the RAN-GTPase Exportin 5. Once in the cytosol, the pre-miRNAs are cleaved by the RNase III-like endonuclease Dicer. Dicer-mediated cleavage produces a transient ~22 nt duplex RNA, of which one strand (the miRNA or “guide” strand) is stably incorporated into the RNA-induced silencing complex (RISC). The other strand, called the “star” [*] or “passenger” strand, is less likely to associate with RISC and consequently is typically found at several-fold lower steady state levels. RISC is a multiprotein complex of which a key component is an Argonaute (Ago) protein. Ago-loaded miRNAs (miRISC) typically bind to target transcripts and repress gene expression. However, notable exceptions including translational activation under stress conditions and modulation of hepatitis C virus (HCV) replication have been reported [24,25].

Mounting evidence suggests that miRISC functions by inducing an initial blockade to translation followed by enhanced turnover of repressed target transcripts [26,27]. Because there are potentially hundreds of miRNAs of biological relevance in any given cell type, each with the potential to regulate many (some studies suggest >100) target transcripts, a model has emerged portraying a complex web of posttranscriptional regulation comprised of numerous interconnected miRNAs and targets (reviewed in [28]). In this model, miRISC complexes loaded with different miRNAs form the “nodes” of the web, with each miRNA regulating numerous transcripts. Conversely, each transcript is capable of being regulated in an additive fashion by different miRISC complexes. Thus, even though a typical miRNA may impart only a modest effect on any single target, the sum total of transcript regulation conveyed by a particular miRNA can combine for significant phenotypic consequences. On the other hand, data exist that for some miRNAs, only a minority of miRISC-miRNA target interactions are of biological importance (reviewed in [29]). Additionally, gene knockout studies in animals demonstrate that numerous miRNAs are not essential for viability [30,31]. This suggests that some miRNAs serve a primary role as subtle regulators to “fine tune” or “balance” levels of gene expression. Consequently, some miRNAs are only essential during stress, serving as key mediators of homeostasis. Therefore, host miRNAs display a spectrum of gene regulatory activities with phenotypic consequences ranging from subtle to profound, and it may be expected that virus-encoded miRNAs will behave the same.

**Virus-Encoded miRNAs**

**Which Types of Viruses Encode miRNAs?**

RNAi likely arose as a primary defense against harmful genetic elements such as viruses, yet in an interesting evolutionary twist, divergent viruses co-opted miRNA expression for pro-viral purposes. DNA viruses account for the majority of known virus-encoded miRNAs with the herpesvirus family encoding most known viral miRNAs. Herpesviruses dominate both in terms of absolute number of known virus-encoded miRNAs and in the average number of miRNAs encoded per virus (typically >10/ genome). Herpesviruses comprise an extended family of large genome DNA viruses whose defining trait is the ability to undergo long-term, and often life-long, latent infections. Latency is a specialized type of persistent infection where only a few viral gene products are expressed allowing for efficient evasion of the immune response. Latency is fully reversible, and with appropriate cues, the virus initiates the lytic mode of infection comprising full viral gene expression and culminating in the production of infectious virus and lysis of the host cell [32]. In this regard, the dual infectious modes of herpesviruses (latent versus lytic) can be thought of as a very simple two-stage model—similar to cell type differentiation that occurs in eukaryotic organisms during development. Both processes integrate extracellular events and subsequent signal transduction. Both ultimately depend on sometimes subtle differences in gene expression that are regulated by transcriptional and/or posttranscriptional mechanisms (Figure 2).

Several aspects of the herpesvirus life cycle are instructive for understanding the other virus families that encode miRNAs. miRNAs are likely invisible to the adaptive immune response—a valuable trait for viruses that undergo persistent infection [7]. Given the often subtle nature of miRNA-mediated regulation, it is likely that most virus-encoded miRNAs may have a diminished role during lytic infection where robust changes in host and viral gene expression dominate even though viral miRNAs are typically detectable at these times. For the most part, natural viruses that encode miRNAs have a DNA component to their replication cycle, replicate in the nucleus where they have full access to the initiating host miRNA biogenesis machinery, and undergo long-term persistent infections. These include viruses with DNA genomes (The Herpesvirus, Polyomavirus, Ascovirus, Baculovirus, Iridovirus, and Adenovirus families) and at least one member of the retrovirus family, bovine leukemia virus (BLV) (Table 1).

Naturally occurring positive or negative sense RNA or dsRNA genome viruses that express miRNAs are not widely accepted. In fact, until recently, it had been speculated that viruses with RNA genomes would not encode miRNAs due to negative effects on fitness that would be incurred with cis cleavage of the genome, antigenome, or miRNAs mediated by the miRNA processing machinery [33,34]. Retroviruses package an RNA genome into the capsid but also contain a DNA stage in their infectious cycle where the reverse-transcribed provirus genome integrates into host DNA. It has been reported that HIV may encode miRNAs, but this is not widely accepted due to low abundance, lack of evolutionary conservation amongst strains, unknown biological relevance, and the discordance of results amongst different labs [35–38]. BLV, however, clearly encodes numerous miRNAs [39]. Interestingly, BLV avoids Drosha-mediated cleavage of its genome and miRNAs, which overlap the miRNA cluster portion of the genome. This occurs because, unlike most known miRNAs, BLV miRNAs are encoded as shorter RNA polymerase III (pol III) transcribed hairpins that can directly serve as Dicer substrates. As a result, BLV transcripts are not cleaved by Drosha, and only subgenomic small RNAs are processed into miRNAs. Thus, at
least one retrovirus encodes miRNAs. Combined with recent reports of laboratory engineered RNA viruses that successfully express miRNA-like RNAs [40–43], it seems likely that additional virus-encoded miRNAs await discovery.

As mentioned above, the viruses most likely to encode miRNAs will have nuclear and DNA components to their lifecycle and have the ability to establish persistent infections. However, it’s clear that not all viruses that meet these criteria encode miRNAs. As least one type of human papillomavirus (HPV, small dsDNA genome viruses some of which are associated with human tumorigenesis) does not encode miRNAs [44]. We note that these findings do not exclude the possibility that other PVs may encode miRNAs. In fact, one study claims that HPV-18 encodes a miRNA but lacks solid proof demonstrating the involvement of the miRNA biogenesis or effector machinery [45]. The preponderance of evidence suggests that at least some PVs, and perhaps many if not all others, do not encode miRNAs. Similarly, although most herpesviruses that have been examined in-depth encode miRNAs, it appears that Varicella Zoster Virus (VZV), the etiologic agent of chicken pox and shingles, does not [46]. This finding is particularly interesting given that other human neurotropic herpesviruses (HSV1 & 2) and, furthermore, other animal Varicelloviruses including Bovine Herpesvirus 1 and Suid Herpesvirus 1 do encode miRNAs [47,48]. This raises the question as to what is different between the VZV and other herpesviruses lifecycles that determines miRNA utilization. As more small RNA sequencing studies are performed, understanding which viruses do and do not encode miRNAs will be informative to the overarching goal of understanding virus miRNA function.

**Virus-Encoded miRNA Functions**

Virus-encoded miRNAs can be grouped into two classes: those that are analogs of host miRNAs and those that are viral specific. Similar to some virus-encoded regulatory proteins, a subset of viral
miRNAs have evolved to mimic host effectors. Viral miRNAs that mimic host effectors are referred to as “analogs.” The 5’ end of a miRNA (~nucleotides 2–8), called the “seed” region, plays an especially important role in directing RISC to mRNA targets. It is estimated that ~60% of regulation by a particular miRNA is due to binding with perfect seed complementary to the target transcript [49]. A fraction of virus-encoded miRNAs share seeds with host miRNAs and at least three viruses: Kaposi’s Sarcoma-associated Herpesvirus (KSHV), Marek’s Disease Virus 1 (MDV1), and BLV have been shown to negatively regulate transcripts via the same target docking sites as their counterpart host miRNAs [28,39]. Mimicking a host miRNA allows a viral miRNA to potentially regulate hundreds of transcripts that have evolved target sites for a particular host miRNA. Presumably such regulatory networks evolve to effect specific functions, for example inhibiting apoptosis. Our estimates suggest ~26% of currently annotated human virus-encoded miRNAs could mimic host miRNAs by possessing identical seed sequences (Figure 3A). However, this likely represents a gross overestimate because it includes star strands for both host and viral miRNAs. Furthermore, this estimate is based on hexameric seeds, whereas heptameric seeds are better predictors of shared targets [50]. Performing the same analysis using heptameric seeds further reduces the overlap of host and viral miRNA seeds to ~15%. Similar results are obtained in other systems including viruses with rodent and avian hosts (Figure 3A). Additionally, based on low abundance, untested biogenesis, and unknown functional relevance, it’s unclear whether all of the currently annotated viral or host miRNAs are bona fide miRNAs, underscoring that some seed matches between host and viral miRNAs arise by chance [28]. Therefore, it seems likely that only a minority of virus-encoded miRNAs truly mimic host miRNAs.

Although more than 250 virus-encoded miRNAs are known [51], an in-depth functional understanding is lacking for most. Part of this stems from the fact that these miRNAs were only relatively recently discovered. Additionally, there is a lack of easily accessible animal models for some viruses that encode miRNAs, and most viral miRNAs encoded by human viruses are not conserved in...
nonprimate animal virus models. Based on what is currently known about virus-encoded miRNAs (reviewed in-depth in [28]), we propose the hypothesis that, despite often being detectable during lytic infection, most will function to foster persistent/latent infections. Accordingly, most functions ascribed to virus-encoded miRNAs can be grouped in the following categories: (1) prolonging longevity of infected cells, (2) evading the immune response, and (3) regulating host or viral genes to limit the lytic cycle.

### Prolonging Longevity of Infected Cells

Preventing cell death seems like an obvious advantage to viruses that take up persistent or latent infections in long-lived cells. Host miRNAs play a major role in maintaining cellular homeostasis, and not surprisingly, numerous host miRNAs are implicated in the regulation of cell death (reviewed in [84]). Several different viruses including KSHV, Epstein Barr virus (EBV), and MDV1 encode miRNAs that can play a subtle role in preventing apoptosis by

| Virus Family or Subfamily | Virus Species | Pre-miR Hairpins | Mature miRs | Proposed Functions Highlighted in this Review |
|---------------------------|---------------|------------------|-------------|------------------------------------------------|
| **Alpha-herpesvirinae**    | Herpes Simplex Virus 1 | 16 | 25 | Prolonging longevity of infected cells [57], host miR-155 mimic [28,67] |
|                           | Herpes Simplex Virus 2 | 18 | 24 | |
|                           | Herpes B virus | >3* | >3* | |
|                           | Herpesvirus of turkeys | 17 | 28 | |
|                           | Infectious laryngotracheitis virus | >7* | >10* | |
|                           | Bovine herpesvirus 1 | 10 | 12 | |
|                           | Marek’s disease virus type 1 | 14 | 26 | |
|                           | Marek’s disease virus type 2 | 18 | 36 | Host miR-29 mimic |
|                           | Pseudorabies virus | 13 | 13 | |
| **Beta-herpesvirinae**     | Human cytomegalovirus | 11 | 17 | Prolonging longevity of infected cells [61] |
|                           | Mouse cytomegalovirus | 18 | 28 | Evasion of the immune response [84] |
|                           | Human herpesvirus 68 | 4 | 8 | |
| **Gamma-herpesvirinae**    | Epstein–Barr virus | 25 | 44 | Prolonging longevity of infected cells [54–56,62], host miR-29 mimic |
|                           | Rhesus lymphocryptovirus | 36 | 50 | Host miR-29 mimic |
|                           | Kaposi’s sarcoma-associated herpesvirus | 12 | 25 | Prolonging longevity of infected cells [58–60], regulating host and viral genes to limit the lytic cycle [87–91], host miR-155 mimic [68,72–74] |
|                           | Rhesus monkey rhadinovirus | 15 | 25 | |
|                           | Herpesvirus saimiri strain A11 | 3 | 6 | |
|                           | Mouse gamma herpesvirus 68 | 15 | 28 | |
| **Polyomaviridae**         | Simian virus 40 | 1 | 2 | Autoregulation of viral early genes [79] |
|                           | JC polyomavirus | 1 | 2 | Autoregulation of viral early genes [96], evasion of the immune response [83] |
|                           | BK polyomavirus | 1 | 2 | Autoregulation of viral early genes [96], evasion of the immune response [83] |
|                           | Mouse polyomavirus | 1 | 2 | Autoregulation of viral early genes [80] |
|                           | Merkel cell polyomavirus | 1 | 2 | Autoregulation of viral early genes [97] |
|                           | SA12 | 1 | 2 | Autoregulation of viral early genes [99] |
| **Retroviridae**           | Bovine leukemia virus | 5 | 8 | Host miR-29 mimic [39] |
| **Iridoviridae**           | Singapore Grouper Iridovirus | 14 | 15 | |
| **Ascoviridae**            | Heliothis virescens ascovirus | 1 | 1 | Targets viral polymerase transcript [101] |
| **Baculoviridae**          | Bombyx mori nucleopolyhedrosis virus | 4 | 4 | |
| **Adenoviridae**           | Human adenoviruses types 2 and 5 (others likely) | 2b | 3 | |
| **Unclassified**           | Bandicoot papillomatisis carcinomatisis virus type 1 | 1 | 1 | Autoregulation of viral early genes [98] |
|                           | Bandicoot papillomatisis carcinomatisis virus type 2 | 1 | 1 | Autoregulation of viral early genes [98] |
|                           | Heliothis zea nudivirus-1 | 2 | 2 | Promotes latency-like state by inhibiting viral gene expression [86] |

*aCurrently annotated miRNAs in miRBase. Recent reports indicate these numbers to be higher [118,119].

*bNote that the Adenoviral miRNAs are derived from inefficient processing of an atypical precursor structure known as the Virus-associated RNAs (vaRNAs).

doi:10.1371/journal.ppat.1003018.t001
targeting pro-apoptotic host genes. EBV is a gamma herpesvirus associated with cellular hyperproliferative disorders such as infectious mononucleosis as well as B cell and solid cell tumors (reviewed in [53]). The EBV-encoded miRNA miR-BART5 targets the transcript of the pro-apoptotic host gene PUMA [54], and members of the EBV BART miRNA cluster also target transcripts of the pro-apoptotic gene Bim [55]. Furthermore, the EBV BHRF1 miRNAs have been implicated in preventing apoptosis during infection of cultured primary B cells [56]. MDV1-encoded miRNA miR-M3 targets the transcript of host gene Smad2 and has been shown to reduce drug-induced apoptosis in cell culture [57]. Interestingly, for some viruses such as KSHV, different viral miRNAs can target independent host transcripts preventing early initiating apoptotic events such as the cytokine signaling receptor TWEAKR as well as late apoptotic effectors such as caspase 3 [58,59]. It is also noteworthy that at least three different human herpesviruses (human cytomegalovirus [HCMV], EBV, and KSHV) have been shown to encode miRNAs that target host pro-apoptotic gene BclAF1. These viral miRNAs utilize different miRNA target sites, which may imply that BclAF1 is an important effector in the life cycle of diverse herpesviruses and viral miRNAs may converge on similar targets without reliance on conserved target sites [60–62]. Alternatively, it remains possible that since BclAF1 has an atypically long 3’ UTR (>4 Kb), it may be a member of a class of hypothetical transcripts that due to abundance or composition of UTRs are hyper-prone to miRNA-mediated regulation. Although the in vivo relevance remains to be determined, some viral miRNAs likely serve to evade cell death.

Several viruses known to encode miRNAs, including herpesviruses KSHV, EBV, MDV1, and the polyomavirus Merkel Cell Carcinoma Polyomavirus (MCPyV), are associated with tumorigenesis. Inducing tumors is likely not a primary advantage for these viruses but rather an accidental off consequence of the need to alter the cell cycle, prevent cell death, and avoid the immune response [63]. EBV, the etiologic agent of various human tumors, encodes miRNAs that have been implicated in cell culture models of transformation [64–66]. In addition, two important in vivo studies have demonstrated a role for MDV1 and KSHV miRNAs in tumorigenesis [67,68]. Both MDV1, an alpha herpesvirus of chickens, and KSHV, a lymphotropic gamma herpesvirus of humans, are associated with tumors. MDV1 causes T-cell lymphomas and KSHV is associated with a subset of primary effusion lymphomas and Kapoisi’s Sarcoma (KS). Amazingly, both viruses encode miRNAs that function as analogs of the host miRNA miR-155. Misexpression of miR-155 alters lymphopoiesis and plays a role in tumorigenesis (reviewed in [69–71]). Infection of chickens with a mutant version of MDV1 that does not express miR-155 results in loss of oncogenicity in most subjects [67]. Several studies have combined to show that the KSHV analog of miR-155 (miR-K12-11) shares overlapping targets with the host miRNA [72–74]. Recently, in an orthotopic humanized mouse model, exogenous expression of KSHV miR-K12-11 was shown to be sufficient to drive hyperproliferation of B cells [68].

We have shown that one of the BLV miRNAs functions as an analog of the host miRNA miR-29. miR-29 has been shown to function as either an oncogene or a tumor suppressor depending on the context [75]. miR-29 is overexpressed in human chronic lymphocytic leukemias (CLLS), which bear a striking phenotypic resemblance to BLV-associated tumors in cattle [76]. When miR-
29 is experimentally overexpressed in B cells, mice develop B cell tumors that strongly resemble CLL [77]. Exactly how BLV causes tumors has remained enigmatic since most BLV tumor cells do not express abundant viral pol II transcripts or proteins. The identification of a BLV pol III–derived potential oncomiR supports a model for miRNAs in BLV-mediated tumorigenesis, but this speculation awaits confirmation in vivo. Also worth noting is that at least three other lymphotropic viruses, EBV (miR-BART1-3p), RLCV (Rhesus Lymphocryptovirus, miR-rL1-6-3p), and MDV2 (Marek’s Disease Virus 2, miR-M21), also encode miRNAs with miR-29 seeds. Thus, a picture is emerging whereby miRNAs encoded by tumor viruses can contribute to increased cell survival and tumorigenesis.

Evading the Immune Response

Akin to nonstructural viral proteins that often function to evade the immune response, it seems likely that some viral miRNAs perform a similar role. Theoretically, miRNAs can contribute to immune evasion indirectly by lowering viral protein levels and consequent antigenicity, or directly by suppressing components of the host immune response [78]. Simian Vacuolating Virus 40 (SV40), a prototypic polyomavirus with a circular genome possessing opposing transcriptional units for the early and late genes, encodes a miRNA that is perfectly complementary to the early viral transcripts. The SV40 miRNA directs cleavage of the early viral transcripts and results in reduced early viral gene expression at late times of lytic infection [79]. When SV40-infected cells are co-cultured with cytotoxic T cells (CTLs), more CTL-mediated lysis is observed in cells infected with a miRNA mutant virus. This suggests a possible role for the SV40 miRNA in evading the adaptive immune response in vivo. Murine polyomavirus (muPyV) also encodes a miRNA that negatively regulates early gene expression in a manner similar to SV40 [80]. However, infection of mice with a muPyV miRNA mutant virus does not support a robust role for the miRNA in evading the adaptive immune response as little difference in CTL response is observed [80]. Although SV40 and muPyV are different viruses, they share numerous similarities in infectious cycles and autoregulatory activity of their respective miRNAs. These results suggest that the underlying purpose of Polyomaviridae miRNA-mediated autoregulation remains to be determined and that caution is warranted when interpreting the in vivo relevance of cell culture experiments. miRNAs from several different human herpesviruses and the star strand derivative of the human polyomavirus JC (JCV) have been implicated in co-culture experiments in evading the Natural Killer (NK) cell innate immune response [81–83]. While these findings await confirmation in vivo, they do suggest a possible shared function in evading NK cells via miRNAs encoded by very different kinds of viruses. To date, the only in vivo evidence providing a link between miRNAs and immune evasion are from studies conducted on murine cytomegalovirus (MCMV). Deletion of two MCMV miRNAs results in reduced titers in the salivary glands of specific genetic backgrounds of mice [84]. The phenotypic, which suggests that these miRNAs function to promote persistent infection, was reverted in mice that were defective in both the adaptive and innate arms of the immune response through the depletion of NK cells and CD4+ T-cells. Taken together, these observations support the hypothesis that some virus-encoded miRNAs serve a subtle role in evading the immune response.

Regulating Host or Viral Genes to Limit the Lytic Cycle

The restricted gene expression of latency and some other forms of persistent infection represents a successful immune evasion strategy. In addition to encoding direct modulators of the immune response, latently infected cells evade the immune response by expressing a limited number of proteins, providing for reduced antigenicity. Several different herpesviruses encode miRNAs that have been implicated in maintaining latent infection and altering the balance between latency/lytic infection [55]. HSV1, KSHV, and HCMV all encode miRNAs that have been reported to subtly regulate either viral and/or host genes that could promote latency/persistent infection (reviewed in-depth [28], discussed briefly below). A recent study demonstrates that miRNA-mediated promotion of latency is not restricted to herpesviruses as Heliothis zea nudivirus-1 (HzNV-1), a large DNA genome insect virus, encodes miRNAs that promote a latency-like state by directly inhibiting viral gene expression [86].

Studies of the KSHV latency system provide some of the most well-documented examples of viral miRNAs regulating the latent/lytic switch (reviewed in-depth in [28]). It should be noted, however, that the effects of individual KSHV miRNAs in this process are invariably subtle. The KSHV encoded miRNAs miR-K12-9-5p and miR-K12-7-5p have been shown to directly regulate the transcript of the master lytic switch protein (RTA) [87,88]. Several KSHV-encoded miRNAs also target host transcripts that result in enhanced latency [89–91]. For example, the KSHV-encoded miRNA, miR-K12-1-5p, directly targets the transcript of host gene IkBz, which modulates the NF-kB pathway and reduces lytic activation [99]. Additionally, miR-K12-3-5p directly targets the transcript of host transcription factor NFIB, which has been shown to be an activator of the RTA promoter [91]. Importantly, a KSHV deletion mutant that removes most of the viral miRNAs or knockdown of KSHV miRNA function results in increased lytic activity [89,91]. As mentioned above, although not as well studied as KSHV, it is likely that virus-encoded miRNAs from some other herpes viruses also regulate entry into or the degree of lytic infection.

Large-scale efforts to identify transcripts directly targeted by herpesvirus miRNAs have implied that at least some putative viral lytic replication-inducing targets are not detectable in association with RISC [62,92,93]. This may suggest that these putative targets are not biologically relevant. On the other hand, the lack of sensitivity of these target identification methods likely would have missed very low abundance “leaky” transcripts. As low abundance lytic-promoting transcripts may be sufficient to initiate feed-forward lytic-inducing loops, a role for viral miRNAs in promoting latency cannot be ruled out from these negative target profiling studies. Indeed, host miRNAs generally display an inverse cell type expression profile with their targets, suggesting that a major role of miRNAs is to enforce homeostasis when inappropriate low-level “leaky” transcripts are expressed [94,95]. By analogy, enforcing latency against low-level gene expression noise could very well also apply to virus-encoded miRNAs and the simple developmental state of latency (Figure 2).

What about those viruses without a well-defined latency? Polyomaviruses generally take up life-long persistent infections in their hosts but the mechanisms that allow for this are not well understood. We have shown that several polyoma and polyoma-like viral miRNAs regulate or have the capacity to regulate early gene expression during late times of lytic infection [79,96–99]. Although regulation during lytic infection could be the main function of these miRNAs, it is also possible that, similar to the herpesviruses and HzNV-1, polyomaviral miRNAs may play a role in tilting the balance between persistent and lytic infection. Similarly, HCMV miRNAs have not been studied in a latent context, but it could be predicted based on known viral targets of HCMV [100] and the limited understanding of MCMV miRNA
function in vivo [84] that some HCMV miRNAs will play a role in maintaining or establishing latency/persistence. The DNA insect virus Heliothis virescens ascovirus (HvAV) encodes a miRNA that targets its own viral polymerase [101]. Although HvAV infection results in death in some insect hosts, it has recently been shown that other insect hosts may undergo an attenuated infection and thus serve as possible reservoirs for persistent infection [102]. Therefore, it is possible that the HvAV miRNA could promote persistent infection in some contexts. Testing a role for the polyomaviral, HCMV, and ascoviral miRNAs in promoting persistence will likely require developing sensitive in vivo assays.

Noncanonical miRNA Functions

Finally, for virus-encoded miRNAs, especially those that are not analogs of host miRNAs, all possible functions must be considered. Several viral miRNAs are found clustered in genomic regions near origins of replication or antisense to ncRNAs. It is possible that completely novel functions in genome replication or regulation of ncRNAs await discovery for viral and host miRNAs. In addition to canonical miRNA repressive activities on trans targets, the biogenesis of some miRNAs can convey cis regulation [103]. Several viruses encode pre-miRNAs embedded in cis within viral mRNA transcripts (Figure 4A). Pre-miRNAs from KSIV and EBV have been shown to function as negative cis regulators of viral protein expression albeit via different mechanisms. In KSIV two pre-miRNAs embedded within the Kaposin B (KapB) 3′UTR are cleaved by the Microprocessor complex resulting in decreased KapB protein expression in latency [104]. Interestingly, this repression is partially alleviated during lytic replication implicating a posttranscriptional mechanism for differential control of viral gene expression in latent versus lytic infection. The EBV BHRF1 transcript is also regulated by cis pre-miRNA elements, but in this situation, docking of the Microprocessor complex combined with an alternative transcription initiation site is hypothesized to promote an altered splicing pattern of this mRNA to a form that is subject to reduced translational efficiency [105]. Thus, although via different mechanisms, the net result is similar to KapB with less BHRF1 protein being produced specifically during latency. As viruses often serve as divining rods pointing towards new host activities, such seemingly atypical activities of viral miRNAs likely apply to some host transcripts as well.

The Future

Currently, specific challenges and goals of the virus-encoded miRNA field overlap extensively with the broader parent fields of both virology and RNAs. Similar to host miRNAs, it will be imperative to determine which of the reported viral miRNAs possess biologically relevant activities. Several reported viral miRNAs are expressed at low levels compared to the other host and viral miRNAs. Are these miRNAs expressed at higher levels in other contexts, or do they possibly function at lower levels by a currently unknown mechanism? With notable exceptions, there is a striking lack of evolutionary conservation of most viral miRNAs. This could imply that viral miRNAs are a site of rapid evolution, perhaps even a driver of speciation. To help better understand which viral miRNAs are most relevant, it would be useful to have a deeper survey of the viruses that encode miRNAs. Perhaps even more informative will be to understand why some members of the same virus subfamilies do and do not encode miRNAs (e.g., HTLV in the Delta Retroviridae or VZV in the Varicello Herpesviridae [36,46]). Of course, the overarching goal of the field is to parlay the survey of bona fide miRNAs into an understanding of their function.

In terms of understanding viral miRNA function, two classes emerge: those that mimic host miRNAs (analogs) and those that are viral specific (Figure 3). Mimicking host miRNAs provides obvious benefits to a virus by allowing it to access a pre-existing target network of numerous host transcripts that may have been selected for a particular functional outcome (e.g., prevention of apoptosis or evasion of immune signaling). In this regard, it seems curious that more examples of viral miRNA analogs of host miRNA do not exist as our estimates suggest that the majority of viral miRNAs do not share seed identity with bona fide host miRNAs (Figure 3A). Despite this, several recent high throughput target identification studies demonstrate that numerous host transcripts are directly bound by nonanalog viral miRNAs [62,92,106]. This raises several questions. How are these miRNAs targeting so many transcripts, and which of these interactions are biologically relevant? Certainly, some of these miRNAs may be tapping into existing miRNA target sites or other trans factor docking site networks by unknown mechanisms. For other nonanalog viral miRNAs, it seems unlikely that all identified targets are advantageous, especially given that introduction of some nonnatural siRNAs into cells will also redirect RISC to a similarly large number of unintended targets [107]. More likely, on an individual basis, some reported viral miRNA targets could be neutral or even provide a negative fitness cost to the virus as long as the sum total of negatively regulated targets remains of overall advantage to the virus. A challenge for the field will be extracting from these very valuable lists of viral miRNA-RISC-bound targets those that are the most functionally relevant.

Another mystery that applies to animal host and viral miRNAs is the observation that most lack perfect complementarity to their target transcripts. It has been hypothesized that miRNAs evolved independently in plants and animals [18]. In plants, for unknown reasons, most of the known miRNA targets are bound with perfect complementarity, resulting in siRNA-like RISC-mediated cleavage of the target transcripts. Some exceptional viral miRNAs do bind with perfect complementarity and direct cleavage of their targets, but this is uncommon and restricted to transcripts that lie antisense to the miRNA as opposed to cleaving host targets (Figure 4B). The reason why miRNA-mediated cleavage is not employed more often by viral or animal host miRNAs is unknown. Clearly, mammalian RISC can be programmed to direct siRNA-like cleavage of mRNA transcripts in a laboratory setting. It is possible that siRNAs work in mammalian cells because they access a vestigial remnant from when RNAi played a predominant antiviral role. Even so, this model would predict that at least some viral miRNAs would have evolved to utilize perfectly complementary miRNAs to more robustly eliminate undesirable host transcripts. On the other hand, it could be that Ago-mediated RNA cleavage is somehow disadvantageous to the virus or host cells. For example, Ago-mediated cleavage could signal a damage response. In this regard, it is interesting to note that siRNAs perfectly complementary to some transposons have been cloned in mammalian cells and various components of the RNAi machinery are linked to sensing and suppressing transposon activity [11,12,108,109].

Finally, a major reason for studying virus-encoded miRNAs is to be able to better develop therapeutic interventions. One of the most exciting stories in the world of viruses and miRNAs comes not from a virus-encoded miRNA but rather a host miRNA, miR-122 [25]. miR-122 is essential for maximal replication of HCV, and strategies to hinder HCV replication based on blocking this miRNA are already showing promise in both in vivo models and preliminary clinical studies [110]. These studies inspire hope that targeting virus-encoded miRNAs may also be clinically viable. As
is true for all miRNA therapeutic applications, the main hurdle will be delivery of inhibitors or mimics to the appropriate tissues. If this hurdle can be surmounted, it is then plausible that blocking viral miRNAs could be used as a strategy to purge the latent reservoir—something of a “holy grail” in the herpesvirus field. Determining if such strategies are possible and uncovering which miRNAs are the most promising targets will require us to greatly advance our current understanding of viral miRNA functions. These could include canonical as well as noncanonical miRNA functions (for example, regulating origins of replication or modifying chromatin). Ultimately, a true understanding of viral miRNA function will require additional animal studies and likely the development of new relevant animal models for persistent infections. With the development of these new tools and models, viruses will continue to provide insights into host miRNA pathways and reveal new targets and functions of interest and possibly clinical relevance.

Notes

While this manuscript was in revision, three additional papers documenting functions of KSHV miRNAs were published [111–113]. These papers further support the model that viral miRNAs contribute to cell longevity and modulation of the immune response. In addition, a paper documenting the Microprocessor complex in regulation of RNA pol II transcription through binding pri-miRNA-like sequences was also published [114]. This paper provides an additional example of a noncanonical cis-regulation mediated by components of the miRNA pathway.
References

1. Ulltsey I, Shukmatava A, Jan CH, Sive H, Bartel DP (2011) Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. Cell 147: 1537–1550.

2. Wang KC, Chang HY (2011) Molecular mechanisms of long noncoding RNAs. Mol Cell 43: 904–914.

3. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, et al. (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391: 806–811.

4. Buchon N, Vaury C (2006) RNAi: a defensive RNA-silencing against viruses and transposable elements. Heredity 96: 195–202.

5. Obbard DJ, Gordon KJH, Buck AH, Jiggins FM (2009) The evolution of RNAi as a defence against viruses and transposable elements. Philos Trans R Soc Lond B Biol Sci 364: 99–115.

6. Rij RP van, Berezikov E (2009) Small RNAs and the control of transposons and viruses in Drosophila. Trends Microbiol 17: 163–171.

7. Cullen BR (2006) Viruses and microRNAs. Nat Genet 38: S25–S30.

8. Umbach JL, Cullen BR (2009) The role of RNAi and microRNAs in animal virus replication and antiviral immunity. Genes Dev 23: 1151–1164.

9. Jeang K-T (2012) RNAi in the regulation of mammalian viral infections. BMC Biology 10: 58.

10. Yang SK, Kazazian HH (2006) L1 retrotransposition is suppressed by evolutionarily conserved small interfering RNAs in human cultured cells. Nat Struct Mol Biol 13: 763–771.

11. Kaur N, Druil H, Saraf T, Tarallo V, Gelfand BD, Powder J, et al. (2011) Dicer1 defect induces Alu RNA toxicity in age-related macular degeneration. Nature 471: 325–330.

12. Tarallo V, Hireno Y, Gelfand BD, Skeru N, et al. (2012) Dicer1 loss and Alu RNA induce age-related macular degeneration via the NLRP3 inflammasome and MyD88. Cell 149: 847–859.

13. Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, et al. (2011) CRISPR-Dicer interaction generates natural double-stranded RNA and mediates adaptive immunity. Nature 471: 602–607.

14. Palmer KL, Shkumatava A, Jan CH, Sive H, Bartel DP (2011) Conserved microRNA evolution and loss of CRISPRs following a host shift in a novel wildlife alphaherpesvirus. PLoS ONE 6: e11519–11524.

15. Lin J, Cullen BR (2007) Analysis of the interaction of primates with microRNA expression in latent HIV infection. Trends Pharmacol Sci 32: 675–681.

16. Varble A, Chua MA, Perez JT, Manicassamy B, Garcia-Sastre A, et al. (2010) Engineered RNA viral synthesis of microRNAs. Proc Natl Acad Sci USA 107: 11519–11524.

17. Cullen BR, Moore MS, Johnson WK, Garrett-Engele P, Lim LP, et al. (2007) The microRNA-cloning vector miR-neo. Mol Cell 25: 267–276.

18. Miska EA, Alvarez-Saavedra E, Abbott AL, Lau NC, Hellman AB, et al. (2007) Most Caenorhabditis elegans microRNAs are individually not essential for viability. Mol Cell 28: 439–449.

19. Lin J, Cullen BR (2007) The integration of primate retroviruses with human RNA interference machinery. J Virol 81: 12218–12226.

20. Zhang M, Cheng J, Sun Z, Zhao J, Wang J, et al. (2012) Novel microRNAs that mimic a B-cell oncomiR. Proc Natl Acad Sci USA 109: 3077–3082.

21. Varble A, Chua MA, Perez JT, Manicassamy B, Garcia-Sastre A, et al. (2010) Engineered RNA viral synthesis of microRNAs. Proc Natl Acad Sci USA 107: 11519–11524.

22. Lee RC, Ambros V (2001) An extensive class of small RNAs in C. elegans. Cell 105: 861–874.

23. Slezak-Prochazka I, Durmus S, Kroesen B-J, Van Den Berg A (2010) Identification of microRNAs from a cytoplasmic RNA virus. Nucleic Acids Res 38: 8329–8337.

24. Vasudevan S, Tong Y, Steitz JA (2007) Switching from repression to activation: Micro RNAs can up-regulate translation. Science 318: 1931–1934.

25. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P (2005) Modulation of picornavirus 2C expression by microRNA. Science 310: 1116–1119.

26. Grimson A, Farh KK-H, Johnston WK, Garrett-Engele P, Lim LP, et al. (2007) MicroRNA targeting specificity in mammalian determinants beyond seed sequence. Mol Cell 27: 91–105.

27. Zhang X, Liu B, Cai Z, Chen J, Wang J, et al. (2012) MicroRNA-200b regulates TWEAKR expression. J Biol Chem 287: 20225–20234.

28. Grundhoff A, Sullivan CS (2011) Virus-encoded microRNAs. Virology 411: 252–263.

29. Mendell JT, Olson EN (2012) MicroRNAs of Epstein-Barr Virus promote cell cycle progression and prevent apoptosis of primary human B cells. PLoS Pathog 6: e1000783. doi:10.1371/journal.ppat.1000783

30. Miska EA, Alvarez-Saavedra E, Abbott AL, Lau NC, Hellman AB, et al. (2007) Most Caenorhabditis elegans microRNAs are individually not essential for viability. Mol Cell 25: 267–276.

31. Xu S, Cao Y, Cui Z, Bi Y, Gao T, Zhang J, et al. (2012) Novel microRNAs that mimic a B-cell oncomiR. Proc Natl Acad Sci USA 109: 3077–3082.

32. Sun Z, Moore MS, Lau NC, Hellman AB, et al. (2007) Most Caenorhabditis elegans microRNAs are individually not essential for viability. Mol Cell 25: 267–276.

33. Manicassamy B, Garcia-Sastre A, et al. (2010) Engineered RNA viral synthesis of microRNAs. Proc Natl Acad Sci USA 107: 11519–11524.

34. Langets RA, Shapiro JS, Pham AM, tenOever BR (2012) In vivo delivery of synthetic microRNA expression vectors to target viral replication. J Virol 86: 10980–10989.

35. Kozomara A, Griffiths-Jones S (2010) miRBase: integrating microRNA annotation and deep-sequence data. Nucleic Acids Res 39: D1152–D1157.

36. Lin J, Cullen BR (2007) Analysis of the interaction of primates with microRNA expression in latent HIV infection. J Virol 81: 10677–10683.

37. Glazov E, Horwood PF, Asavalapaksakul W, Kongwanguk M, Mitchell RW, et al. (2010) Characterization of microRNAs encoded by the bovine herpesvirus 1 genome. J Gen Virol 91: 32–41.

38. Guo W, An J, Ye P, Zhao K-N, Antonsson A (2011) Prediction of conserved microRNAs from skin and mucosal human papillomaviruses. Arch Virol 156: 1161–1171.

39. Cordingley JL, Patel MA, Cole RJ, Gilden DH, Cullen BR (2009) Analysis of human alphaherpesvirus microRNA expression in latently infected human trigeminal ganglia. J Virol 83: 10677–10683.

40. Anselmo A, Kier L, Jaffrezic R, Rastigiano T, Cecere M, et al. (2011) Coexpression of host and viral microRNAs in porcine dendritic cells infected by the pseudorabies virus. PLoS ONE 6: e13734. doi:10.1371/journal.pone.0013734

41. Zouolou DG, Lovci MT, Hil I, Huang YY, et al. (2010) Comprehensive discovery of endogenous Argonaute binding sites in Caenorhabditis elegans. Nat Struct Mol Biol 17: 179–184.

42. Varble A, Chua MA, Perez JT, Manicassamy B, Garcia-Sastre A, et al. (2010) Engineered RNA viral synthesis of microRNAs. Proc Natl Acad Sci USA 107: 11519–11524.
82. Stern-Ginossar N, Saleh N, Goldberg MD, Prichard M, Wolf DG, et al. (2009)
81. Nachmani D, Stern-Ginossar N, Sarid R, Mandelboim O (2009) Diverse
79. Sullivan CS, Grundhoff AT, Tevethia S, Pipas JM, Ganem D (2005) SV40-
77. Santanam U, Zanesi N, Efanov A, Costinean S, Palamarchuk A, et al. (2010)
76. Gillet N, Florins A, Boxus M, Burteau C, Nigro A, et al. (2007) Mechanisms of
75. Pekarsky Y, Croce CM (2010) Is miR-29 an oncogene or tumor suppressor in
73. Skalsky RL, Samols MA, Plaisance KB, Boss IW, Riva A, et al. (2007) Kaposi's
71. Tili E, Croce CM, Michaille J-J (2009) miR-155: on the crosstalk between
68. Boss IW, Nadeau PE, Abbott JR, Yang Y, Mergia A, et al. (2011) A Kaposi's
67. Zhao Y, Xu H, Yao Y, Smith LP, Kgosana L, et al. (2011) Critical role of the
66. Marquitz AR, Mathur A, Shair KHY, Raab-Traub N (2012) Infection of
62. Riley KJ, Rabinowitz GS, Luna JM, Darnell RB, et al. (2012) EBV and human microRNAs co-target oncogenic and apoptotic viral and human genes during latency. EMBO J 31: 2207–2221.
60. Moore FS, Chang Y (2010) Why do viruses cause cancer? Highlights of the first century of human tumour virology. Nat Rev Cancer 10: 876–889.
57. Freederle R, Haar J, Bernhardt K, Linselstadt SD, Bannert H, et al. (2011) The members of an Epstein-Barr Virus microRNA cluster cooperate to transform B lymphocytes. J Virol 85: 9801–9810.
54. Freederle R, Linselstadt H, Bannert H, Lips Bencic M, et al. (2011) A viral microRNA cluster strongly potentiates the transforming properties of a human herpesvirus. PLoS Pathog 7: e1001294. doi:10.1371/journal.ppat.1001294.
50. Marquita AR, Mathur A, Shair KHY, Raab-Traub N (2012) Epstein-Barr Virus in a gastric carcinoma cell line induces anchorage independence and global changes in gene expression. Proc Natl Acad Sci USA 109: 9593–9598.
47. Zhao Y, Xu H, Yao Y, Smith LP, Kgosana L, et al. (2011) Critical role of the herpesvirus-encoded microRNA-155 ortholog in the induction of the Marek's disease lymphomas. PLoS Pathog 7: e1001305. doi:10.1371/journal.ppat.1001305.
43. Boss IW, Nadeau PE, Abbott JR, Yang Y, Mergia A, et al. (2011) A Kaposi's sarcoma-associated herpesvirus-encoded ortholog of microRNA miR-155 induces human splenic B-cell expansion in NOD/LttSeid Il2Rnull mice. J Virol 85: 9872–9886.
40. McClure LV, Sullivan CS (2008) Kaposi's sarcoma herpes virus tapis into a host microRNA regulatory network. Cell Host Microbe 3: 1–3.
37. Li J, Toney FC, Croce J, Bearnaschi E (2009) miR-155 gene: a typical multifunctional microRNA. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1792: 497–505.
34. Tili E, Croce CM, Michaille J-J (2009) miR-155: on the crosstalk between immunity and cancer. Int Rev Immunol 28: 264–284.
31. Gevrey M, Khachatrian N, Sache C, Fuentes C, Majores WH, et al. (2007) A viral microRNA functions as an orthologue of cellular miR-155. Nature 450: 1096–1099.
28. Skalsky RL, Samols MA, Plaisance KB, Boss IW, Riva A, et al. (2007) Kaposi's sarcoma-associated herpesvirus encodes an ortholog of miR-155. J Virol 81: 12836–12845.
25. Santanam U, Zanesi N, Efanov A, Costinean S, Palamarchuk A, et al. (2010) Chronic lymphomatis leukemia modeled in mouse by targeted miR-29 deficiency. Proc Natl Acad Sci USA 107: 12210–12215.
22. Sullivan CS (2008) New roles for large and small viral RNAs in evading host defenses. Nat Rev Genet 9: 505–507.
19. Sullivan CS, Grundhoff AT, Tevethia S, Pipas JM, Ganem D (2005) SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to antiviral agents. EMBO J 24: 1732–1741.
16. Sullivan CS, Sung CK, Park CD, Grundhoff A, Lukacher AE, et al. (2009) Murine polyomavirus encodes a microRNA that cleaves early RNA transcripts but is not essential for experimental virology. Virolology 387: 157–167.
13. Nakamura D, van-Ginossar N, Sarid H, Bandelhosch O (2009) Diverse herpesvirus microRNAs target the stress-induced immune ligand MICB to escape recognition by natural killer cells. Cell Host Microbe 3: 376–385.
10. Stern-Ginossar N, Saleh N, Goldfeld MD, Prichard M, Wolf DG, et al. (2009) Analysis of human cytomegalovirus-encoded microRNA activity during infection. J Virol 83: 10684–10693.
7. Bauman Y, Nachmani D, Vitenstein A, Tsukerman P, Drayman N, et al. (2011) An identical microRNA of the human JC and BK polyomavirus integrates in human lymphocytes. J Virol 85: 9801–9810.
4. Döhlen I, Krompich A, Kothe S, Tuddenham L, Tangay M, et al. (2010) Cytomegalovirus microRNAs facilitate persistent virus infection in salivary glands. PLoS Pathog 6: e1001150. doi:10.1371/journal.ppat.1001150.
1. Murphy E, Vainich J, Robins H, Sheik T, Levine AJ (2008) Suppression of immediate-early viral gene expression by herpesvirus-coded microRNAs implicates for latency. Proc Natl Acad Sci USA 105: 5453–5458.
