microRNAs (miRNAs) represent a class of noncoding RNA species, believed to be regulating gene expression by binding to complementary sites in the 3′ UTRs of target mRNAs. They play important regulatory roles in various metabolic pathways in most eukaryotes. The recent discovery of virus encoded miRNAs suggests that viruses may be using them to regulate host and viral gene expression. Another class of closely related small interfering RNAs (siRNAs) also has been found within the HIV-1 genome and shown to be exerting a limited impact on virus reproduction. Additionally, an additional type of viral noncoding RNAs named small noncoding RNAs (sncRNAs) ranging from a few tens to a few hundred nucleotides in length, has also been identified. sncRNAs have a wide phylogenesis and high levels of expression, suggesting they may play an important roles in different species. Here we discuss...
the genomic organization, expression, conservation as well as potential function of virally encoded miRNA, siRNA, and sncRNAs.

**Keywords** miRNA; siRNA; Small noncoding RNA; Viruses

**INTRODUCTION**

MicroRNAs (miRNAs) are a class of noncoding RNAs (ncRNA). With transcripts ranging between 21–24 nt in length, miRNAs play important roles, regulating translation and processing mRNAs through base pairing with mRNA 3′-untranslated regions (3′-UTRs) (Lagos-Quintana et al. 2001). Since lin-4 and let-7 first discovered in *Caenorhabditis elegans*, several hundred miRNAs have been identified in different organisms (Lagos-Quintana et al. 2003; Lagos-Quintana et al. 2002; Lau et al. 2001; Lee and Ambros 2001; Llave, Xie et al. 2002; Sempere et al. 2003). And their substantial effect on various regulatory pathways, i.e., embryonic development, apoptosis, cell division, differentiation, death, hematopoiesis, and cancer development, is widely recognized (Alvarez-Garcia and Miska 2005; Ambros 2004).

Moreover, another class of closely related ncRNAs, namely the small interfering RNA (siRNA), has emerged. These RNAs are similar in length to miRNAs and enable the regulation of gene expression through binding to complementary sites of mRNAs, thereby causing mRNA cleavage or its translational repression (Ruvkun 2001). Endogenously encoded siRNAs involved in the silencing of translatable elements have been observed in plants and animals (Aravin et al. 2001; Llave, Kasschau et al. 2002). Recently, viruses have also been found to encode miRNAs and siRNAs and their functions in the regulation of host and viral genes have also been analyzed.

MiRNAs and endogenous siRNAs mainly differ in their biogenesis (Figure 1): miRNAs are transcribed from specific genomic loci, whereas siRNAs are generated from double-stranded RNAs (dsRNAs). In animals, miRNA genes are generally transcribed by RNA polymerase II (pol II) forming long primary transcripts (pri-miRNA) (Cai, Hagedorn, and Cullen 2004; Lee, Kim et al. 2004). The pri-miRNAs are processed by the RNase III enzyme Drosha and its cofactor DGCR8 to an approximately 60–80 nt long imperfect hairpin called the pre-miRNA (Han et al. 2004; Lee et al. 2003). This hairpin is then exported to the cytoplasm by Exportin-5, by recognition of the stem and the 3′ overhang of pre-miRNAs, with transportation being also facilitated through the action of the Ran co-factor, which act as GTPase that contribute to hydrolysis of Ran-GTP to Ran-GDP (Lund et al. 2004; Yi et al. 2003; Zeng and Cullen 2004). In the cytoplasm the pre-miRNAs are further processed by the RNase III enzyme Dicer and the cofactor TRBP, to which generation of the miRNA duplex intermediate follows (Chendrimada et al. 2004).

![Biogenesis of viruses encoded miRNA, siRNAs and sncRNAs](image-url)

*FIG. 1. Biogenesis of viruses encoded miRNA, siRNAs and sncRNAs. Viral miRNAs are transcribed by RNA polymerase II or polymerase III from viral genome in nucleus. The long transcript pri-miRNAs are processed into pre-miRNAs of about 70 nt long by Drosha and DGCR8, the pre-miRNAs are transported to cytoplasm by Exportin-5 and processed into mature miRNAs by Dicer and TRBP. miRNAs regulate gene expression through two different mechanisms: translation inhibition or mRNA cleavage. Double-stranded regions of HIV genome can also be processed by Dicer into siRNAs that have restricted effects on viral reproduction. Viral sncRNAs usually transcribed by polymerase III. '?' represent sncRNAs are transported into cytoplasm by unknown mechanism where they exert their functions.*
of specific host genes. The discovery of miRNAs and sncRNAs could offer an additional tool to viruses in the regulation of gene expression, miRNA and sncRNAs are mainly encoded by DNA Viruses.

Small noncoding RNAs (sncRNAs) are another form of short ncRNAs other than mi- and siRNAs, usually dozens to thousands nt in length. They have been identified in different species such as E. coli, Arabidopsis thaliana, Drosophila melanogaster, Macaca mulatta, and Homo sapiens (Huttenhofer et al. 2001; Marker et al. 2002; Saetrom et al. 2005; Venter et al. 2001; Yuan et al. 2003). These sncRNAs play a large number of important roles in structural, catalytic and especially regulatory role processes (Huttenhofer, Brosius, and Bächlerlee 2002; Storz, Opdyke, and Zhang 2004). Many act either as positive or negative regulators of translation or mRNA stability through complementary base pairing. Such pairing can change the structures of target RNAs by recruiting proteins to the target RNAs (Lankenau, Corces, and Simons 1994; Morfeldt et al. 1995; Wagner and Simons 1994). Some regulatory sncRNAs also interact with proteins by simulating/resembling the protein’s cellular target, hence competing with the protein binding to that target (Romeo 1998). The biological roles of other RNA species involve various aspects of metabolism, including protein secretion, tRNA processing, splicing, and rRNA biogenesis (Tollervey and Kiss 1997).

The broad phylogenies of sncRNAs and their important biological roles lead to the hypothesis that viruses, having limited coding capacity due to their small genome size, may be employing sncRNAs in order to regulate the expression of viral and host cell genes during replication. Indeed, over the last two decades many viruses have been found to carry sncRNA genes, therefore identifying viral sncRNAs would greatly expand our knowledge on the many functions that noncoding RNAs may exhibit in a cell.

**miRNA, siRNA, and sncRNAs are Mainly Encoded by DNA Viruses**

Viruses are ubiquitous and abundant in nature and infect and parasitize all living organisms. They are very simple biological entities composed of a small number of macromolecules produced from the organism they infect. Allowing for a fast and effective way to regulate gene expression, miRNA and sncRNAs could offer an additional tool to viruses in the regulation of specific host genes. The discovery of miRNAs and sncRNAs in viruses lead to a surprising discovery in the realm of gene regulation, suggesting that also such simple and primitive infectious agents may be capable of gene regulating mechanisms through the action of ncRNAs. To date, miRNA, siRNA, and other sncRNAs encoded by viruses have been classified into four families: herpesviridae, polyomaviridae, adenoviridae, and retroviridae (Table 1). The fact that all of them are either DNA viruses or retroviruses, confirmed the hypothesis that miRNAs and sncRNAs are mainly transcribed from DNA sequences and in retroviruses they use DNA as an intermediate for replication. Although some other virus families include Poxviridae and some RNA viruses such as the +ssRNA virus Yellow Fever Virus of the Flaviviridae, and the –ssRNA viruses Measles Virus of the Paramyxoviridae, which also predicted to encode miRNAs, none of them have been so far validated. It’s difficult for RNA viruses, mainly confined to the cytoplasm, to approach the Drosha in the nucleus and it is known that infection of RNA viruses commonly results in an antiviral RNA interference response and viral genome degradation in plants and invertebrates. Recent research indicates that infection with HIV-1 can also induce sequence-specific siRNA silencing (Bennasser et al. 2005). While searching for conserved regions with a 19 bp internal duplex, five putative sncRNAs in HIV-1, HIV-2, and SIV were identified. These retroviral duplexes can be processed by Dicer into functional siRNAs and exhibit virus-restricting activity in infected cells.

**Viruses Encoded miRNAs and siRNAs**

The identification of five miRNAs in the B95-8 strain of Epstein-Barr virus (EBV), which belongs to the γ-Herpesvirus family, was the first proof supporting the existence of virus encoded miRNAs (Pfeffer et al. 2004). In other EBV strains, a total of 18 novel pre-miRNAs has been identified within a genomic region deleted from a B95-8 strain (Cai et al. 2004; Cui et al. 2006; Gupta et al. 2006). Herpesviruses can be classified into three subfamilies (α, β and γ) based on their genomic structure and viral biology. To date, all three Herpesvirus subfamilies have been found to encode miRNAs and sncRNAs. Three research groups have found a total of 31 distinct miRNAs in the Herpes simplex viruses type 1 (HSV-1) and 18 distinct miRNAs in HSV-2, all belonging to the α-Herpesvirus subfamily (Cui et al. 2006; Gupta et al. 2006). Marek’s disease virus (MDV-1, an oncogenic α-Herpesvirus in chicken) and MDV-2 encoded miRNAs have also been identified, however, no sequences similarities were found between them (Burnside et al. 2006; Venter et al. 2001). Cloning and bioinformatic analysis have identified 11 miRNAs along the human cytomegalovirus (HCMV, α-herpesvirus) genome (Dunn et al. 2005; Greer et al. 2005; Pfeffer et al. 2005). Evidence has confirmed that other γ-Herpesviruses besides the EBV, such as Kaposi’s Sarcoma-associated herpesvirus (KSHV) (Cai et al. 2005; Grundhoff, Sullivan, and Ganem 2006; Pfeffer et al. 2004; Samols et al. 2005), Murine gammaherpesvirus 68 (MHV-68) (Pfeffer et al. 2005; Huttvagner et al. 2001; Lee, Nakahara et al. 2004). Subsequently, one strand of the siRNA or miRNA products is assembled into the effector complex, termed RNA-induced silencing complex (RISC) (Hammond et al. 2000; Mourelatos et al. 2002), with the miRNAs within these complexes interacting with their target mRNA by base pairing. Whether the target mRNAs will be translationally repressed or degraded by cleavage is determined by the degree of complementarity between the miRNA and the target mRNAs. In contrast, siRNAs result from cleavage of very long precursor dsRNAs by the enzyme Dicer, and can also be introduced into cells by transfection of synthetic siRNA duplexes. The siRNAs can program RISC to cleave mRNAs bearing perfectly complementary target sites. This process was referred to as RNA interference (Elbashir et al. 2001).

Some regulatory sncRNAs also interact with proteins by simulating/resembling the protein’s cellular target, hence competing with the protein binding to that target (Romeo 1998). The biological roles of other RNA species involve various aspects of metabolism, including protein secretion, tRNA processing, splicing, and rRNA biogenesis (Tollervey and Kiss 1997).

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## TABLE 1

virus-encoded miRNA, siRNA, and snmRNAs

<table>
<thead>
<tr>
<th>Category</th>
<th>Species</th>
<th>Function</th>
<th>Origins</th>
<th>RNA polymerase</th>
<th>Total number</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>siRNA</td>
<td>HIV</td>
<td>Transcriptional regulation</td>
<td>Cleavage of long ds RNA</td>
<td>RNA pol II</td>
<td>5</td>
<td>Bennasser et al. 2005</td>
</tr>
<tr>
<td>t’RNA-like-ncRNA</td>
<td>MHV68</td>
<td>Unknown</td>
<td>Trnscribed from genomic sequence</td>
<td>RNA pol III</td>
<td>8</td>
<td>Bowden et al.1997, Simas et al.1998</td>
</tr>
<tr>
<td>Satellite-like small RNA</td>
<td>Trichomonas vaginalis T1 virus</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>1</td>
<td>Tai et al. 1995</td>
</tr>
</tbody>
</table>

Note: snmRNA: small non-messenger RNA; HSV: Herpes simplex virus; MDV: Marek’s disease virus; HVP: Herpesvirus papio; HCMV: Human cytomegalovirus; EBV: Epstein-Barr virus; KSHV: Kaposi’s Sarcoma-associated herpesvirus; MHV-68: Murine gammaherpesvirus 68; rLCV: Rhesus lymphocryptovirus; HVS: Herpesvirus saimiri; YFV: Yellow fever virus.
Viruses Encode Small Noncoding RNAs (sncRNAs)

So far, several different forms of small noncoding RNAs have been found in viruses: EBV-encoded RNA (EBERs) and the virus-associated RNA (VA RNAs) encoded by adenoviruses, enabling the suppression of translational inhibition through their interaction with protein kinase (PKR) (Lerner et al. 1981; Ma and Mathews 1996). EBERs are also found in other herpesviruses such as Rhesus lymphocryptovirus (rLCV) and Herpesvirus papio (HVP) (Howe and Shu 1988; Rao, Jiang, and Wang 2000). The MHV68 genome contains eight tRNA-like sncRNAs encoded by a 6 kb cluster, which also includes nine miRNAs found immediately downstream of the tRNA-like genes. Unlike cellular tRNAs, these tRNA-like transcripts are not aminocytlated (Bowden et al. 1997; Simas et al. 1998). Herpesvirus saimiri (HVS) encode a group of seven transcripts resembling spliceosomal U snRNAs (Albrecht and Fleckenstein, 1992; Lee et al. 1988; Wassarman, Lee, and Steitz 1989). These sncRNAs, named Herpesvirus saimiri U RNAs (HSURs), are the only known virus encoded U RNAs to date. They range in length from 75 to 143 nt and possess the 5′ terminal m3G caps, characteristic of U snRNAs, nevertheless, their sequences differ from those of cellular U RNAs. Two other HSV encoded ncRNAs known as HVR-1 and HVR-2, have also been identified (Bachmann et al. 2005). Adenoviruses express two virus-associated RNAs (VA RNAs), which are highly structured VA RNAs bearing relatively long double-stranded and stem-loop regions. VA-RNAs (VA RNAs), which are highly structured VA RNAs bearing relatively long double-stranded and stem-loop regions. VA-RNAs (VA RNAs), which are highly structured VA RNAs bearing relatively long double-stranded and stem-loop regions.

Genomic Organization and Expression of Viral miRNAs and sncRNAs

Like their cellular counterparts, most viral miRNAs locate outside the transcribed regions of other genes in the viral genome. All of the 23 miRNAs encoded by EBV, for example, are located within the 5′ or 3′ UTR and intron region. Five of the miRNAs were initially discovered in the EBV B95–8 strain and were found to be located within two genome clusters. One cluster includes miR-BHRF-1, miR-BHRF-2 and 3, with former being located within the 5′UTR of the BHRF-1 gene while the latter two are both found within the 3′UTR of the same gene. Other clusters include EBV miRNAs (miR-BART-1 and 2) located within the BART gene. In other EBV strains, a total of 18 novel pre-miRNAs has been identified within a genomic region that has been deleted from the B95–8 strain. These newly identified miRNAs locate in two groups within the first intron of the BART locus (Cai et al. 2006; Grundhoff, Sullivan, and Ganem 2006). This was also shown to occur for HSV, MHV-68, SV40, but not the case of KSHV (Cui et al. 2006; Grundhoff, Sullivan, and Ganem 2006; Pfeffer et al. 2005; Sullivan et al. 2005). A total of 11 miRNAs were found along the HCMV genome, five of which are situated within an intergenic region, one in an intron, while three were found to be complementary to known viral Open reading frames (ORFs), suggesting that these miRNAs may direct the cleavage of the viral transcripts of their own (Dunn et al. 2005; Grey et al. 2005).

miRNAs encoded by HSV, HCMV, and EBV separate along the genome while the MHV-68 and KSHV miRNAs are found in clusters (Cui et al. 2006; Grey et al. 2005; Grundhoff, Sullivan, and Ganem 2006; Pfeffer et al. 2005). In KSHV, miR-K12-1 through to −9 as well as −11 locates within the intron region of the kaposin primary transcript, and miR-K12-12 locates within the 3′UTR, while miR-K12-10 resides within the ORF K12. Although the miR-K12-10 precursor processing does not appear to
substantially inhibit the production of kaposin mRNA, whether this phenomenon plays a role in the regulation of kaposin levels still remains unknown. The kaposin gene is known to encode for at least three proteins (kaposin A, B, and C), translated from overlapping ORFs that include a sequence known as K12 as well as two adjacent direct repeat elements termed DR1 and DR2. Three major latency clusters consisting of ORFs 71, 72 and 73 are located immediately upstream of the pre-miRNA cluster. Transcripts corresponding to the pri-miRNA cluster and the kaposin gene have been identified (Cai and Cullen 2006; Pearce, Matsumura, and Wilson 2005). The promoter that initiates the transcription of the pri-miRNA cluster and the kaposin gene is also able to initiate the transcription of ORFs 71, 72, and 73. Whether this locus is transcribed as a shorter transcript encoding only ORFs 71, 72, and 73 or as a longer form also encoding for the pri-miRNA cluster and the kaposin gene depends on the use of a polyadenylation site downstream of ORF 71. Involvement of this site results in the production of an mRNAs encoding for ORFs 71, 72, and 73, while the read-through transcripts can generate either the pri-miRNAs cluster or the kaposin pre-mRNA. Moreover, another transcript initiated from a promoter located within ORF 72, encodes only for pri-miRNA transcription also requiring the read-through of the ORF 71 polyadenylation site.

**Transcriptional Characteristic of Viral sncRNAs**

As viruses generally lack RNA polymerases, expression of viral miRNAs and sncRNA depends on the cellular transcriptional machinery. Most viral miRNAs are transcribed by pol II, however the processes involved in the development and maturation of a number of viral miRNAs differ from that of cellular miRNAs. These exceptions are found with MHV68 miRNA, originating from tRNA-like-sncRNA transcripts and some adenovirus miRNAs, processed from snoRNAs, thought to be transcribed by RNA polymerase III (Pfeffer et al. 2005). The short hairpin of MHV68 miRNA indicates that their processing mechanism may be different from other miRNAs. After being transported to the cytoplasm by Exportin-5, a number of adenovirus miRNAs are directly processed from double-stranded structures of VA RNAs by Dicer (Aparicio et al. 2006).

Almost all of the sncRNAs found in the above mentioned viruses are transcribed by pol III. The VA RNA promoter strongly resembles the initiation elements of tRNA genes, composed of A and B boxes responsible for the recruitment of transcription factor TFIIC. All of the VA RNAs have both boxes at similar position except for the Ad10 VA RNA II that lacks the discernible B box. The A box of the VA RNA II gene is also found to be less conserved than that in the VA RNA I genes (Fowlkes and Shenk 1980; Guilfoyle and Weinmann 1981; Ma and Mathews 1996). These discrete promoter elements are also found in the CMER encoded by HCMV (Marschalek et al. 1989). In addition to the internal transcription signals, some conserved flanking regions have also been observed, e.g., an A-rich sequence between the protein coding region pTP splicing site and the VA RNA genes is required for proper initiation of transcription, its deletion altering the selection of transcriptional start sites (Thimmappaya, Jones, and Shenk 1979). EBER1 and EBER2 genes encoded by the Epstein-Barr virus are similar to the human 7S L RNA, containing internal promoter structures including A and B boxes, as well as upstream elements similar to pol II promoter elements. Nucleotides −23 to −28 are analogous to a TATA box, −40 to −55 bind activating transcription factor (ATF), while −56 to −77 combine with the Sp1 protein. In vivo, these elements stimulate a 50-fold increase in the levels of expression (Howe and Shu 1989). Newly synthesized EBER2 can regulate transcription of its own gene and efficient transcription of the EBER2 gene depends on the folded structure of the completed transcript, a stem-loop structure, consisting of the 5′- and 3′-end of the RNA, being an essential element for its regulation (Dumpelmann, Mittendorf, and Benecke, 2003).

Since most human snRNAs are transcribed by RNA pol II, the HSUR genes were expected to contain similar expression signals. The enhancer and the TATA elements, which make up the HSUR promoters, were found through alignment of genomic sequences flanking the transcription start site, suggesting that these U RNAs are transcribed by RNA pol II (Lee et al. 1988).

**Conservation of Viral miRNA and sncRNAs**

Comparative genomics shows that, only with a few exceptions among closely related viruses, viral miRNAs have little homology to each other or to known host cell miRNAs. Of the 24 miRNA candidates predicted within HSV-1, eight were conserved in the HSV-2 genome in terms of both sequence and location (Cui et al. 2006), and screening for stem-loop structures in the HCMV and CCMV genomes also revealed that only about one quarter of the predicted miRNAs were more than 60% identical (Grey et al. 2005). The SV40 miRNAs appear to be conserved in other polyomaviruses, including JCV, BKV and SA12 and both the pri-miRNA and mature miRNA were detected by northern blotting in BSC40 monkey kidney epithelial cells after infection with SA12. Also the predicted hairpin structures of the SV40 pre-miRNA were found to be conserved in other related polyomaviridae including BKV, JCV and SA12 (Cantalupo et al. 2005; Sullivan et al. 2005).

To investigate sequence conservation among viral sncRNAs, we used all the sequences as queries to perform a BLAST search against the sequence collection in Genbank (Benson et al. 2004). The majority of the sncRNAs did not show significant sequence similarity with those from other viruses except in the case of a few closely related species. For example, sequences similar to the EBER1 and EBER2 encoded by EBV could only be found in Cercopithecine herpesvirus and Herpesvirus papio. CMER encoded by HCMV is also observed in the genome of Chimpanzee cytomegalovirus and Tupaia herpesvirus. sncRNAs were also compared with other genomes without finding any significant similarities. Since sncRNAs function depends more on their secondary structure rather than on a specific sequence, their secondary structures, as predicted by the software RNAdo,
FIG. 2. Secondary structures of EBER1 and EBER2 encoded by EB viruses, the VA RNA encoded by adenoviruses and the CMER encoded by Cytomegalovirus. A: secondary structure of CMER. B and C: secondary structure of EBER1 and EBER2. La protein region was indicted by a green line. D: secondary structure of VA RNA1, the stem-loop region of VA RNA strongly suggests that it can be processed by Dicer to generate miRNAs. All the structures were predicted by the software RNAfold. The similarity between CMER and VA RNA suggests that they may have the same functions.

Function of Viral miRNAs and sncRNAs

Bioinformatic analysis indicates that viral miRNAs targets include both cellular as well as self-mRNAs, however, most targets remain to be confirmed and so far the function of only a few viral miRNAs has been determined. The first was the HSV-1 latency-associated transcript (LAT) encoded miR-LAT located in the first exon of LAT that has been shown to confer resistance to apoptosis. The 3'-UTR of transforming growth factor-β1 (TGF-β1) and SMAD3 protein both contain sequences with partial homology to miR-LAT. TGF-β is a multifunctional polypeptide-signaling factor that can inhibit cell growth and promote cell differentiation. It induces phosphorylation of SMAD2 and SMAD3, which then bind to each other, as well as with SMAD4 within the nucleus, where they then activate transcription of the TGF-β responsive genes involved in a number of cellular processes. miR-LAT therefore inhibit apoptosis through inhibition of TGF-β signaling, thereby maintaining the cell under a state of latent infection and hence allowing for expression of viral proteins (Gupta et al. 2006). The LAT was shown to encode totally four miRNAs recently, and one of which, the miR-H2–3p was transcribed from the antisense orientation of viral immediate-early transcriptional activator: ICP0, another miR-H6 derived from another transcript can inhibit the transcription factor ICP4 (Umbach et al. 2008). Another LAT-related miRNA encoded by HSV-2, which designated miR-1, can reduce the expression of ICP34.5, their own neurovirulence factor (Tang et al. 2008).
Details regarding the function of another viral miRNA have been investigated: the EBV-miR-BHRF1-3, for example, was shown to inhibit the cellular IFN-inducible T-cell attracting chemokine CXCL-11/IFN-γ (Xia et al. 2008), and the EBV miR-BART2, located in the fourth intron of Bam HI-A region rightward transcript (BART) gene, fully complementary to the viral DNA polymerase gene BALT5 on its counterpart chain. Consistent with a previously predicted processing mRNA of the viral DNA polymerase gene, miR-BART-2 was shown to result in cleavage of this transcript (Barth et al. 2008). Interestingly, an insertion of up to 24 nucleotides, resulting from mechanisms yet to be elucidated but probably occurring downstream of the cleavage site, has been observed (Fumari, Adams, and Pagano 1993). This inserted sequence may serve as a noncanonical polyadenylation signal and may be responsible for the repair or recovery of the cleaved transcript. However the biological role of this cleavage as well as why these cleaved transcripts are only found in the B95–8 strain remains still unclear. Recent researches may reveal most miRNAs encoded by herpesvirus was to suppress expression of their own genes so as to regulate their life cycle (Grey et al. 2007; Murphy et al. 2008).

The miRNAs located within the SV40 late transcript’s 3′ UTR have also been identified. A previously discovered sncRNA, designated as SV-associated sncRNA (SAS-RNA), encoded by SV40 and located within the region where early and late transcripts overlap, was later identified as a pre-miRNA (Sullivan et al. 2005). This hypothesis strongly supported by the length and biogenesis of SAS-RNA as well as the association with early mRNAs (Alwine 1982; Alwine, Dhar, and Khoury 1980; Alwine and Khoury 1980; Hay, Amster-Choder, and Aloni 1986). The SV40 genome is circular and part of the region encoded by the late transcript overlaps with the early transcript from the opposite strand, including the pre-miRNA. Viral miRNA appears to target the early mRNA and then cleave it. As the major product of the early mRNA, the viral T-antigen, which is the target of the cytotoxic T lymphocyte (CTL) response, was down regulated by the miRNA during the later phase of infection, thus the miRNAs may be assisting SV40 in evading the immune system (Sullivan et al. 2005). Like the SV40, miRNAs encoded by the JC Virus and BK Virus also regulate early gene expression when expressed late during infection (See et al. 2008). Recently it was observed that another viral miRNA, the hcmv-miR-UL112 encoded by HCMV, is responsible for down regulating the expression of human major histocompatibility complex class I–related chain B (MICB) genes, thus giving the impression of a reaction by the immune system (Stern-Ginossar et al. 2007). New bioinformatics research reveals that about 14 viral genes may be the targets of hcmv-miR-UL112 and at least 3 of them were validated (Grey et al. 2007).

A miRNA derived from the HIV-1 nef gene, designated as miR-N367, was identified both in cells infected with HIV-1 and in cells transduced with a nef small hairpin RNA (shRNA) (Omoto et al. 2004). miR-N367 efficiently down regulates viral transcription through the U3 region negative responsive element (NRE) in the genome and nef sequences in the 3′-UTR. A possible role of miR-N367 in regulation of gene expression through promoter interference at the transcription level was suggested, which differs from the miRNA modes of action normally observed. Thus HIV-1 may regulate its own transcription and replication by miR-N367.

So far, most of the sncRNA information available deals with EBERs encoded by EBV and the VA RNAs encoded by adenoviruses. Two sncRNA molecules, EBER1 and EBER2, 167 and 172 nucleotides long respectively, are actively expressed in primate B-lymphocytes infected by EBV (Arrand, Young, and Tugwood 1989; Lerner et al. 1981; van Santen, Cheung, and Kieff 1981). Both RNAs are transcribed by RNA pol III, possess 5′pppA termini and lack poly(A)-tails (Glickman, Howe, and Steitz 1988). They are found both in the cytoplasm and nuclei of EBV-infected cells, and exist as ribonucleoprotein (RNP) complexes with a cellular La antigen (Lerner et al. 1981). The interaction site was shown in Figure 2B and 2C. Another cellular protein, an EBER-associated protein (EAP) quantitatively binding EBER1 was further designated as the ribosomal protein L22, suggesting that EBER RNP may participate in some aspect of translation in EBV-transformed cells (Tochyski et al. 1994). The EBERs bind the RNA-dependent protein kinase (PKR) and prevent inhibition of translation by PKR in vitro (Clarke, Sharp, and Clemens 1990). PKR is an interferon-induced serine/threonine protein kinase enabling the phosphorylation of the α-subunit of the eukaryotic translation initiation factor 2 (eIF-2α). It is activated by double-stranded RNA and once this occurs, it in turn inactivates the translation machinery, resulting in the dramatic reduction of protein synthesis. PKR plays an important role in interferon-mediated antiviral defense, has been implicated in cell growth control and differentiation and might function as a tumor suppressor. Over expression of PKR leads to apoptosis and therefore EBERs appears to confer interferon-induced apoptosis resistance by binding to PKR and hence inhibiting its activation (Nanbo et al. 2002). This may constitute one of the mechanism through which viruses evade cellular defense. It has been partially confirmed by the observation how L22 can competes with PKR for a common EBER1 binding site, thereby attenuating the biological effects of this viral RNA (Elia et al. 2004).

Like EBER1, VA I’s main function is to block RNA-dependent protein kinase (PKR) activity and thereby prevent inhibition of protein synthesis (O’Malley et al. 1986), while enhancing protein levels by stabilizing ribosome-associated viral mRNAs (O’Malley et al. 1989). EBER1 and EBER2 are capable of functionally substituting for the VAI RNA in adenoviruses depleted of VAI and VAII genes in infected cells (Bhat and Thimmappaya 1985). The VA RNA secondary structure is characterized by three main components: an apical stem-loop, essential for PKR binding, a central domain, involved in the inhibition of PKR activation, and a terminal stem, required for binding to Exportin-5 and transport of VA RNA to cytoplasm (Ma and Mathews 1996). VA RNA also acts as an RNA interference
noncoding RNA initial strand separation (Huang, Zhu, and Anders 1996). The synthesis by cooperating with the Y-block in order to produce SRT may play a role in initiating or regulating initiation of DNA end of SRT, is essential to the HCMV that viruses may also encode ncRNAs bearing a large variety of functions including structural, catalytical functions, as well as gene expression regulation and more. Our current understanding of ncRNAs in different organisms as well as their function and classification is progressively increasing. This reports deals with the information available on various types of ncRNAs: miRNAs, siRNAs, and small noncoding RNAs encoded by viruses. Research on ncRNAs encoded by viruses will provide deeper knowledge on sequence, structure and evolution of virus, as well as host-viruses ncRNA dependent interaction.

So far, more than one hundred miRNAs have been identified belonging to four virus families, including herpesviridae, polyomaviridae, adenoviridae and retroviridae. Like cellular miRNAs, most viral miRNAs were transcribed by Pol II. However, for a number of viral miRNAs, biogenesis may differ from that of cellular miRNAs. For example, The miRNA encoded by MHV-68 are processed from tRNA-like-sncRNA transcripts, which was transcribed by Pol III (Borchert, Lanier, and Davidson 2006; Pfeffer et al. 2005), and VA RNA can also be processed to generate miRNAs (Aparicio et al. 2006). The identification of microRNA-encoding long non-coding RNA suggests that some noncoding RNAs with unknown function can act as pri-miRNAs, thus the number of miRNAs may be larger than previously anticipated (He, Su et al. 2008). Due to their similarities to snRNAs, most of the snRNAs were considered to be transcribed by Pol III.

Different from their host counterparts, viral miRNAs were poorly conserved, showing little homology to each other as well as known host cell miRNAs, except among closely related viruses. The majority of the snRNAs did not show significant sequence similarity with those from other viruses except in the case of a few closely related species. This suggests that viral miRNAs and snRNAs may have evolved rapidly. For this reason new algorithms that do not rely on sequence conservation are needed to predict novel viral miRNAs and snRNAs. But exceptions were also found: the first eight nucleotides of miR-K12-11 encoded by HCMV are similar to those of hsa-miR-155 (Pfeffer et al. 2005). Thus viral miRNAs may function as regulators of cellular regulatory network (Gottwein et al. 2007; Skalsky et al. 2007). Another example is the miRNAs encoded by the JC Virus and BK Virus, They are conserved and like the SUV40 miRNA, may regulate early gene expression of the viral life cycle (Seo et al. 2008).

CONCLUSION AND PERSPECTIVES

In the past few years, the number of known ncRNA genes that do not encode proteins, except the infrastructural RNAs, has increased dramatically, with snRNAs, snoRNAs, miRNAs, siRNAs, and piRNAs being identified (Backofen et al. 2007). The establishment of sequences databases such as NONCODE, RNAdb, greatly facilitated researches around ncRNAs (He, Liu et al. 2008; Pang et al. 2005), constituting a valuable tool when investigating the complexity of genomes, ranging from bacteria to mammals. During the last two decades, studies have revealed that viruses may also encode ncRNAs bearing a large variety of
(Jopling et al. 2005), while miR-32 expressed in human cells limits the accumulation of primate foamy virus type 1 (PFV-1) (Lecellier et al. 2005). Moreover bioinformatic and experimental research revealed that HIV-1 genes could be targeted by a group of cellular miRNAs (Harbiraran et al. 2005; Huang, Zhu, and Anders 1996). Involvement of miRNAs in viral and hosts gene regulation has uncovered a new aspect of host-viruses interactions.

Viruses can also fight against the action of host miRNAs through a number of mechanisms. The Tas protein encoded by PFV, for example, can suppress the inhibitory effect of human miRNAs (Lecellier et al. 2005), while HIV-1 encoded Tat protein was shown to function as a suppressor of RNA silencing (SRS) (Benmasset et al. 2005). Tat was initially identified as a transcriptional activator, whose major function is to stimulate the expression of HIV-1 gene products. A sequence known as the Tat responsive region (TAR) located downstream of the transcriptional start site is essential to its function. TAR RNA inhibits the initiation of translation by activating the double-stranded RNA activated inhibitor of protein synthesis. Interestingly, the secondary structure of the TAR sequence can recruit Dicer and can be processed. HIV may use TAR RNA in order to suppress cellular RNAi mechanisms during viral infection (Klase et al. 2007). The same mechanism can also be observed with the adenoviruses encoded VA RNA (Lu and Cullen 2004). Although this further supports the hypothesis that miRNAs may be originating from Pol III transcripts, it also points out to the fact that viruses can use sncRNAs to counteract cellular restriction during viral reproduction.

Small noncoding RNAs may play an important role both in the regulation of gene expression, as well as functioning as structural elements in a cell. In the past few years, an increasing number of sncRNAs have been identified in many different organisms, ranging from prokaryotes to more complex eukaryotes (Huttenhofer et al. 2001; Marker et al. 2002; Saetrom et al. 2005; Venter et al. 2001; Yuan et al. 2003). In the E. coli more than one hundred sncRNAs have already been identified (Saetrom et al. 2005). The broad phylogenies and high expression levels of the sncRNAs suggest that they may be carrying out important task within a given organism. In Burkitt’s lymphoma, for instance, EBERs induce the expression of IL-10, which can act as an autocrine growth factor (Komano et al. 1999), and similarly in the secretion of insulin-like growth factor 1 in EBV-induced gastric carcinoma cells (Iwakiri et al. 2003), revealing the role of EBERs in both lymphoid and epithelioid carcinogenesis.

Most sncRNAs loci display RNA polymerase III promoter elements and are thus likely to be transcribed by pol III. Their secondary structures commonly show more conservation than their actual sequences and like their cellular counterparts, some of them can interact with proteins. The EBER and VA RNAs can bind to PKR to confront the translational inhibition (Clarke, Sharp, and Clemens 1990; O’Malley et al. 1986). Others act by base pairing with cellular RNAs, the HSUR1 and HSUR2, for example, bear sites complementary to the miRNA polyadenylation signal, suggesting its function in mRNA maturation (Lee et al. 1988). But most sncRNAs exert their action outside of the nucleus.

miRNA, siRNA, and other small noncoding RNA species are involved at various steps during a number of cellular processes. They may represent a novel gene regulatory network in the genetic regulation of complex organisms. The discovery that miRNAs can regulate messenger-like noncoding RNAs confirmed this hypothesis (Zhao et al. 2008). We noticed that most known viral ncRNA have been also found in animal viruses and in some cases also in plant viruses. Viral ncRNAs play important functions in safeguarding viral reproduction by opposing cellular defense mechanisms. Because of their specific and accurate miRNA/siRNA-mRNA identification mechanism, miRNA/siRNA can be used as a tool for inhibit of viral gene expression. For example: Transfection with synthesized plasmid expressing pre-miRNAs that specific for viral genes efficiently inhibits viral replication when these cells were infected with the target virus (Dang et al. 2008). Artificial miRNA targeting conserved sequences of HIV genes contribute to effective and durable inhibition of HIV replication (Liu et al. 2008; Son et al. 2008). Similar results also can be observed during the infection of HBV and Marek’s disease virus (Chen et al. 2008; Ely et al. 2008). Thus efforts to identify novel viral sncRNAs and their function may have important implications in the treatment of virus-related diseases.

REFERENCES
THE PROPERTIES AND FUNCTIONS OF VIRUS ENCODED miRNA, siRNA, AND OTHER SMALL NONCODING RNAs


