Parasitol Res (2010) 107:501–507
DOI 10.1007/s00436-010-1927-6

REVIEW

MicroRNAs of parasites: current status and future perspectives

Quan Liu · Wenbin Tuo · Hongwei Gao · Xing-Quan Zhu

Received: 10 May 2010 / Accepted: 18 May 2010 / Published online: 8 June 2010

Abstract MicroRNAs (miRNAs) are a class of endogenous non-coding small RNAs regulating gene expression in eukaryotes at the post-transcriptional level. The complex life cycles of parasites may require the ability to respond to environmental and developmental signals through miRNA-mediated gene expression. Over the past 17 years, thousands of miRNAs have been identified in the nematode *Caenorhabditis elegans* and other parasites. Here, we review the current status and potential functions of miRNAs in protozoan, helminths, and arthropods, and propose some perspectives for future studies.

Introduction

MicroRNAs (miRNAs) are a class of endogenous non-coding small RNAs encoding 22-nucleotide (nt) long RNAs that regulate target mRNAs in plants and animals. The first miRNA, *lin-4*, was identified in a genetic screening for mutations involved in developmental timing in the nematode *Caenorhabditis elegans* in 1993 (Lee et al. 1993). Surprisingly, *lin-4* does not encode a protein, but a novel 22-nt small RNA, which regulates *lin-14* translation through RNA–mRNA interaction, and plays an important role in developmental timing in *C. elegans* (Lee et al. 1993; Wightman et al. 1993). Seven years later, a second 22-nt small RNA of this type, *let-7*, a gene also involved in *C. elegans* developmental timing was discovered (Reinhart et al. 2000). *let-7* regulates developmental timing in *C. elegans* by translational repression of *lin-41* and *hbl-1* through RNA–RNA interactions with their 3′ untranslated regions (Slack et al. 2000; Abrahante et al. 2003; Roush and Slack 2008).

The discovery of *lin-4* and *let-7* small regulatory RNAs was very exciting in the field of life sciences for several reasons. First, homologs of the *let-7* gene were identified in other animals including humans (Lagos-Quintana et al. 2001). The conservation of *let-7* across species suggested an important and fundamental biological role for this small RNA. Second, this type of small RNAs regulated gene expression through specific base-paring with 3′ untranslated regions of target mRNAs (Lee and Ambros 2001; Grosshans and Slack 2002). Third, the mechanism of RNA interference (RNAi), which was mediated by small interfering RNAs (siRNAs) derived from cleavage of long endogenous or exogenous dsRNA, was discovered at that time, and it became clear that miRNA and RNAi pathways share common components (Banerjee and Slack 2002; Hutvagner and Zamore 2002; Tang and Zamore 2004).

Within the following 5 years from the discovery of *let-7*, more than 100 additional small regulatory RNAs similar to *lin-4* and *let-7* were identified in the worm *C. elegans*, the fruit fly *Drosophila*, and humans (Lau et al. 2001; Lai et al.

Q. Liu · H. Gao (✉)
Institute of Military Veterinary,
AMMS Key Laboratory of Jilin Province for Zoonosis Prevention and Control,
1068 Qinglong Road,
Changchun 130062 Jilin Province, People's Republic of China
e-mail: ccltdz@sohu.com

W. Tuo
Animal Parasitic Diseases Laboratory,
Animal and Natural Resources Institute, Agricultural Research Service, United States Department of Agriculture,
Beltsville, MD 20705, USA

X.-Q. Zhu (✉)
Department of Parasitology,
State Key Laboratory of Veterinary Etiological Biology,
Lanzhou Veterinary Research Institute, CAAS,
Lanzhou 730046, Gansu Province, People's Republic of China
e-mail: xingquanzh@scau.edu.cn
e-mail: xingquanzhu1@hotmail.com

2003; Abbott et al. 2005; Bentwich et al. 2005). These small non-coding RNAs were named microRNAs. Subsequently, many more short regulatory RNAs were identified in almost all multicellular organisms, including plants (Rhoades et al. 2002), worms (Ambros 2003), flies (Ambros 2003), fish (Schier and Giraldez 2006), frogs (Tang and Maxwell 2008), and mammals (Smibert and Lai 2008) and in single cellular algae (Zhao et al. 2007) and viruses (Omoto and Fujii 2005; Simon-Mateo and Garcia 2006; Hussain et al. 2008). miRNAs are now considered as a key mechanism of post-transcriptional control within the networks of gene regulation.

Biogenesis of miRNAs

Although mature miRNAs are only about 22-nt long, their biogenesis is complicated. miRNAs are transcribed by RNA polymerase II in larger precursors as primary miRNAs (pri-miRNAs) containing characteristic stem–loop structures that are processed in the nucleus by a complex of the RNase III enzyme Drosha (Borchert et al. 2006). Drosha products are about 65-nt long hairpins called pre-miRNAs. pre-miRNAs are exported into the cytoplasm by Exportin-5 and Ran-GTP, where they are cleaved by the RNase III enzyme Dicer to release the mature miRNAs that are RNA duplexes of about 22-nt in length (Kim 2004). One miRNA strand of the duplex, called the miR strand, is selectively loaded onto an argonaute (AGO) protein, the RNA-induced silencing complex is formed and can now bind to, and repress target mRNAs containing sites of partially complementary to the miRNA (Ronemus et al. 2006; Ding et al. 2009). These miRNAs perform a variety of significant functions of cells such as in growth, metabolism, development, and cell differentiation (Wienholds and Plasterk 2005; Du and Zamore 2007).

miRNAs in protozoan parasites

miRNAs can regulate gene expression in eukaryotes at the post-transcriptional level. miRNAs exist in some protozoan parasites based on following considerations. First, this mechanism is very important for protozoan parasites, by which they could regulate gene expression in host cells to improve their abilities to infect and proliferate via inhibition of host immune responses, and change their gene expression to escape immunologic surveillance (Ong et al. 2006). Second, protozoan parasites belong to eukaryotes with big genome encoding many genes, and abundant antisense RNAs have been identified in certain species (Pascolo et al. 1993; Zamore 2002). Third, RNAi pathway sharing common components with miRNA is positive for some protozoan parasites (Lemos and Menezes 1978; Blackman 2003). Finally, the complex life cycles of many protozoan parasites require the ability to respond to environmental and developmental signals through regulating gene expression (Jolly et al. 2007).

In fact, the miRNA pathway requires AGO and Dicer proteins involved in its biogenesis. AGO- and Dicer-like proteins have been identified in *Trypanosoma congolense*, *Leishmania braziliensis*, *Giardia lamblia*, *Entameoba histolytica*, *Trichomonas vaginalis*, and *Toxoplasma gondii* by a comparative genomics approach (Best et al. 2005; Krautz-Peterson and Skelly 2008; Prucca et al. 2008). However, no AGO- and Dicer-like proteins have been found in the genomes of *Leishmania major*, *Leishmania infantum*, *Trypanosoma cruzi*, *Plasmodium* spp., *Cryptosporidium* spp., *Theileria* spp., *Babesia bovis*, and *Eimeria tenella* (Militello et al. 2008). Thus, some but not all protozoan parasites have the miRNA regulating pathway.

miRNAs in *Trypanosoma brucei*

T. brucei develops chronic infection in mammalian hosts due to antigenic variation. Most of the gene regulation takes place at the post-transcriptional level, which reflects the role of miRNAs in mRNA metabolism (Aitcheson et al. 2005). A total of 1,162 potential miRNAs have been found in *T. brucei* using bioinformatics approaches. The entire surface of the parasite is covered with a dense coat made up of approximately five million dimers of a single antigen, the variant surface glycoprotein (VSG). Individual trypanosomes have hundreds of VSG genes, but only one of which is expressed at a time. The predicted 16 miRNAs are considered to regulate expression of VSGs. Furthermore, a number of miRNA hairpins have been found in clusters of multiple identical copies. The target proteins, 20S proteosome, GM6, and GRESAG 4.2 corresponding to these clustered miRNAs, play essential role in trypanosomiasis (Berberof et al. 1996; To and Wang 1997). These miRNAs can act as genetic switches modulating host–parasite interaction and provide useful clue toward control of trypanosomiasis (Mallick et al. 2008).

miRNAs in *E. histolytica*

E. histolytica is an anaerobic protozoan parasite causing dysentery and liver abscess, and killing an estimated 2.5 million people around the world each year, mostly in tropical countries. *Entamoeba* requires controlled regulation of gene expression for switching between cyst and trophozoite forms not only for its survival inside and outside the host, but also for growth in the liver at high oxygen tension. The presence of miRNA machinery in *E. histolytica* inspired researchers to investigate the presence

of putative miRNAs and their targets in this protozoan parasite (Abed and Ankri 2005; Solis and Guillen 2008). Seventeen putative candidate miRNAs in *E. histolytica* were recently identified using the bioinformatics approaches based on its genome (De et al. 2006). However, the putative miRNA candidates and their functions need further experimental validation.

miRNAs in *G. lamblia*

G. lamblia, one of the earliest branching eukaryotes, is a unicellular and binucleated protozoan responsible for giardiasis in humans. Gene expression is usually regulated at transcriptional and translational levels in higher eukaryotes. However, few consensus promoters have been identified and a simple AT-rich region was sufficient to initiate transcription in *Giardia*, and its mRNAs have exceedingly short 3′ and 5′-UTRs, thus greatly reducing the availability of regulatory sites for translational regulation (Adam 2001). Therefore, *Giardia* represents a unique model for studying the evolution of eukaryotic translational regulation.

The genome analysis showed no homologs of Drosha or Exportin-5 in *Giardia*, which is not necessary for miRNA pathway due to non-complete nuclear envelope of *Giardia* (Adam 2001). However, Dicer and AGO homologs were found in *Giardia* (Prucca et al. 2008). These data raised the possibility that miRNA-mediated translational repression could be one mechanism of gene regulation of this protozoan. Small RNAs of *G. lamblia* were cloned and sequenced, and four miRNAs derived from small nucleolar RNA (snoRNA) were identified, suggesting that the snoRNAs can be precursors of miRNAs in *Giardia* (Saraiya and Wang 2008). To date, miRNA precursors have been identified among non-coding cellular transcripts, 3′-UTRs of mRNAs, introns, transposable elements, and viral transcript (Cullen 2004; Klase et al. 2007; Piriyapongsa and Jordan 2007; Ruby et al. 2007). This unique phenomenon reflects the potential evolutionary significance of *Giardia*. The presence of a snoRNA-derived miRNA-mediated translational repression in *Giardia* has been demonstrated by increase and decrease of miR2 expression (Saraiya and Wang 2008). Additional 50 potential miRNAs, which are unique to *G. lamblia*, have been identified using computational programs (Zhang et al. 2009). This is the first report of cloning and experimental validation of miRNA in protozoan parasite, which provides a novel way for identification and functional analysis of miRNA in other parasites.

miRNAs in *T. vaginalis*

T. vaginalis, belonging to a highly diverged eukaryotic lineage, is the causative agent of trichomoniasis, one of the most common sexually transmitted diseases. *T. vaginalis* contains 59,672 protein-coding genes, 1,136 RNA-coding genes, and 38,201 repeat genes (Aurrecoechea et al. 2009), the largest number of genes among all protozoan genomes, and there is a very stringent control of differentially expressed genes under variable environments, which makes it a very interesting model organism to study cellular processes.

The *Trichomonas* genome encodes at least two AGO proteins which contain AGO specific Piwi domains (Carlton et al. 2007). The expression of Tv-AGO1 (TVAG-453810) and Tv-AGO2 (TVAG-411040) genes were determined by quantitative real-time PCR, suggesting that functional miRNA machinery exists in *T. vaginalis*. Thereafter, a total of nine tva-miRNA (tva-miR-001~009) ranging from 17 to 23 nucleotides long have been identified using direct cloning of miRNA tags and bioinformatics analysis (Lin et al. 2009). However, the exact length of the cloned miRNAs in *T. vaginalis*, target prediction and miRNA–target interaction are not yet known.

miRNAs in other protozoan parasites

Database mining of the predicted coding regions of *T. gondii*, *T. congolense*, and *L. braziliensis* revealed the existence of putative ORFs with convincing homology to the classical RNAi genes, namely potential homologues of AGO and Dicer, which are also the key enzymes in miRNA regulatory pathway (Militello et al. 2008). Furthermore, the expression of the *T. gondii* AGO-like protein (TgAGO) is supported by a number of tachyzoite cDNAs and exhibits unique features. For example, TgAgo is smaller than reported AGO proteins derived from higher eukaryotic organisms, but has a similar size to those from archaeal bacteria. TgAgo contains a conserved PiWi domain and non-conserved PAZ domain, and is mainly localized in the cytoplasm (Al Riyahi et al. 2006). However, the AGO-like proteins of *T. congolense* and *L. braziliensis* have not yet been identified.

However, AGO- and Dicer-like proteins have not been found in *L. major*, *L. infantum*, *T. cruzi*, *Plasmodium* spp., *Cryptosporidium* spp., *Theileria* spp., *B. bovis*, and *E. tenella* using bioinformatics analysis. Recently, the absence of miRNAs in *Plasmodium falciparum* has been experimentally validated by small RNA sequencing, which confirms with the absence of AGO/Dicer genes (Xue et al. 2008b). We can therefore conclude that the miRNA-mediated gene regulation may be absent in protozoan without AGO/Dicer proteins.

miRNAs in helminth parasites

Parasites of the class trematodes, especially *Schistosoma mansoni* and *Schistosoma japonicum* with a complex life

cycle and a unique repertoire of genes expressed at different life cycle stages, are important eukaryotic pathogens of humans. miRNAs may be involved in growth, development, and cell differentiation of these parasites.

miRNAs in *S. mansoni*

Genomics and transcriptomics studies have revealed several ESTs in schistosomes with homology to Dicer and AGO, protein components of the miRNA silencing pathway. miRNA pathway in *S. mansoni* has recently been proven positive by bioinformatic approaches. A total of 13 putative proteins related to miRNA pathway in *S. mansoni* were found using amino acid sequences of well-known proteins involved in the miRNA pathway against *S. mansoni* genome and transcriptome databases. These proteins participate in processing of miRNA precursor (SmDicer1), processing of primary miRNA transcripts (SmDrosha1/2), short RNA binding (SmAgo1/2/3/4), and nuclear export of miRNA precursors (Exportin-5-like protein; Gomes et al. 2009).

The expression of SmDicer1 and SmAgo2/3/4 transcripts is significantly different in different developmental stages of *S. mansoni*. The expression of SmDicer1 increases significantly from cercariae to schistosomula stage following the mechanical transformation. For SmAgo2/3/4 transcripts, a significant decreases from cercariae to MTS-8.5 followed by an increase in MTS-18.5 peaking at MTS-48 stage and then declining in the MTS-72. SmDicer1 and SmAgo2/3/4 transcripts reach their high expression levels in the egg stage (Krautz-Peterson and Skelly 2008; Gomes et al. 2009). In addition, various groups have used the RNAi technique to regulate gene expression in sporocysts, schistosomula, and adult worms (Boyle et al. 2003). All data have demonstrated that *S. mansoni* has miRNA-mediated gene regulation mechanism. However, the specific miRNAs in this parasite have not been reported.

miRNAs in *S. japonicum*

In *S. japonicum*, five novel miRNAs were identified and designated as sja-let-7, sja-miR-71, sja-bantam, sja-miR-125, and sja-miR-new1. The expression patterns of these miRNAs are highly stage-specific, especially sja-miR-71 and sja-bantam, where expression reaches a peak in the cercaria stage and then drops quickly in the schistosomulum stage. These findings suggest that these miRNAs are involved in schistosome infection, growth, and development (Xue et al. 2008a). Further work is required to identify the target mRNAs of these novel miRNAs and elucidate the functions of newly identified miRNAs in *S. japonicum*.

miRNAs in arthropods

The mosquitoes *Anopheles gambiae* and *Anopheles stephensi* are the principal vectors of malaria in Africa and Asia. Understanding the functions of mosquito miRNAs will contribute to a better understanding of mosquito biology, such as longevity, reproduction, and mosquito–pathogen interactions, which are important to disease transmission.

About 91 candidate miRNAs, along with their pre-miRNAs, were identified in *A. gambiae* by searching the homologues of known *Drosophila melanogaster* miRNAs, in which 41 predicted miRNAs are known *D. melanogaster* miRNAs and the remaining 50 miRNAs are potential novel *A. gambiae* miRNAs (Chatterjee and Chaudhuri 2006). In *A. stephensi*, the four novel miRNAs (miR-x1-miR-x4) have been identified. The expression of miR-x2 is restricted to adult females and predominantly in the ovaries. A significant reduction of miR-x2 level is observed 72 h after a bloodmeal. Therefore, miR-x2 is likely to be involved in female reproduction and its function may be conserved among divergent mosquitoes (Mead and Tu 2008). A mosquito homolog of miR-14, a regulator of longevity and apoptosis in *D. melanogaster*, represented 25% of all sequenced miRNA clones from 17-day-old *A. stephensi* female mosquitoes. *A. stephensi* miR-14 expression is consistent during the adult lifespan regardless of age, sex, and blood-feeding status (Mead and Tu 2008). Thus, miR-14 is likely to be important across all mosquito life stages.

Potential functions of miRNAs in parasites

The discovery of miRNAs is one of the major scientific breakthroughs in recent years, and hundreds of miRNAs have been identified in various eukaryotic organisms. These tiny regulators of gene expression have unique tissue-, developmental-stage-, and disease-specific patterns. Although the functions of most miRNAs discovered have not been determined, primary functions of miRNAs suggest that miRNAs regulate some kinds of physiological and pathological processes in parasites.

miRNAs regulate a variety of developmental and physiological processes in parasites. For example, sja-let-7 might take part in the transformation from miracidium to sporocyst in the snail intermediate host; sja-bantam might take part in developmental processes throughout the lifespan of *S. japonicum* (Xue et al. 2008a); miR-14 is likely important across all mosquito life stages from embryos to aged adults (Mead and Tu 2008). Nevertheless, miRNAs may take part in pathogenic process of parasites. *T. brucei* miRNAs modulate expression of VSGs related to immune evasion, and *T. brucei* miRNA target proteins 20S proteosome, GM6, and GRESAG 4.2 are involved in

trypanosomiasis (Mallick et al. 2008). In *Giardia*, miR2 may contribute to the pathogenicity by regulating expression of the 22 VSP genes (Saraiya and Wang 2008).

While parasite miRNAs can regulate their own gene expression, it is likely that parasite miRNAs also regulate expression of the host genes. Both parasites and hosts encode miRNAs, by which the parasites could make cellular environment highly susceptible to their propagation and survival, and hosts could make cellular environment unsusceptible to parasite survival. For example, *let-7i* regulates TLR4 expression in cholangiocytes and contributes to epithelial immune responses against *Cryptosporidium parvum* infection (Chen et al. 2007); knocking down Dicer1 and AGO1 mRNAs led to an increased sensitivity to *Plasmodium* infection (Winter et al. 2007).

Perspectives

To date, approximately 1,300 potential miRNAs have been identified in parasites and their number is still increasing. Due to the approaches used for their discovery, miRNAs with abundant and widespread expression have been found first. Further work aimed at identifying the target mRNAs of these novel miRNAs will be needed to interpret functions of newly identified miRNAs.

Because of the complex life cycles of parasites with several developmental stages in vertebrate and invertebrate hosts and a unique repertoire of genes expressed at different developmental stages, it is particularly important to elucidate the roles of miRNAs in the growth and development of parasites and their abilities to regulate infection of mammalian hosts. Clarifying of gene regulation based on miRNAs will contribute to dissection of the biological basis of antigenic variation and immune evasion for parasites.

Recent findings that antisense oligonucleotides can specifically block miRNA will trigger efforts to explore miRNAs as a potential new class of therapeutics (Zhang and Farwell 2008). We can anticipate the discovery of more and more cell-type- or developmental-stage-specific miRNAs with highly specialized functions related to the cellular processes in parasites in the coming years, which may provide novel therapeutics for parasitic diseases.

Acknowledgments This work is supported, in part, by grants from the National Basic Research Program of China (Grant no. 2007CB513104), National Natural Science Foundation of China (Grant no. 30972178), the National Special Research Program for Non-Profit Trades (Agriculture; Grant no. 200803017), the Program for Changjiang Scholars and Innovative Research Team in University (Grant no. IRT0723), National Key Technology R&D Program of China (Grant no. 2010BAD04B01), and "Gold Idea" Foundation of Institute of Military Veterinary, Academy of Military Medical Sciences (Grant no. YCX0901).

References

Abbott AL, Alvarez-Saavedra E, Miska EA, Lau NC, Bartel DP, Horvitz HR, Ambros V (2005) The let-7 microRNA family members mir-48, mir-84, and mir-241 function together to regulate developmental timing in *Caenorhabditis elegans*. Dev Cell 9:403–414

Abed M, Ankri S (2005) Molecular characterization of *Entamoeba histolytica* RNase III and AGO2, two RNA interference hallmark proteins. Exp Parasitol 110:265–269

Abrahante JE, Daul AL, Li M, Volk ML, Tennessen JM, Miller EA, Rougvie AE (2003) The *Caenorhabditis elegans* hunchback-like gene lin-57/hbl-1 controls developmental time and is regulated by microRNAs. Dev Cell 4:625–637

Adam RD (2001) Biology of *Giardia lamblia*. Clin Microbiol Rev 14:447–475

Aitcheson N, Talbot S, Shapiro J, Hughes K, Adkin C, Butt T, Sheader K, Rudenko G (2005) VSG switching in *Trypanosoma brucei*: antigenic variation analysed using RNAi in the absence of immune selection. Mol Microbiol 57:1608–1622

Al Riyahi A, Al-Anouti F, Al-Rayes M, Ananvoranich S (2006) Single argonaute protein from *Toxoplasma gondii* is involved in the double-stranded RNA induced gene silencing. Int J Parasitol 36:1003–1014

Ambros V (2003) MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. Cell 113:673–676

Aurrecoechea C, Brestelli J, Brunk BP, Carlton JM, Dommer J, Fischer S et al (2009) GiardiaDB and TrichDB: integrated genomic resources for the eukaryotic protist pathogens *Giardia lamblia* and *Trichomonas vaginalis*. Nucleic Acids Res 37: D526–D530

Banerjee D, Slack F (2002) Control of developmental timing by small temporal RNAs: a paradigm for RNA-mediated regulation of gene expression. Bioessays 24:119–129

Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O et al (2005) Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 37:766–770

Berberof M, Pays A, Lips S, Tebabi P, Pays E (1996) Characterization of a transcription terminator of the procyclin PARP A unit of *Trypanosoma brucei*. Mol Cell Biol 16:914–924

Best A, Handoko L, Schluter E, Goringer HU (2005) In vitro synthesized small interfering RNAs elicit RNA interference in african trypanosomes: an in vitro and in vivo analysis. J Biol Chem 280:20573–20579

Blackman MJ (2003) RNAi in protozoan parasites: what hope for the Apicomplexa? Protist 154:177–180

Borchert GM, Lanier W, Davidson BL (2006) RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol 13: 1097–1101

Boyle JP, Wu XJ, Shoemaker CB, Yoshino TP (2003) Using RNA interference to manipulate endogenous gene expression in *Schistosoma mansoni* sporocysts. Mol Biochem Parasitol 128:205–215

Carlton JM, Hirt RP, Silva JC, Delcher AL, Schatz M, Zhao Q et al (2007) Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*. Science 315:207–212

Chatterjee R, Chaudhuri K (2006) An approach for the identification of microRNA with an application to *Anopheles gambiae*. Acta Biochim Pol 53:303–309

Chen XM, Splinter PL, O'Hara SP, LaRusso NF (2007) A cellular micro-RNA, let-7i, regulates Toll-like receptor 4 expression and contributes to cholangiocyte immune responses against *Cryptosporidium parvum* infection. J Biol Chem 282:28929–28938

Cullen BR (2004) Derivation and function of small interfering RNAs and microRNAs. Virus Res 102:3–9

De S, Pal D, Ghosh SK (2006) *Entamoeba histolytica*: computational identification of putative microRNA candidates. Exp Parasitol 113:239–243

Ding XC, Weiler J, Grosshans H (2009) Regulating the regulators: mechanisms controlling the maturation of microRNAs. Trends Biotechnol 27:27–36

Du T, Zamore PD (2007) Beginning to understand microRNA function. Cell Res 17:661–663

Gomes MS, Cabral FJ, Jannotti-Passos LK, Carvalho O, Rodrigues V, Baba EH, Sa RG (2009) Preliminary analysis of miRNA pathway in *Schistosoma mansoni*. Parasitol Int 58:61–68

Grosshans H, Slack FJ (2002) Micro-RNAs: small is plentiful. J Cell Biol 156:17–21

Hussain M, Taft RJ, Asgari S (2008) An insect virus-encoded microRNA regulates viral replication. J Virol 82:9164–9170

Hutvagner G, Zamore PD (2002) A microRNA in a multiple-turnover RNAi enzyme complex. Science 297:2056–2060

Jolly ER, Chin CS, Miller S, Bahgat MM, Lim KC, DeRisi J, McKerrow JH (2007) Gene expression patterns during adaptation of a helminth parasite to different environmental niches. Genome Biol 8:R65

Kim VN (2004) MicroRNA precursors in motion: exportin-5 mediates their nuclear export. Trends Cell Biol 14:156–159

Klase Z, Kale P, Winograd R, Gupta MV, Heydarian M, Berro R, McCaffrey T, Kashanchi F (2007) HIV-1 TAR element is processed by Dicer to yield a viral micro-RNA involved in chromatin remodeling of the viral LTR. BMC Mol Biol 8:63

Krautz-Peterson G, Skelly PJ (2008) *Schistosoma mansoni*: the dicer gene and its expression. Exp Parasitol 118:122–128

Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T (2001) Identification of novel genes coding for small expressed RNAs. Science 294:853–858

Lai EC, Tomancak P, Williams RW, Rubin GM (2003) Computational identification of *Drosophila* microRNA genes. Genome Biol 4: R42

Lau NC, Lim LP, Weinstein EG, Bartel DP (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. Science 294:858–862

Lee RC, Ambros V (2001) An extensive class of small RNAs in *Caenorhabditis elegans*. Science 294:862–864

Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–854

Lemos MV, Menezes H (1978) The effect of an immune RNA (RNAi) against *Trypanosoma cruzi* infection in mice. Tropenmed Parasitol 29:119–126

Lin WC, Haraguchi T, Ozaki Y, Iba H (2009) Identification of microRNA in the protist *Trichomonas vaginalis*. Genomics 93:487–493

Mallick B, Ghosh Z, Chakrabarti J (2008) MicroRNA switches in *Trypanosoma brucei*. Biochem Biophys Res Commun 372: 459–463

Mead EA, Tu Z (2008) Cloning, characterization, and expression of microRNAs from the Asian malaria mosquito, *Anopheles stephensi*. BMC Genomics 9:244

Militello KT, Refour P, Comeaux CA, Duraisingh MT (2008) Antisense RNA and RNAi in protozoan parasites: working hard or hardly working? Mol Biochem Parasitol 157:117–126

Omoto S, Fujii YR (2005) Regulation of human immunodeficiency virus 1 transcription by nef microRNA. J Gen Virol 86:751–755

Ong SJ, Hsu HM, Liu HW, Chu CH, Tai JH (2006) Multifarious transcriptional regulation of adhesion protein gene ap65-1 by a novel Myb1 protein in the protozoan parasite *Trichomonas vaginalis*. Eukaryot Cell 5:391–399

Pascolo E, Blonski C, Shire D, Toulme JJ (1993) Antisense effect of oligodeoxynucleotides complementary to the mini-exon sequence of the protozoan parasite *Leishmania amazonensis*. Biochimie 75:43–47

Piriyapongsa J, Jordan IK (2007) A family of human microRNA genes from miniature inverted-repeat transposable elements. PLoS one 2:e203

Prucca CG, Slavin I, Quiroga R, Elias EV, Rivero FD, Saura A, Carranza PG, Lujan HD (2008) Antigenic variation in *Giardia lamblia* is regulated by RNA interference. Nature 456:750–754

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G (2000) The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature 403:901–906

Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP (2002) Prediction of plant microRNA targets. Cell 110: 513–520

Ronemus M, Vaughn MW, Martienssen RA (2006) MicroRNA-targeted and small interfering RNA-mediated mRNA degradation is regulated by argonaute, dicer, and RNA-dependent RNA polymerase in Arabidopsis. Plant Cell 18:1559–1574

Roush S, Slack FJ (2008) The let-7 family of microRNAs. Trends Cell Biol 18:505–516

Ruby JG, Jan CH, McConkey GA (2007) Intronic microRNA precursors that bypass Drosha processing. Nature 448:83–86

Saraiya AA, Wang CC (2008) snoRNA, a novel precursor of microRNA in *Giardia lamblia*. PLoS Pathog 4:e1000224

Schier AF, Giraldez AJ (2006) MicroRNA function and mechanism: insights from zebra fish. Cold Spring Harb Symp Quant Biol 71:195–203

Simon-Mateo C, Garcia JA (2006) MicroRNA-guided processing impairs Plum pox virus replication, but the virus readily evolves to escape this silencing mechanism. J Virol 80:2429–2436

Slack FJ, Basson M, Liu Z, Ambros V, Horvitz HR, Ruvkun G (2000) The lin-41 RBCC gene acts in the *C. elegans* heterochronic pathway between the let-7 regulatory RNA and the LIN-29 transcription factor. Mol Cell 5:659–669

Smibert P, Lai EC (2008) Lessons from microRNA mutants in worms, flies and mice. Cell Cycle 7:2500–2508

Solis CF, Guillen N (2008) Silencing genes by RNA interference in the protozoan parasite *Entamoeba histolytica*. Methods Mol Biol 442:113–128

Tang GQ, Maxwell ES (2008) Xenopus microRNA genes are predominantly located within introns and are differentially expressed in adult frog tissues via post-transcriptional regulation. Genome Res 18:104–112

Tang G, Zamore PD (2004) Biochemical dissection of RNA silencing in plants. Methods Mol Biol 257:223–244

To WY, Wang CC (1997) Identification and characterization of an activated 20S proteasome in *Trypanosoma brucei*. FEBS Lett 404:253–262

Wienholds E, Plasterk RH (2005) MicroRNA function in animal development. FEBS Lett 579:5911–5922

Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell 75:855–862

Winter F, Edaye S, Huttenhofer A, Brunel C (2007) *Anopheles gambiae* miRNAs as actors of defence reaction against Plasmodium invasion. Nucleic Acids Res 35:6953–6962

Xue X, Sun J, Zhang Q, Wang Z, Huang Y, Pan W (2008a) Identification and characterization of novel microRNAs from *Schistosoma japonicum*. PLoS One 3:e4034

Xue X, Zhang Q, Huang Y, Feng L, Pan W (2008b) No miRNA were found in *Plasmodium* and the ones identified in erythrocytes could not be correlated with infection. Malar J 7:47

Zamore PD (2002) Ancient pathways programmed by small RNAs. Science 296:1265–1269

Zhang B, Farwell MA (2008) microRNAs: a new emerging class of players for disease diagnostics and gene therapy. J Cell Mol Med 12:3–21

Zhang YQ, Chen DL, Tian HF, Zhang BH, Wen JF (2009) Genome-wide computational identification of microRNAs and their targets in the deep-branching eukaryote *Giardia lamblia*. Comput Biol Chem 33:391–396

Zhao T, Li G, Mi S, Li S, Hannon GJ, Wang XJ, Qi Y (2007) A complex system of small RNAs in the unicellular green alga *Chlamydomonas reinhardtii*. Genes Dev 21:1190–1203