Host–virus genome interactions: macro roles for microRNAs

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Summary

MicroRNAs are recently discovered small endogenous non-coding RNAs. These small RNAs of ~22 nucleotide length are crucial post-transcriptional regulators of gene expression in a wide spectrum of normal and abnormal biological processes including antiviral defence, oncogenesis and development in higher eukaryotes. Of late, a number of viruses have also been shown to encode for microRNAs. The host- and virus-encoded microRNAs and their targets together thus form a novel regulatory layer of genetic interactions between the host and the virus. Recent reports have thrown light on this new regulatory layer. A clear understanding of the cross-talk between the host and virus would not only enable us to understand the molecular basis of viral pathogenesis, but also enable us to develop better therapeutic strategies. This review discusses the intricacies of host–virus cross-talk mediated by microRNAs. Recent trends in this field and the challenges that need to be addressed are also discussed.

Introduction

MicroRNAs (miRNAs) are endogenous small non-coding RNAs, which have recently attracted immense attention as tiny regulators with profound impact on eukaryotic gene expression. MicroRNA-mediated repression, initially discovered in Caenorhabditis elegans about a decade ago, was initially thought to be an unusual form of gene regulation. The discovery of conserved let-7 homologues (Lee et al., 1993; Reinhart et al., 2000) in a number of animal species 7 years ago paved the way for discovery of new miRNAs in higher eukaryotes including humans. Recent studies have demonstrated that microRNAs are involved in several major biological processes.

Apart from higher eukaryotes, very recently, viruses have also been shown to encode for microRNAs (Nair and Zavolan, 2006). As per miRBase (Griffiths-Jones, 2006), the registry of microRNAs, at least eight viral species encode for microRNAs and majority of them are herpesviruses. The number of miRNA sequences encoded also varies widely, ranging from 23 in Epstein–Barr virus (EBV) to one in herpes simplex virus (HSV). The count of virus-encoded microRNAs is expected to go up with better computational methods for prediction of putative microRNA precursor candidates and high-throughput experimental validation of the candidates.

The present understanding of the biological functions of virus-encoded microRNAs remains sketchy, with evidence mainly derived from studies on individual or a small set of microRNAs encoded by the viruses and their cognate hosts. Survival strategies of the virus and counter strategies of host cells through miRNAs of host and viral origin and their respective targets form the crux of host virus interactions mediated by microRNAs. Thus microRNAs form a complex link between the regulatory networks of the host and the pathogen. A thorough understanding of the microRNA-mediated host–pathogen interaction is essential in understanding the basic pathophysiological changes associated with viral infections.

Each report of host–virus interaction through miRNA action till date has presented a different scenario ranging from pro-viral and antiviral host microRNAs to viral defence strategies (Table 1). Therapeutic strategies cannot be effective without addressing all these possibilities in totality. In this review we discuss four logical models of miRNA-mediated host–virus interaction to understand the relevance of miRNA in viral diseases in a comprehensive fashion. Based on the model we suggest likely strategies that can be explored for development of diagnostics and therapeutics. Potential challenges and grey areas that need to be addressed are also discussed. Emerging areas like artificial microRNAs, microRNA engineering, etc. which would have therapeutic applications are also discussed.
MicroRNA biogenesis and action

MicroRNAs are classically thought to be derived from non-coding RNA, either transcribed as non-coding transcripts or spliced off from introns of coding RNA [see Du and Zamore (2005) for an overview of microRNA biogenesis]. Vertebrate microRNAs seem to be transcribed by RNA Polymerase II as long primary microRNA (pri-miRNA) harbouring a single microRNA or a cluster of microRNAs analogous to an operon in prokaryotes. Recently it has been shown that some microRNAs, especially those associated with repeats, could be transcribed by Polymerase III (Borchert et al., 2006) The nuclear RNase III enzyme, Drosha, along with its partner DGCR8 identifies and cleaves hairpin precursors (Han et al., 2006). The identification and cleavage by Drosha DGCR8 complex is determined by structural features which differentiate microRNA precursors from other hairpins. The resulting stem-loop structures (pre-miRNAs) are then exported out of the nucleus by the Exportin class of nuclear transporters (Yi et al., 2003; Kim, 2004; Zeng and Cullen, 2004; Yi et al., 2005).

In the cytoplasm, the hairpins undergo further processing, and are chopped into duplex RNA of ~22 nucleotides in length by a cytoplasmic RNase III enzyme Dicer (Tijsterman and Plasterk, 2004). The duplex is then unwound, and a single strand selected for incorporation into a protein complex termed as the miRNA–Ribonucleo–protein complex (miRNP). One of the strands in the duplex is selected preferentially over the other, based on structural and thermodynamic features like stability of the ends. The strand with the least stability at the 5’ end (guide strand) would be preferentially selected to be associated with RNA-induced silencing complex (RISC) (Krol et al., 2004). Besides the highly conserved Argonaute (Liu et al., 2004; Okamura et al., 2004) protein which binds to the miRNA–mRNA duplex, miRNPs are expected to contain other uncharacterized protein components (Sontheimer and Carthew, 2004). The structure–function relationship of small RNAs (Rana, 2007) and mechanism of microRNA function (Pillai et al., 2007) have been recently reviewed.

The mature ~22-nucleotide microRNA in association with the RISC then binds to 3’ untranslated region (UTR) of transcripts and causes translational block. Although the mechanism by which microRNAs mediate post-transcriptional regulation is not known, it has been shown that complete complimentarity causes target degradation while imperfect complimentarity causes translational block. Recent evidence suggests that a microRNA may be employed to clear large number of target RNAs during critical developmental stages (Giraldez et al., 2006) by affecting transcript stability through de-adenylation. MicroRNA-mediated de-adenylation has also been reported independently (Wu et al., 2006) suggesting de-adenylation as one distinct way of post-transcriptional regulation mediated by microRNAs.

Recent studies also reveal that microRNA-mediated post-transcriptional regulation is indeed reversible. Bhattacharyya et al. (2006) reported stress-associated reversibility of miR-122-mediated repression of cationic amino acid transporter 1 (CAT-1) mRNA in hepatocarcinoma cells. The overall implication of this is striking as it supports the role of microRNAs as dynamic regulators responding to external and internal cues. Figure 1 summarizes the steps of microRNA biogenesis and action.

Models of miRNA-mediated host–virus interaction

Understanding the intricacies of microRNA-mediated host–virus cross-talk would not be possible without a comprehensive view of the entire set of interactions encompassing microRNAs encoded by the host, virus, their respective targets and their regulatory mechanisms (Scaria et al., 2006). Here we try to explore the intricacies of microRNA-mediated host–virus cross-talk through a simplistic model as summarized in Fig. 2. The possible interactions are discussed in detail with evidence derived from recent reports, besides an overview of virus-encoded RNA interference (RNAi) silencers which have recently been explored in detail.
Host microRNAs targeting viral transcripts

This is one of the most explored mechanisms of microRNA-mediated antiviral defence. The mammalian viruses for which host microRNA targets have been discovered include primate foamy virus (PFV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV). Each case by itself brought to light one or another interesting aspect of microRNA-mediated host–virus interaction.

The first instance of a cellular microRNA mediating antiviral defence was seen in PFV (Lecellier et al., 2005). It also brought to light counter strategies evolved by viruses to silence the miRNA pathway. PFV replication is efficiently restricted by the cellular microRNA miR-32 targeting viral transcripts. Interestingly the virus encodes for a suppressor of RNAi (Tas), which enhances the accumulation of viruses by offsetting the effects of the cellular microRNA.

In the case of hepatitis C, a cell type-specific microRNA (miR-122) targeting the 5′UTR of the viral transcript has been shown to modulate the viral levels. This is also an elegant example of how tissue or cell type-specific microRNAs can influence tropism of viruses. Using computational predictions involving consensus prediction, we have shown five cellular microRNAs could potentially target a set of accessory genes in HIV including *nef* (Hariharan et al., 2005). *nef* is not only known to be auto-regulated by its own microRNA, but also implicated as a major determinant of long-term non-progression of the disease (Joseph et al., 2005). The microRNAs targeting the viral transcripts were variable in individuals, suggesting that variation in expression levels may define the prognosis of infection. We have also experimentally validated the finding using reporter constructs fused to the target site in the 3′UTR (our unpublished results). Similarly many human microRNAs would have antiviral effect against a number of viruses. Results of a large computational screen for host microRNAs targeting viruses have been recently made available online (Hsu et al., 2007).

**Fig. 1.** Schematic overview of the biogenesis and action of microRNAs. The pri-miRNAs transcribed from Pol-II DNA-dependent RNA polymerases are processed to pre-miRNAs. The hairpin-shaped structures are recognized by Drosha-DGCR8/Pasha RNase III enzymes to trim the pri-miRNAs. These pre-miRNAs are transported to the cytoplasm by the enzyme Exportin-5 where they are further acted upon by another class of RNase enzymes called the Dicer which chops off the loop region of the pre-miRNA to release an imperfectly complementary double-stranded structure called miRNA–miRNA*. This duplex is unwound by a helicase to release the strands. One of them (the guide strand) gets incorporated to a protein complex whose individual components include Ago, and a host of yet to be discovered proteins. This complex is now termed as the RNA-induced silencing complex (RISC). The RISC attaches itself to the 3′UTR of other transcripts in two different modes: (i) by binding perfectly with the target transcript, which results in the transcript degradation in the P-bodies, and (ii) by imperfect complementarity due to which the translational efficiency is reduced.
Viral microRNAs targeting host transcripts

The regulatory role of virus-encoded microRNAs on cellular transcripts was initially explored by computational means in HIV (Bennasser et al., 2004). Computational analysis showed that HIV-encoded microRNAs could potentially target critical genes involved in the pathophysiological changes associated with HIV infection (Couturier and Root-Bernstein, 2005). Another recent study also suggests the role of HIV-encoded microRNAs in establishing latency in HIV infection (Weinberg and Morris, 2006).

Another elegant example of virus-encoded microRNAs effectively modulating cellular defence mechanisms to its benefit has been reported in simian virus 40 (SV40). SV40-encoded microRNAs are produced during the late phase in the life cycle and help the virus to evade cytotoxic T cells by targeting early transcripts including those encoding T cell antigens (Sullivan et al., 2005). Although computational predictions span various classes of viruses (Pfeffer et al., 2005) currently most of the experimentally validated microRNAs encoded by viruses arise from the Herpesvirus family of viruses (Pfeffer et al., 2005; Grundhoff et al., 2006), including a unique set of human pathogens like EBV, cytomegalovirus (CMV), Kaposi sarcoma herpesvirus (KSHV) (Cai et al., 2005; Samols et al., 2005; Cai and Cullen, 2006) which are all gamma-herpesviruses and recently HSV-1 (Cui et al., 2006) which is an alpha-herpesvirus. The only two functionally validated microRNA from HSV arise from the latency-associated transcript (LAT) (Cui et al., 2006; Gupta et al., 2006) and one of them is thought to target transcripts associated with apoptosis in the host (Gupta et al., 2006).

Recent evidence has supported the hypothesis that virus-encoded microRNAs can target critical genes associated with disease pathogenesis. We predicted using computational methods employing consensus predictions (our unpublished results); the cellular targets of EBV-encoded microRNAs. We observe that the target genes are particularly enriched in pathways associated with apoptosis and tumour suppression. This observation substantiates the proposed involvement of virus-encoded microRNAs in mediating pathogenesis of conditions like...
neoplasia. Many viruses like human papilloma virus, EBV, human T lymphotropic virus, hepatitis B virus are very well known to be linked to neoplasia. It would be worthwhile to explore the role of microRNAs encoded by oncogenic viruses in mediating oncogenesis. This would not only provide a better understanding of the process, but would also help in designing novel therapeutics based on microRNAs (see below).

Viral microRNAs targeting viral transcripts

This model constitutes an elegant regulatory loop wherein a virus-encoded microRNA generated with borrowed host machinery regulates its own transcripts. This model of gene regulation is seen in HIV where a nef-encoded microRNA has been shown to target its own transcript in in vivo and in vitro experiments (Omoto et al., 2004; Omoto and Fujii, 2005). The role of nef in disease progression and the inability of nef mutants to establish disease leading to long-term non-progression of disease following HIV infection (Kirchhoff et al., 1995; Salvi et al., 1998) suggest that anti-nef miRNAs may have a role in delaying disease progression.

Host microRNAs targeting host transcripts

An elegant mechanism of viruses modulating host microRNA expression and thereby modulate host genes have been recently reported by Triboulet et al. (2007). Analysis of microRNAs differentially expressed in HIV-infected cells revealed that the miR-17/92 cluster of microRNAs was downregulated following HIV infection. The members of the cluster miR-17-5p and miR-20a were also shown to repress the translation of PCAF protein which has been previously shown to be a cofactor of Tat in modulating HIV expression. A set of 11 microRNAs were also shown to be upregulated during HIV infection but the functional role of these microRNAs in modulating viral or host expression is not yet known (Kumar, 2007). It is possible that the host genes modulated by these microRNAs may be important in latency and other pathophysiological changes associated with HIV infection.

Another related example has also been recently reported in Arabidopsis where a peptide derived from the plant pathogen Pseudomonas syringae induces the expression of host microRNAs which target auxin receptor and thus inhibit the growth of the bacteria (Navarro et al., 2006). Genomes of a number of human viruses encode transcription factors, which potentially regulate microRNA expression. The possibility of virus-encoded transcription factors modulating microRNA expression in the host has not been explored. Such a mechanism if proven would provide immense insights and explanations on how viruses can modulate complex regulatory networks in the host with their minimal genomes. This would be possible by simultaneously monitoring the transcriptional outputs of the host and virus during various stages of infection.

Silencing the silencers: virus-encoded suppressors of RNAi

Interestingly viruses have also devised methods to overcome RNAi-mediated antiviral response initiated by the host. The molecular mechanisms of many of these are yet to be understood, and initial reports show the mechanisms are diverse and often unique to the virus. The well-explored mechanisms include secretion of proteins that bind to RNA and thus interfere with the formation of RISC. Examples of this type of regulation have been demonstrated in PFV (Lecellier et al., 2005) and HIV (Browne et al., 2005) and many other viruses. Another mechanism is to encode for RNAs which mimic microRNA precursors in an attempt to overwhelm the cellular microRNA processing machinery. This mechanism has been extensively studied in adenoviruses (Sano et al., 2006).

Discovery of novel microRNAs

Understanding the host–pathogen regulatory networks in a comprehensive way would necessitate the discovery of the entire repertoire of virus- as well as host-encoded microRNAs, their expression profiles and high-throughput validation of targets. Computational prediction (Yoon and De, 2006) and experimental validation of novel microRNAs (Bentwich, 2005) have been the mainstay in the discovery of microRNAs. In the following section we discuss the computational as well as experimental methods for discovery of microRNAs, their expression profiling and validation of targets.

Computational tools for ab initio prediction of microRNA

A major cause for the paucity of information on microRNAs encoded by viruses was the lack of efficient computational methods for prediction of candidates from genomic sequences. This was primarily due to the fact that virus-encoded microRNAs share little homology with that of the host, and there is little conservation of microRNAs across viral classes. This proved to be a major challenge as most of the microRNA prediction algorithms relied heavily on sequence conservation, which fared well in discovering eukaryotic microRNAs. The first major computational prediction and experimental validation of virus-encoded microRNAs utilized a machine-learning approach-based method to classify viral microRNA hairpin precursors based on structural determinants of human precursor sequences (Pfeffer et al., 2005).
Recently *ab initio* prediction methods have emerged which take into consideration structural (Hertel and Stadler, 2006) as well as sequence determinants.

We recently developed a novel method for *ab initio* prediction of microRNA precursors from genome sequences. We created a machine-learning model based on Support Vector Machine (SVM) algorithm trained to discriminate human microRNA precursor hairpins and sequences which have the potential to form hairpins but does not encode for microRNAs. The SVM model is based on a large number of sequence and structure features which would enable to efficiently discriminate the two data sets. As the prediction is not dependent on the conservation of microRNA precursors, unlike the previously reported methods of microRNA precursor prediction, this could predict microRNA precursors which share very less homology between related species. We rationalized that as the model discriminates true microRNA precursors from other hairpin forming sequences based on sequence and structure features rather than by conservation, this could be effectively used to predict viral microRNA precursors as they share the same processing machinery with that of the host and do not share homology with that of the host. A comparison of the efficacy of recently reported *ab initio* prediction methods on the data set of experimentally validated viral microRNA precursors in miRBase revealed that our algorithm offered very high sensitivity and accuracy. A recent independent experimental validation of microRNAs encoded by HSV-1 (Cui et al., 2006; Gupta et al., 2006) also confirms our predictions for that organism substantiating the usefulness of our method for prediction of microRNAs in viral genomes.

**High-throughput experimental validation and expression profiling**

Although the first miRNAs discovered from *C. elegans* were identified by genetics, high-throughput methods of detecting hundreds of miRNAs have become the major route to miRNA discovery today. The methods employed to identify large number of miRNAs simultaneously also provide semi-quantitative information on their expression levels in the source tissue. High-throughput methods of miRNA identification, validation and expression analysis therefore go hand in hand and cross-validate each other.

The two main approaches to large-scale identification of non-coding RNA molecules are, first, large-scale cloning of size-restricted RNA subpopulations separated from total RNA pools and, second, chip-based hybridization methods. In the former method (Lee et al., 1993; Lagos-Quintana et al., 2001; Pfeffer et al., 2005) total RNA isolated from a source of interest is first size fractionated usually by separating it in a polyacrylamide gel matrix. However, the discovery of newer classes of non-coding RNAs of larger than the conventional 21–23 nt limit (Lau et al., 2006) may eventually prove that this restriction is too arbitrary to be of functional significance. The RNA subpopulations are tagged at either end through RNA ligation or polyadenylation (Fu et al., 2005) to adaptors which act as anchors for priming reverse transcriptase-based complimentary strand synthesis. Libraries prepared by large-scale cloning of the reverse-transcribed products allow identification of novel miRNAs. The frequency of occurrence of a miRNA in the library is an estimate of its abundance in the original pool although liable to be affected by factors like the clonability and secondary structure of the RNA. Ligation of the reverse-transcribed fragment into concatamers allows a certain degree of parallelization in miRNA identification. Improvements in sequencing technology like Massive Parallel signature sequencing is expected to support high-throughput miRNA discovery. Currently, at least three high-throughput methods have been applied to identify the expression profiles of miRNAs; in addition to the information on abundance generated indirectly from cloning. These include microarray-based, RNA-primed, Array-based, Klenow Enzyme (RAKE) assay (Nelson et al., 2004; 2006) and Bead based assays.

A combination of computational prediction and microarray-based validation was employed to discover clusters of miRNAs in the BART and BHRF genes of Herpesvirus (Grundhoff et al., 2006). miRNA profiles have been generated using RAKE from HeLa cells transfected with an infectious molecular clone of HIV-1. Forty-three per cent of all miRNAs were downregulated in the infected cells. However, the functional relevance of this downregulation is not understood (Yeung et al., 2005). A subsequent microarray study (Triboulet et al., 2007) in HIV-infected Jurkat cells revealed that upregulation of 11 miRNAs and downregulation of the cancer-associated miR-17/92 cluster could influence virus replication.

**MicroRNAs as biomarker**

The potential of microRNAs as biomarkers has been largely restricted to studies on cancers (Croce and Calin, 2005; He et al., 2005). microRNA expression profiles have been shown to be discriminative of the cancer type (Calin et al., 2004; 2005; Ciafre et al., 2005; Iorio et al., 2005) and also of high prognostic value (Calin et al., 2005) in a number of cancers (Caldas and Brenton, 2005; Eder and Scherr, 2005; Gregory and Shiekhattar, 2005; Esquela-Kerscher and Slack, 2006). For example, miR-155 (BIC) expression is upregulated in Hodgkin’s lymphoma and is downregulated in Burkitt’s lymphoma (Kluiver et al., 2006).

In viral infections, human microRNAs have been thought to be of prognostic value on the basis of differ-
ences in expression levels of a potential antiviral human microRNA between individuals (Hariharan et al., 2005). These microRNAs have the potential to target nef a critical determinant of progression of the disease.

Herpes simplex virus-1 LAT has been shown to encode for microRNAs (Cui et al., 2006; Gupta et al., 2006). EBV, a major oncogenic virus of the Herpesvirus family, also has been shown to encode stage-specific microRNAs (Cai et al., 2006). Of late, Marek’s disease virus (MDV), an oncogenic alpha-herpesvirus of chickens, has been shown to encode microRNAs from latency-associated transcripts (Burnside et al., 2006). The stage-specific expression of microRNAs by viruses could be effectively exploited as a marker to delineate the stage of virus infection especially in neoplastic transformation caused by these viruses (Burnside et al., 2006).

All these reports suggest that microRNAs have good potential to be biomarkers (Cummins and Velculescu, 2006). However, an exhaustive study of their susceptibility to confounding factors would be necessary to critically evaluate their potential as biomarkers. Improvements in methodology have enabled high-throughput profiling of very small quantities of microRNAs, and are a logical step forward for its widespread application as a potential diagnostic.

Anti-microRNAs

Anti-microRNAs emerged as a research tool to delineate functional roles of microRNAs by selectively knocking down microRNAs. The strategy was to design oligonucleotides which are perfectly complementary to the mature miRNA sequence that could titrate away natural miRNAs (Scaria et al., 2007). Backbone modified nucleotides (see below) are a better choice to design the anti-miRNAs which are not only more efficient in blocking the miRNA than the non-modified anti-miRNAs, but also highly specific for specific microRNAs (Krutzfeldt et al., 2005). Anti-microRNAs can be used effectively as a therapeutic and more so against virus-encoded microRNAs as they share little homology with those of human and thus offer few chances for off-target events.

Artificial microRNAs

Artificial microRNAs form the logical next step as an antiviral therapeutic. This may also prove more advantageous than siRNAs as a therapeutic strategy as microRNAs do not need perfect complementarity and can thus tolerate mutations, which would be advantageous given the extreme rate of variations in viral genomes. The strategy would be to logically build microRNAs against ultra conserved regions in the viral transcripts. We have recently developed an algorithm which designs microRNAs for a given sequence using iterative scoring and optimization based on microRNA binding rules (our unpublished results). The nucleotide level scoring and optimization methodology helps in designing highly specific microRNAs, which is a vital consideration while designing therapeutics.

Recent reports on the use of siRNA (Haasnoot and Berkhout, 2006) and short-hairpin RNAs (shRNAs) against viral pathogens like HIV (Konstantinova et al., 2007) show promising results thus opening up a bright future for artificial microRNA therapeutics. Very recently artificial microRNAs have been tried in plants to target groups of genes (Schwab et al., 2006). They have also been shown to be modulated by tissue-specific promoters. Also shRNA libraries modelled on miRNA precursors have been made available encompassing almost the whole transcriptome of human and mouse (Chang et al., 2006) which could be employed as a versatile tool for inducible silencing of particular sets of transcripts.

MicroRNA engineering

Viruses have been classically used for creating stable transfects in gene therapy applications. In the era of synthetic biology, viruses are becoming a promising candidate for microRNA engineering, i.e. to create stable upregulation or downregulation of microRNA levels in cells. The application of microRNA engineering is obvious in stem cell research, especially as microRNAs are known to be critical determinants of cell type (Cheng et al., 2005; Palakodeti et al., 2006; Zhang et al., 2006).

Second application of microRNA engineering would be in synthetic biology to create modular regulatory loops. As microRNAs effects are dependent on nucleotide complementarity, and could be easily included in introns, this offers a new tool to create a simplified protein-free regulatory loop. Recent studies have shown that short RNAs could be engineered into introns (Ying and Lin, 2006). This could easily translate to an effective laboratory tool for elucidation of microRNAs and repression of particular sets of genes as well as may find use as a regulator in engineered pathways in synthetic biology and gene therapy.

Stabilized oligonucleotides

During last couple of decades, exploration and examination of novel structurally modified oligonucleotides, acting as potent and selective therapeutic agents, has gained momentum and led to the development of analogues with desired properties and minimum toxicity. Backbone modifications of nucleic acids offer flexibility in the design and utility of these molecules (Karkare and Bhatnagar, 2006). Among the most widely used modified oligonucleotide
analouges is phosphorothioate (PS) representing an important class of synthetic oligonucleotides where one of the non-bridging oxygens is replaced by sulfur. The sulfurization of the internucleotide bond considerably reduces the action of endo- and exonuclease (Dias and Stein, 2002).

Another class of widely studied modified DNA analogues are the peptide nucleic acids (PNAs) (Nielsen, 2000) that consist of a synthetic peptide backbone formed from \(N-(2\text{-amino-ethyl})\)-glycine units, resulting in an achiral and uncharged molecules. Nevertheless, PNAs can bind to RNA to form sequence-specific hydrogen-bonded structures that are chemically stable and resistant to enzymatic cleavage (Elayadi and Corey, 2001). Another strategy to synthesize modified oligonucleotides is to modify the sugar chemistry. These mainly comprise of nucleotides with alkyl modification at the 2' position of the ribose, e.g. 2'-O-methyl and 2'-O-methoxy ethyl RNA. These are less toxic than PS-oligodeoxynucleotides, possess a higher binding affinity to respective target sequence, can effectively mediate antisense effects by steric block hindrance of translation but lack the ability to activate RNase H (Crooke and Bennett, 1996). Morpholino oligonucleotides (MF), examples of non-ionic DNA analogues in this category, have ribose replaced by a morpholino moiety and phosphoroamidate intersubunit linkages instead of phosphodiester bonds. Most work on morpholino compounds has focused on gene regulation during the development of zebrafish (Nasevicius and Ekker, 2000). MFs do not activate RNase H but can be targeted towards the 5'UTR, or the first 25 bases down-stream of the start codon, to block translation by preventing ribosome binding. Because their backbone is uncharged, MFs are unlikely to form unwanted interactions with nucleic acid-binding proteins. One of the most promising candidates of chemically modified nucleotides developed in the last few years is the locked nucleic acid (LNA) (Kauppinen et al., 2006). LNA bases are ribonucleotide analogues containing a methylene linkage between 2'-oxygen and 4'-carbon of the ribose ring. The constraint on the sugar moiety results in a locked 3'-endo conformation that prepares the base for high-affinity hybridization (Alvarez-Garcia and Miska, 2005; Castoldi et al., 2006). Its close structural resemblance to DNA, high affinity and specificity towards the target strand, high in vivo stability, lack of toxicity, ease of transfection into cells have contributed to its success as a promising tool in therapeutics and functional genomics.

The applications of these backbone modified oligonucleotides in microRNA research are multiple. Backbone modified oligonucleotides have been extensively used as probes for microRNAs. For example, LNA-based probes have been used in Northern blot for efficient detection of microRNAs. DNA oligonucleotides with several positions substituted by LNA residues significantly increased the detection of low abundant miRNAs by at least one order of magnitude. Second, they have been used as stable ant-sense microRNAs (antagomirs) to specifically downregulate one or a set of closely related microRNAs (Krutzenfeldt et al., 2005). The third potential application would be in designing therapeutics.

RNA delivery and cell/tissue type-specific targeting

Targeting of RNA is a major challenge in the way of exploiting the full potential of microRNA-mediated regulation as a therapeutic target (Scaria et al., 2007). The present scenario offers two major methods of targeting small RNAs, which can be effectively exploited in two types of viral infection. Viral vectors could be used to target microRNA or anti-microRNAs in the cell in the case of chronic viral infections and in oncogenic viruses associated with latency as this needs stable and continuous expression, while in the case of other viruses like HIV, HSV, etc., RNA could be targeted to the site of entry of the organism, more so the mucosal membrane.

Retroviral vectors (Brummelkamp et al., 2002) has emerged the mainstay for effective delivery of RNA hairpin precursors compared with plasmid-based vectors as the former has been shown to have stable expression levels in the cell. Recent HIV-based viral transfection vectors can infect both dividing as well as non-dividing cells. Adenovirus-based vectors have been thought to be more safe than HIV-based vectors as they integrate specifically in the AAVS1 region of chromosome 19 (Hamilton et al., 2004).

Recent reports have suggested novel ways to deliver small molecules into the cell. RNA mixed with cationic lipids (Zelphati and Szoka, 1996) has been suggested to be effective enough as they would be easily absorbed through mucosal surfaces (Porteous et al., 1997; Kim et al., 2005). This would be even more advantageous as a large number of human pathogenic viruses infect through mucosal surfaces and could be exploited effectively. For example, in mice models, HSV-2 infection has been shown to be abrogated by topical application of RNAi (Palliser et al., 2006).

Conclusions

The role of microRNAs in cross-talks between the host and the virus is just emerging. A better understanding of the models of the host–virus interaction would enable us not only to have a deeper insight into the complexity of host–virus interaction, but also would aid in designing potential strategies to counter viral infections and their consequences like neoplasia. This would necessitate the integration of better, accurate and fast computational

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algorithms as well as highly accurate and high-throughput techniques for experimental validation. A comprehensive understanding of host–virus interactions mediated by microRNAs would also enable us to make rapid strides into emerging fields like synthetic biology.

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