JOURNAL OF CELLULAR PHYSIOLOGY 210:279–289 (2007)

MINI REVIEW

MicroRNAs and Their Regulatory Roles in Animals and Plants

BAOHONG ZHANG,[1,2]* QINGLIAN WANG,[2] AND XIAOPING PAN[1]

[1]The Institute of Environmental and Human Health, and Department of Environmental Toxicology, Texas Tech University, Lubbock, Texas

[2]Henan Institute of Science and Technology, Xinxiang, Henan, P.R. China

microRNAs (miRNAs) are an abundant class of newly identified endogenous non-protein-coding small RNAs. They exist in animals, plants, and viruses, and play an important role in gene silencing. Translational repression, mRNA cleavage, and mRNA decay initiated by miRNA-directed deadenylation of targeted mRNAs are three mechanisms of miRNA-guided gene regulation at the post-transcriptional levels. Many miRNAs are highly conserved in animals and plants, suggesting that they play an essential function in plants and animals. Lots of investigations indicate that miRNAs are involved in multiple biological processes, including stem cell differentiation, organ development, phase change, signaling, disease, cancer, and response to biotic and abiotic environmental stresses. This review provides a general background and current advance on the discovery, history, biogenesis, genomics, mechanisms, and functions of miRNAs. J. Cell. Physiol. 210: 279–289, 2007. © 2006 Wiley-Liss, Inc.

MicroRNAs (miRNAs) are an abundant class of newly identified endogenous non-protein-coding small RNAs with 20–25 nucleotide length (Ambros, 2001, 2004; Carrington and Ambros, 2003; Bartel, 2004). A majority of identified miRNAs are highly evolutionarily conserved among many distantly related species, some from worms to human in animals (Pasquinelli et al., 2000), and mosses to high flowering eudicots in plants (Axtell and Bartel, 2005; Zhang et al., 2006c), suggesting that miRNAs play a very important role in essential biological processes, including developmental timing (Lee et al., 1993), stem cell differentiation (Houbaviy et al., 2003; Hatfield et al., 2005; Zhang et al., 2006b), signaling transduction (Guo et al., 2005; Karp and Ambros, 2005; Kwon et al., 2005), disease (Labourier et al., 2004; Alvarez-Garcia and Miska, 2005), and cancer (Hayashita et al., 2005; Lu et al., 2005b). Currently, miRNAs have been considered one of the most important regulatory molecules, which regulate gene expression at the posttranscriptional levels by targeting mRNAs for direct cleavage of mRNAs or repression of mRNA translation.

HISTORY

One decade ago, two research groups surprisingly discovered that a small 21-nucleotide RNA molecule called *lin-4* controls developmental timing in *Caenorhabditis elegans* by the posttranscriptional regulation of the heterochronic gene *lin-14* (Lee et al., 1993; Wightman et al., 1993) which plays an important role in controlling the temporal pattern formation (Ambros and Horvitz, 1987). They also observed that *lin-4* didnot code protein, and contained antisense sequences complementary to a repeated sequence element in the 3′ untranslated region (UTR) of the *lin-14* mRNA (Lee et al., 1993; Wightman et al., 1993). Although they hypothesized that *lin-4* downregulated *lin*-14 expression via an antisense RNA–RNA interaction (Lee et al., 1993; Wightman et al., 1993), almost all scientists did not pay any attention on this new class of small RNAs, and a majority of them considered it as an oddity in *C. elegans* genome. Seven years later, *let-7* was discovered as another small regulatory RNA in *C. elegans* with an exactly same regulatory mechanism as *lin-4*, and *let-7* regulates gene expression which controls developmental timing and cellular differentiation in *C. elegans* (Reinhart et al., 2000). More interestingly, both sequence and developmental expression pattern of *let-7* were highly conserved in a wide range of animal species, including vertebrate, ascidian, hemichordate, mollusc, annelid, and arthropod (Pasquinelli et al., 2000), suggesting that *let-7* plays more important roles in biological processes than our previous thought. This finding attracted lots of attention from scientists, evidence by three papers related to this class of small RNA were published in a same issue of the most famous journal *Science* in the following year. In the three papers, an extensive number of small RNAs similar to *lin-4* and *let-7* were identified in invertebrates and vertebrates as well as in *C. elegans* (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001). It was also the first time to recognize this abundant class of newly identified small RNAs as miRNAs. At that time, miRNAs *lin-4* and *let-7* were recognized as the founding members of miRNAs.

Since miRNAs were recognized in 2001, the broad significance of these new identified small RNAs is becoming clear and is being fully appreciated, because more and more evidences suggest that miRNAs play an essential role in multiple biological processes. In the past 5 years, a huge amount of papers related to miRNA research have been published, and miRNA-related research has become one of the hottest research fields in biology. Currently, about 4,000 of miRNAs have been identified in a variety of animals, plants, and viruses, and have been deposited in publicly available databases, such as miRBase (Griffiths-Jones et al., 2006). Computational approaches have estimated that organisms probably contain about 1–5% miRNA genes of the total protein-coding genes (Lai et al., 2003; Lim et al., 2003b;

*Correspondence to: Baohong Zhang, The Institute of Environmental and Human Health, and Department of Environmental Toxicology, Texas Tech University, Lubbock, TX 79409-1163.
E-mail: baohong.zhang@ttu.edu

Received 12 August 2006; Accepted 17 August 2006

DOI: 10.1002/jcp.20869

Lewis et al., 2005), and about 30% of protein-coding genes may be regulated by miRNAs (Lewis et al., 2005). Right now, the mechanisms of miRNA-guided gene regulation and the functions of miRNAs in animals and plants are becoming clear. All of these achievements benefit from the following technological progress.

First, cloning technology makes it possible to identify new miRNAs or to confirm miRNAs predicted by computational approaches. Although the founding members of miRNAs, *lin-4* and *let-7*, were identified by a genetic screening technology (Lee et al., 1993; Wightman et al., 1993; Pasquinelli et al., 2000), the application of this method is limited because it is expensive, time consuming, and dominated by chance. Recently, scientists have improved the cloning technology to better clone small RNAs including miRNAs from different organisms. Using the modified method, lots of new miRNAs have been identified in animals (Lagos-Quintana et al., 2001; Fu et al., 2005) and plants (Lu et al., 2005a; Sunkar et al., 2005). One of the most important advantages of direct cloning is that this is a unique method to clone new miRNAs, especially for tissue-specific or species-specific miRNAs, no matter whose genomes are encoded or not. Currently, several companies have developed commercial kits for miRNA isolation and cloning. The modified cloning method usually includes the following five steps: isolating small RNAs from biological samples, ligating the isolated small RNAs to an adaptor of oligonucleotides, reverse transcription of the ligated small RNAs, amplification by PCR, and sequencing.

Second, computational approaches provide a useful complement method to direct cloning, and the application of computational approaches has dramatically increased the numbers of identified miRNAs. At present, several computational programs, such as miRScan (Lim et al., 2003b), miRseeker (Lai et al., 2003), findMiRNA (Adai et al., 2005), and miRAlign (Wang et al., 2005b), have been developed and successfully applied to predict miRNAs in a various animal and plant species.

Third, expressed sequence tag (EST) and genomic sequence survey (GSS) analysis enhances the traditional computational approaches and genetic approaches to find miRNA homologs. Although computational approaches are good approaches to predict miRNAs, the application of these methods are limited because they require genome sequences which are only available for several model organisms, such as human and *C. elegens* in animals, and *Arabidopsis thaliana* and rice in plants. For many identified miRNAs, they are evolutionarily conserved from species to species, suggesting a powerful tool to identify miRNA homologs using the huge EST and GSS databases which are publicly available. EST analysis has been employed to identify 100s of miRNAs in animals (Weber, 2005) and plants (Zhang et al., 2005, 2006c; Dezulian et al., 2006). EST analysis was also used to find some evidences that miRNAs are conserved among different species (Floyd and Bowman, 2004; Jones-Rhoades and Bartel, 2004). One important advantage of EST analysis is that mining EST databases in a systematic way could provide a deeper insight in the distribution and conservation of miRNAs, and EST analysis can be used to identify miRNAs in any species no matter their genome is encoded or not. Using EST and GSS analysis, Zhang and colleagues (2006) identified a total of 481 miRNAs, belonging to 37 miRNA families in 71 different plant species, and found that many miRNA families were evolutionarily conserved across all major lineages of plants, including mosses, gymnosperms, monocots, and eudicots (Zhang et al., 2006a,c). This finding suggests that regulation of gene expression by miRNAs appears to have existed at the earliest stages of plant evolution and has been tightly constrained (functionally) for more than 425 million years (Zhang et al., 2006c).

Fourth, quantitive PCR and microarray technology make it possible to better study the expression patterns and functions of miRNAs. Hundreds of miRNAs have been identified in a single cell, it is impossible to detect the expression patterns of all miRNAs in a single time using traditional Northern blotting, which is important to study the functions of miRNAs in multiple biological processes, especially in disease and cancer. miRNA-specific quantitive PCR and microarray technology make this study become easier. In the past 1 year, several quantitive PCR (Chen et al., 2005; Raymond et al., 2005; Shi and Chiang, 2005) and microarray technology (Babak et al., 2004; Barad et al., 2004; Nelson et al., 2004b; Thomson et al., 2004; Liang et al., 2005) have been developed, and successfully used to study the functions of miRNAs in organism development (Miska et al., 2004), disease and cancer (Lu et al., 2005b). At present, several companies have developed miRNA chips for studying the expression profiles at a specific situation.

MicroRNA biogenesis

Mature miRNAs only contain ~20–22 nucleotides. However, the genes coding miRNAs are much longer than mature miRNAs; they usually contain several 10s of nucleotides even 100s of nucleotides. The length of miRNA genes varies from miRNAs to miRNAs and from species to species. For example, miRNA genes in plant species are usually longer than in animals.

It is a multiple-step biological process to generate a mature miRNA from a miRNA gene, and several enzymes play critical roles in the process. First, miRNA genes are transcribed to primary miRNAs (pri-miRNAs). This process is facilitated by polymerase II enzyme (pol II), and a polymerase II-depend promoter was identified in several miRNA genes (Lee et al., 2004). Lots of miRNA genes exist in plants and animals. Computational approaches establish that the number of miRNA genes may be more than 1–5% of the total protein-coding genes (Lai et al., 2003; Lim et al., 2003a,b; Lewis et al., 2005). Generally, miRNA genes exist in any location of a genome, including introns and exons. However, a majority of the characterized miRNA genes are located at the internal space of two protein-coding genes and they have a different transcribed direction to neighboring protein-coding genes. This indicates that miRNA genes may be transcribed as independent units. However, there is still some evidence indicating that miRNAs may be transcribed together with protein-coding genes. Smalheiser (2003) found that pri-miRNA-mRNA transcripts existed in human and mouse EST databases. Like protein-coding mRNAs, pri-miRNAs can be spliced, may be caped by 7-methyl guanosine at its 5′ site, and added a polyadenylated tail at its 3′ site (Cai et al., 2004) although they may not contain an open reading frame (ORF). After pol II transcribes miRNA genes, the 5′ capped and 3′ polyadenylated pri-miRNA forms a specific hairpin-shaped stem-loop secondary structure and enters a large complex called microprocessor complex (500–650 kDa) which constitutes of a Drosha (a RNase III endonuclease) and an essential cofactor DGCR8/Pasha (a protein contains two double-stranded

RNA binding domains) (Denli et al., 2004; Gregory et al., 2004; Han et al., 2004a; Landthaler et al., 2004). In this new identified microprocessor complex, DGCR8 first recognizes the distinct stem-loop structures and binds to the pri-miRNAs (Denli et al., 2004; Gregory et al., 2004; Han et al., 2004a; Landthaler et al., 2004), then Drosha asymmetrically and specifically cut the both strands of the hairpin-shaped stem at the sites near the base of the stem loop; and finally release a 60- to 70-nt pre-miRNAs that have a 5′ phosphate and a 3′ 2 nt overhang. This mechanism has been deeply understood in animals (Denli et al., 2004; Gregory et al., 2004; Han et al., 2004a; Landthaler et al., 2004). The pre-miRNAs are then transported to the cytoplasm by Exportin-5 (Exp5) (a member of the Ran transport receptor family) (Bohnsack et al., 2004; Yi et al., 2003; Lund et al., 2004). This transport process requires energy, a specific hairpin secondary structure, and another important factor Ran (Yi et al., 2003; Bohnsack et al., 2004; Lund et al., 2004). Once in the cytoplasm, pre-miRNAs are further processed by Dicer, a second RNase III endonuclease (Grishok et al., 2001; Hutvagner et al., 2001; Ketting et al., 2001). In this step, the PAZ domain of Dicer was thought to first recognize the 2-nucleotide 3′ overhang, then Dicer cuts off the about 20 ~ 22 nucleotide length double strand miRNA:miRNA* duplex with 5′ phosphate and a 3′ 2 nt overhang from the end of the hairpin structure stem (Zhang et al., 2004). Finally, miRNA:-miRNA* duplex is unwound by helicase into two single strands, mature miRNA and miRNA*, and miRNA* is degraded by an unknown enzyme nuclease while mature miRNA is incorporated into an ribonucleoprotein effector complex known as RNA-induced silencing complex (RISC) which induces gene silence at a posttranscriptional level (Schwarz et al., 2003; Hammond, 2005; Khvorova et al., 2003).

Although miRNA biogenesis in plants is similar within animals, both are transcribed from miRNA genes by pol II, and sequentially produce pri-miRNAs and pre-miRNAs, and finally form mature miRNAs. However, there are several differences between animal miRNA biogenesis and plant miRNA biogenesis. First, no homolog of Drosha and DGCR8/Pasha have been identified in plants, so the similar mechanism processing pri-miRNAs to pre-miRNAs in plants is still unclear. However, evidences have demonstrated that plant pre-miRNA formation is mediated by the Dicer-like protein 1 (DCL1) (Park et al., 2002; Reinhart et al., 2002; Papp et al., 2003; Kurihara and Watanabe, 2004). With the helps of other factors, such as HYL1 (a two dsRBD-containing nuclear protein) (Han et al., 2004b; Vazquez et al., 2004) and HEN1 (a protein with a dsRBD and a methyltransferase domain) (Boutet et al., 2003; Park et al., 2005; Yu et al., 2005), DCL1 further cleaves a pre-miRNA to a miRNA:miRNA* duplex in nucleus instead of in cytoplasm which usually happen in animal miRNA biogenesis (Park et al., 2002; Reinhart et al., 2002; Papp et al., 2003; Kurihara and Watanabe, 2004). Recent study shows that it is a crucial step in plant miRNA biogenesis to methylate miRNA:miRNA* duplex at the 2′ hydroxyl groups on the 3′ most nucleotides (Yu et al., 2005). However, the biochemical function of the 2′ hydroxyl groups on miRNA biogenesis and similar mechanism existing in other organisms are still unclear. Following the formation of miRNA: miRNA* duplex, the duplex is transported to cytoplasm by HASTY, the plant ortholog of *exp5* (Park et al., 2005), and then unwound by helicase and release mature miRNAs for mediating gene expression.

In RISC complex, miRNAs bind to targeted messenger RNA (mRNA) and inhibit gene expression by direct cleavage of targeted mRNAs or repression of translation through perfect or near-perfect complementarity between the miRNAs and the targeted mRNAs.

Mechanisms of microRNA-mediated gene regulation

Three mechanisms have been described for miRNA-mediated gene regulation: mRNA degradation, translational repression, and miRNA-mediated mRNA decay. No matter what kind of mechanisms, all miRNAs regulate gene expression at the posttranscriptional level.

Translational repression and mRNA degradation are two common mechanisms for miRNA-mediated gene regulation. In most cases, it is governed by the complementarity between miRNAs and targeted mRNAs. When an miRNA perfectly or near-perfectly pairs to the targeted mRNAs, it was thought that mRNA cleavage is the primary mechanism for miRNA-mediated gene regulation (Rhoades et al., 2002; Bartel, 2004). Otherwise, if a miRNA imperfectly pairs to its targeted mRNAs, translational repression is thought to be occurred. The degree of repression is associated with the number of miRNA-binding sites in a targeted mRNA (Cuellar and McManus, 2005). In translational repression, a majority of miRNAs bind to their targeted mRNAs at the 3′ UTRs; however, some miRNAs can also bind to the 5′ UTR and/or the ORF (Zeng et al., 2002; Doench and Sharp, 2004). Although the precise mechanism of miRNA-mediated translational repression has not been elucidated, studies suggest that miRNA may hamper ribosome movement along the mRNAs, and repress protein translation (Carrington and Ambros, 2003). However, not all miRNAs follow this role to regulation gene expression. For example, *miR 172* regulates gene expression by repressing translation although it can perfectly complement to the targeted *APETALA2* (*AP2*) mRNA (Aukerman and Sakai, 2003; Chen, 2004).

A majority of miRNAs downregulate gene expression by translational repression in animals while by mRNA degradation in plants. However, some miRNAs downregulates gene expression by translational repression in plants. For example, *miR 172* regulate *AP2* through translational repression despite *miR 172* can perfectly complement with *AP2* mRNA (Aukerman and Sakai, 2003; Chen, 2004). In animals, there are also miRNAs which directly degrade their targeted mRNAs. For example, *miR-196* directly cleaves the mRNA of *HOXB8* (Mansfield et al., 2004; Yekta et al., 2004; Hornstein et al., 2005), which play important role in animal development (van den Akker et al., 1999; Greer and Capecchi, 2002; Reilly, 2002; El-Mounayri et al., 2005).

Recently studies suggest a third mechanism of miRNA-mediated gene expression. After mRNA transcription, a ploy(A) tail always is added to the 3′ of the mRNA to keep mRNA more stable and avoid the occurrence of mRNA decay (Jacobson and Peltz, 1996; Coller and Parker, 2004). Recently, Wu et al. (2006) found that miRNAs are involved in mRNA decay by directing rapid deadenylation of mRNAs. Their finding indicates that miRNAs destabilize mRNAs by accelerating poly(A) tail removal as an initial step in mRNA degradation. Giraldez et al. (2006) also observed the same phenomena. In their study, they found that *miR-430* accelerated the deadenylation of target mRNAs in zebrafish, and facilitated the deadenylation and clearance of maternal mRNAs during early embryogenesis

and affected embryo development. This conclusion has been confirmed by several in vitro and in vivo studies (Bagga et al., 2005; Jing et al., 2005; Wu and Belasco, 2005), in which the cellular concentrations of target mRNAs were reduced by miRNAs that do not perfectly or near-perfectly complement to their targeted mRNAs.

Targets of microRNAs

miRNAs regulate gene expression through targeting mRNA for cleavage, translational repression or mRNA decay. Thus, identifying miRNA targets is a very important step to study miRNA functions in animal and plant development. In the past years, several approaches have been employed to identify miRNA targets. A genetic approach is the first approach to identify miRNA targets, which is based on the abnormal expression of targeted mRNAs in the miRNA loss-of-function mutants. This approach has been used to identify several miRNA targets that play an important role in worm development (Lee et al., 1993; Wightman et al., 1993; Reinhart et al., 2000; Johnston and Hobert, 2003). For all known miRNA targets, they have conserved perfect or near-perfect complementary sites of miRNAs (Llave et al., 2002; Pasquinelli and Ruvkun, 2002; Saxena et al., 2003; Bartel, 2004; Mallory et al., 2004a; Meister et al., 2004b; Ota et al., 2004; Vella et al., 2004; Bagga et al., 2005; Brown and Sanseau, 2005; Millar and Waterhouse, 2005), especially for plant miRNAs (Aukerman and Sakai, 2003; Wang et al., 2005a; Williams et al., 2005b). This suggests a powerful strategy to predict miRNA targets by computational approaches. Based on this characteristic, several laboratories have developed different computational strategies to predict miRNA targets in available genome database, and successfully identified 100s of miRNA targets for given miRNAs (Rhoades et al., 2002; Enright et al., 2003; Lewis et al., 2003; Stark et al., 2003; Axton, 2004; Bonnet et al., 2004; John et al., 2004; Jones-Rhoades and Bartel, 2004; Kiriakidou et al., 2004; Lai, 2004; Rajewsky and Socci, 2004; Rehmsmeier et al., 2004; Wang et al., 2004; Axtell and Bartel, 2005; Bentwich, 2005; Brennecke et al., 2005; Brown and Sanseau, 2005; Burgler and Macdonald, 2005; Grun et al., 2005; Hariharan et al., 2005; Kawasaki and Taira, 2005; Krek et al., 2005; Legendre et al., 2005; Lewis et al., 2005; Li and Zhang, 2005; Nakahara et al., 2005; Robins et al., 2005; Saetrom et al., 2005; Williams et al., 2005a; Xie et al., 2005; Yoon and De Micheli, 2005; Zhang, 2005). These computer software programs include TargetScan (Lewis et al., 2003), TargetScanS (Lewis et al., 2005), miRanda (Enright et al., 2003; John et al., 2004), MovingTargets (Burgler and Macdonald, 2005), PicTar (Grun et al., 2005; Krek et al., 2005), RNAhybrid (Rehmsmeier et al., 2004), DIAN-AmicroT (Lim et al., 2005) for animals; and MIRcheck (Jones-Rhoades and Bartel, 2004), findMiRNA (Adai et al., 2005), miRU (Zhang, 2005), and PatScan* (Dsouza et al., 1997; Rhoades et al., 2002) for plants. Microarray technology was also recently employed to identify miRNA targets, and successfully identified 100s of miRNA targets (Lim et al., 2005).

Predicting and identifying miRNA targets by computational approaches is much easier in plants than in animals. This is due to the fact that complementarity between miRNAs and targeted mRNAs is much higher in plants than in animals for a majority of targets (Carrington and Ambros, 2003; Ambros, 2004; Bartel, 2004). Thus, for a majority of plant miRNAs have predicted targets although some of them have not been validated by experimental approaches. However, there are no targets found for a majority of animal miRNAs although more miRNAs are identified in animals than in plants. There are only one or a few targets for a majority of plant miRNAs (Rhoades et al., 2002), while there are lots, even 100s of targets for each animal miRNA (Enright et al., 2003; Lewis et al., 2003, 2005; Stark et al., 2003; John et al., 2004; Lai, 2004; Rajewsky and Socci, 2004; Bentwich, 2005; Krek et al., 2005).

Currently, a majority of plant miRNAs have identified targets. For example, *miR 172* targets *AP2* in *Arabidopsis thaliana* and *gossy15* in maize for regulating flower development and developmental timing switch (Aukerman and Sakai, 2003; Chen, 2004; Lauter et al., 2005). For animal miRNAs, a majority of miRNAs have not been experimentally identified their targets although 100s of targets are predicted for each miRNA. However, about 30% of protein-coding genes are predicted to be negatively regulated by miRNAs (Lewis et al., 2005). This suggests miRNAs are the biggest regulator in gene regulation.

Functions of microRNAs in plants

Plant miRNAs play an important role in many aspects, including organ development, phage change, signal transduction, and response to environmental stress.

miRNAs regulate organ development in plants. Dicer-like enzyme 1 (DCL1) is an important enzyme that processes pri-miRNAs to pre-miRNAs then to miRNA:miRNA* duplexes (Park et al., 2002; Reinhart et al., 2002; Papp et al., 2003; Kurihara and Watanabe, 2004; Liu et al., 2005; Kurihara et al., 2006). Loss-of-function of the *dcl1* gene reduced the expression level of mature miRNAs and consequently caused many developmental abnormalities (Liu et al., 2005; Kurihara et al., 2006). These developmental abnormalities include arrested embryos at early stages, altered leaf shape and morphology, delayed floral transition, and female sterility (Park et al., 2002; Reinhart et al., 2002; Liu et al., 2005). HASTY, an ortholog of exportin 5, is also an important protein in mature miRNA biogenesis, which transports the miRNA:miRNA* duplex from the nucleus to the cytoplasm in *Arabidopsis* (Park et al., 2005). Loss-of-function of *hasty* gene also caused pleiotropic developmental abnormalities, such as disrupting leaf shape and flower morphology, accelerating phase change, and reducing fertility (Bollman et al., 2003). All these finding indicate that miRNAs play an important role in a variety of developmental process in plants, and miRNAs regulate plant development at different organ level, including roots, stems, shoots, and flowers.

miRNAs control leaf development by regulating the expression of class-III homeodomain leucine zipper (HD-ZIP) transcription factor genes, which control leaf asymmetry pattern along adaxial/abaxial (upper/lower) axis (Juarez and Timmermans, 2004; Juarez et al., 2004). PHABULOSA (PHB), PHAVOLUTA (PHV), and REVOLUTA (REV) are three closely related *Arabidopsis* HD-ZIP transcription factors. Dominant mutations in any of these three transcription factor genes (*phb*, *phv,* and *rev*) results in radialization and adaxialization of leaf and vascular bundles in the stem (McConnell et al., 2001; Emery et al., 2003). Recently, several laboratories demonstrated that all of the three transcription factors are the targets of *miR165* and *miR166*, and are regulated by these two miRNAs (Emery et al., 2003; Bao et al., 2004; Bowman, 2004; Juarez et al., 2004; Mallory et al., 2004b; Zhong and Ye, 2004; Kim

et al., 2005; Williams et al., 2005b; Ko et al., 2006). *miR165* and *miR166* are evolutionarily conserved in all lineages of land plants, including mosses, ferns, gymnosperms, and angiosperms (Zhang et al., 2006c). Misexpression of *miR165* and *miR166* resulted in leaf developmental abnormalities in many plant species, including *Arabidopsis* and corn (Juarez et al., 2004). Except *miR165* and *miR166*, *miR159*/*Jaw* also controls leaf development by regulating a subset of TCP transcription factor genes (Palatnik et al., 2003). Loss-of-function of *miR159*/*Jaw* caused uneven leaf shape and curvature (Palatnik et al., 2003).

miRNAs control apical meristem development by targeting several members of the NAM/ATAF/CUC (NAC)-domain transcription factors that play an important role in both embryogenic, floral, shoot and root development (Aida et al., 1997; Takada et al., 2001; Hibara et al., 2003). Inappropriate expression of *miR164* resulted in abnormal expression of NAC-domian transcription factors, and caused many developmental abnormality, including shoot and root development (Laufs et al., 2004; Mallory et al., 2004a; Guo et al., 2005).

miRNAs regulate plant development most likely by regulating signal transduction. *miR164* regulates auxin signals for lateral root development by targeting *NAC* mRNA cleavage (Guo et al., 2005). *miR167* regulates plant development by negatively regulating the expression of several AUXIN RESPONSE FACTORS (ARF), including ARF 2, ARF 3, ARF 4, ARF 10, ARF 16, and ARF 17 (Mallory et al., 2005; Sorin et al., 2005; Williams et al., 2005a; Yang et al., 2006).

miRNAs regulate phase change in plants. It is an important transition in plants from vegetable growth to reproductive growth. This is always evidenced by the appearance and formation of appropriate floral organs. More and more evidences show that plant phase change is controlled by miRNAs. *APETALA 2* (*AP2*) is one of the class A genes that control plant flowering time and floral morphology (Lohmann and Weigel, 2002). Overexpression of *miR172* inhibited translation of *AP2* and *AP2*-like mRNAs, and resulted in early flowering and disrupting the specification of floral organ identity in *Arabidopsis* and maize (Aukerman and Sakai, 2003; Chen, 2004; Lauter et al., 2005).

miR159, *miR156,* and *miR171* are also involved in phase change and floral development (Llave et al., 2002; Achard et al., 2004; Schwab et al., 2005). *miR171* is predominantly expressed in inflorescence and floral tissues instead of in leaf and other vegetative tissues (Llave et al., 2002). Overexpression of *miR159* results in a low level expression of *LEAFY* and further perturbed anther development and delayed flowering in a short-day photoperiod (Achard et al., 2004). *miR156*-affected plant phase transition, including quickly initiating the formation of rosette leaves, by negatively regulating the expression of a Squamosa promoter binding protien like (SPL) plant-specific transcription factor (Schwab et al., 2005).

miRNAs regulate plant responses to environmental stresses. Abiotic and biotic stresses are a big issue for plant growth and development, lots of field studies show that environmental stress caused about 20–30% yield loss, and some may destroy crop yield (Zhang et al., 2000; Mahajan and Tuteja, 2005; Vinocur and Altman, 2005; Yamaguchi and Blumwald, 2005). In the evolution, crops have evolved different mechanisms to resist different environmental stress, including salinity, cold, drought, and pests. Although several genes have been identified and isolated from plants (Zhu, 2001; Nakashima and Yamaguchi-Shinozaki, 2006; Valliyodan and Nguyen, 2006), the principle mechanism of plant resistance still remains unknown. Recently, increasing evidences suggest that miRNAs may play an important role in plant response to biotic and abiotic stresses (Zhang et al., 2006d).

miRNAs are involved in plant diseases. Some of these miRNAs may get involved in virus-induced gene silencing. Helper component-proteinase (HC-Pro), p19, p21, and p69, are unrelated viral suppressors of gene silencing, and they play important roles in the virus response to plant antiviral silencing response (Plisson et al., 2003; Chapman et al., 2004). Several investigations demonstrated that several miRNAs are related the activity of these viral suppressors (Kasschau et al., 2003; Chapman et al., 2004; Chen et al., 2004; Llave, 2004). HC-Pro inhibited the expression level and activity of *miR171*, and caused *miR171*-related developmental deficiency (Kasschau et al., 2003). P69 enhanced the expression and activity of miRNAs, and caused rapid degradation of miRNA-targeted mRNAs, and consequently enhance plant resistance to pathogens (Chen et al., 2004). A recent study demonstrated that bacterial flagellin-derived peptide induced an overexpression of *miR393* in *Arabidopsis* (Navarro et al., 2006). *miR393* negatively regulated F-box auxin receptors (TIR1, AFB1, AFB2, and AFB3), and resulted in inhibit bacteria *Pseudomonas syringae* growth and increased plant resistance to pathogens (Navarro et al., 2006).

miRNAs are also involved in plant response to other stresses. Our study indicated that 25.8% of ESTs containing miRNAs were identified in stress-induced plant tissues, including pathogen-, salt-, drought-induced tissues (Zhang et al., 2005). *miR395* was overexpressed under sulfate starvation conditions (Jones-Rhoades and Bartel, 2004). *miR319* was induced by either cold or other stress (Sunkar and Zhu, 2004). *miR402* was strongly induced by drought, cold, and/or salinity (Sunkar and Zhu, 2004). Recently, Lu et al. (2005c) identified 48 miRNA sequences from the *Populus* genome, and some of them were induced by mechanical stress and may function in a critical defense system for structural and mechanical fitness.

Functions of microRNAs in animals

Increasing evidences suggest that miRNAs have versatile multiple biological functions in animals, although only few targets of animal miRNAs have been identified and the function of very few miRNAs have been worked out in details. The function of animal miRNAs has been studied by several approaches. As we know, Dicer and Argonaute2 are two important enzymes in biogenesis and functions of miRNAs (Hutvagner et al., 2001; Tijsterman and Plasterk, 2004; Chendrimada et al., 2005). Loss-of-function of these two enzymes decreases the expression of global miRNAs, and can be useful to study the global functions of miRNAs (Meister et al., 2004b; Karube et al., 2005). Knockdown and/or knockout or overexpression of specific miRNAs is a good approach to investigate the specific function of an unique miRNA (Hutvagner et al., 2004; Meister et al., 2004a; Lee et al., 2005). Recently developed miRNA microarray technology and miRNA-specific real-time PCR also provide useful information on miRNA functions (Miska et al., 2004; Baskerville and Bartel, 2005; Liang et al., 2005).

miRNAs regulate developmental timing. Two founding members of miRNAs, also the two best-studied

miRNAs, *lin-4* and *let-7*, regulate developmental timing in *C. elegans* (Lee et al., 1993; Wightman et al., 1993; Reinhart et al., 2000). Loss-of-function of *lin-4* and *let-7* result in retarded development. However, *lin-4* controls worm development at an early stage at the first larval stage (L1) and controls worm developmental transition from the L1 stage to the L2 stage (Lee et al., 1993); *let-7* controls worm development at a late stage and controls worm developmental transition from the L2 stages to the L3 stage (Reinhart et al., 2000). *Lin-4* control worm development by negatively regulating the expression of two genes *lin-14* and *lin-28*, in which both have antisense complementary sites to *lin-4* (Lee et al., 1993; Wightman et al., 1993; Moss et al., 1997).

miRNAs regulate animal development. miRNAs regulate animal development at multiple tissues and at multiple developmental stages. Emerging evidences suggest that miRNAs are essential for the normal development of almost all animal tissues (Lagos-Quintana et al., 2002), including stem cell (Houbaviy et al., 2003; Suh et al., 2004; Forstemann et al., 2005; Hatfield et al., 2005; Kanellopoulou et al., 2005; Lechman et al., 2005; Lee et al., 2005; Murchison et al., 2005), embryo (Kloosterman et al., 2004; Aboobaker et al., 2005; Alvarez-Garcia and Miska, 2005; Biemar et al., 2005; Kanellopoulou et al., 2005; Schulman et al., 2005; Wienholds et al., 2005; Wienholds and Plasterk, 2005; Yang et al., 2005), brain (Krichevsky et al., 2003; Miska et al., 2004; Rogelj and Giese, 2004; Sempere et al., 2004; Giraldez et al., 2005; Lugli et al., 2005; Nelson and Mourelatos, 2005; Rogaev, 2005; Rowan, 2005; Wu and Belasco, 2005; Nelson et al., 2006; Schratt et al., 2006), heart (Lagos-Quintana et al., 2002; Schubert, 2005; Zhao et al., 2005b), and limb (van den Akker et al., 1999; Harfe et al., 2005; Hornstein et al., 2005; Lancman et al., 2005; Maatouk et al., 2005), liver and other tissues (Lagos-Quintana et al., 2002). Specific miRNAs control specific tissue development at a specific developmental stage (Lagos-Quintana et al., 2002). *Dicer* mutant in zebrafish embryos developed normal at the beginning, but embryo development arrested ~8 days after fertilization (Wienholds et al., 2003). This suggests that embryo development need appropriate expression of certain miRNAs. *HOX* is an important gene in animal development, and it is negatively regulate by *miR-196* and *miR-181* (Yekta et al., 2004; Naguibneva et al., 2006). Misexpression of these miRNAs caused abnormal expression of *HOX*, and results in animal developmental abnormality (Mansfield et al., 2004; Yekta et al., 2004; Guenther et al., 2005; Naguibneva et al., 2006). One of the muscle-specific miRNAs, *miR-1*, controls the balance between differentiation and proliferation during cardiogenesis by negatively regulating the critical cardiac regulatory proteins (Zhao et al., 2005a).

miRNAs and cancer. One of the biggest progresses on miRNAs is to find that miRNAs play an important role in cancer pathogenesis. Cancer is the most difficult cured human disease. Although several genes, including oncogenes and tumor suppressor genes, have been identified in human and/or other model animal genomes, to solve the mechanism of cancer formation is still far away from what we thought. However, more and more evidences suggest that miRNAs play an important role in cancer, and better understanding the function of miRNAs in cancers will provide a new insight for cancer research.

The function of miRNAs in cancers is initially recognized from two finding: one is that a majority of miRNAs were located in the cancer-associated genomic regions or in fragile sites (Calin et al., 2004); another is that miRNAs were differential expression between cancer cells and the related normal tissue cells (Lu et al., 2005b). The first evidence for miRNAs involved in cancer came from a molecular study characterizing the 13q14 deletion in human chronic lymphocytic leukemia (CLL) (Calin et al., 2002), the most common form of adult leukemia in the Western world (Dohner et al., 2000). With the development of mature miRNA microarray technology (Babak et al., 2004; Barad et al., 2004; Liu et al., 2004; Nelson et al., 2004a,b; Thomson et al., 2004; Liang et al., 2005; Lu et al., 2005b), more and more evidences become available to connect miRNAs with cancers.

Almost all cancers have been found to be related with the abnormal expression of miRNAs, and some miRNAs may play a critical role in many common and important cancers, including cancers in lung, brain, breast, blood, liver, colon, lymphomas, thyroid, and testicular germ cell (see review Zhang et al., 2006f). One good example is lung cancer. Lung cancer is one of the common cancers and the leading cause of cancer-related deaths, especially in many economically developed countries. Several evidences indicates that miRNA *let-7* may controls lung cancer development, or at least plays an essential role in the pathogenesis of lung cancer (Takamizawa et al., 2004; Eder and Scherr, 2005; Johnson et al., 2005; Morris and McManus, 2005). The expression of *let-7* was significantly reduced in lung cancer, and reduced *let-7* expression was associated with shortened postoperative survival (Takamizawa et al., 2004). In contrast, overexpression of *let-7* inhibited lung cancer cell growth (Takamizawa et al., 2004). Following study indicates that *let-7* regulated lung cancer pathogenesis by negatively regulating the expression of oncogene *RAS* (Johnson et al., 2005). *RAS* is a well-studied oncogene which interferes with p53 pathway in lung cancer, and it has multiple complementary sites to *let-7* in its 3′UTR (Johnson et al., 2005). Thus, miRNA *let-7* inhibited *RAS* mRNA translation, and inhibited lung tumor cell differentiation and growth (Johnson et al., 2005). Recent study demonstrated that the expression of *let-7* was significantly reduced while the expression of *RAS* was dramatically increased in lung tumor tissues (Johnson et al., 2005). This suggests that miRNA *let-7* may functions as a tumor suppressor gene in human and other mammalians.

miRNAs and disease. Except cancers, miRNAs are also involved in a broad spectrum of human disease. A link between miRNAs and human disease came from the identification of an essential multisubunit protein complex termed microprocessor that is necessary and sufficient for processing miRNA precursor RNAs (Denli et al., 2004; Gregory et al., 2004; Han et al., 2004a; Landthaler et al., 2004; Gregory and Shiekhattar, 2005). In the microprocessor complex, an essential cofactor is DiGeorge syndrome critical region gene 8 (DGCR8) (Denli et al., 2004; Gregory et al., 2004; Han et al., 2004a; Landthaler et al., 2004), which is located at chromosomal region 22q11.2 and is commonly deleted in DiGeorge syndrome (Denli et al., 2004; Gregory et al., 2004; Han et al., 2004a; Landthaler et al., 2004). DiGeorge syndrome is a rare congenital disease. Although the symptoms vary greatly between individual patients, the common symptoms include a history of recurrent infection, heart defects, and characteristic facial features (Baldini, 2004).

miRNAs maybe also involved in virus-related or -induced disease and immune defense. Lecellier et al.

(2005) found that a cellular miRNA, miR-32, mediated antiviral defense in human cells, and regulated primate foamy virus type I (PFV-1) proliferation. Several mammalian viruses, including the herpesvirus family, such as Epstein-Barr virus (EBV), simian virus 40 (SV40), and Kaposi sarcoma-associated virus, have been found to code miRNAs (Pfeffer et al., 2004, 2005; Cai et al., 2005; Grey et al., 2005; Omoto and Fujii, 2005; Sullivan et al., 2005; Sullivan and Ganem, 2005a,b; Cai and Cullen, 2006; Jiang et al., 2006; Schuetz and Sarnow, 2006; Simon-Mateo and Garcia, 2006). Although the function of these coding miRNAs is still unknown, it may play a role in human disease development.

Except these functions mentioned, miRNAs also regulate programmed cell death (Brennecke et al., 2003; Xu et al., 2003; Cheng et al., 2005; Cimmino et al., 2005), miRNA and siRNA biogenesis (Bartel, 2005), insulin secretion (Poy et al., 2004), and metabolic processes (Xu et al., 2003; Esau et al., 2006).

CONCLUSIONS AND FUTURE PERSPECTIVES

miRNAs have attracted lots of interests from scientists due to their versatile functions in development, signaling, disease and cancers, and become one of the most important gene regulators. However, miRNA-related research and their application are still in infants, and several mysteries are still in secret, such as what is the origin of miRNAs, what regulates miRNA expression. Although thousands of miRNAs have been identified, their targets are still unclear, especially for a majority of animal miRNAs. The future application of miRNA-related researches will become brighter if miRNA targets are identified and the regulatory mechanisms are clear.

Recently, Zhang and colleague proposed that designed artificial miRNAs may be used to suppress gene expression for knockdown of targeted genes and studying gene function (Zhang et al., 2006d). This hypothesis was quickly confirmed in multiple plant species by two recent studies at two individual laboratories (Alvarez et al., 2006; Schwab et al., 2006). Schwab et al. (2006) designed a range of artificial miRNAs to target various endogenous protein-coding genes in *Arabidopsis thaliana*. Their results indicated that artificial miRNAs were highly expressed while their proposed targets were downexpressed in *Arabidopsis thaliana*. This finding was also confirmed in other plant species, tobacco and tomato (Alvarez et al., 2006). Both studies indicated that artificial miRNAs only knocked down their predicted targets. This suggests that artificial miRNAs could be widely used to knock down gene expression in a range of organisms. One advantage is that artificial miRNAs not only knock down one single gene, but also they can knock down several related, but not identical, targeted genes simultaneously. This provides a powerful tool to study gene function with multiple genes in a gene family, which usually exist in animals and plants. Both groups also found that artificial miRNAs were expressed at an inducible and/or tissue-specific manner with limited nonautonomous effects by using inducible or tissue-specific promoters which is an advantage over siRNAs (Alvarez et al., 2006; Schwab et al., 2006). These studies suggest that artificial miRNAs may become a new genetic approach to study and improve gene function in organisms. Due to the fact that miRNAs are highly conserved from species to species in animals (Pasquinelli et al., 2000) and plants (Zhang et al., 2006c), the similar strategy may also be used to cancer clinic and prevention study, such as preventing cancer development by knockout of oncogene.

LITERATURE CITED

Aboobaker AA, Tomancak P, Patel N, Rubin GM, Lai EC. 2005. *Drosophila* microRNAs exhibit diverse spatial expression patterns during embryonic development. Proc Natl Acad Sci U S A 102:18017–18022.

Achard P, Herr A, Baulcombe DC, Harberd NP. 2004. Modulation of floral development by a gibberellin-regulated microRNA. Development 131:3357–3365.

Adai A, Johnson C, Mlotshwa S, Archer-Evans S, Manocha V, Vance V, Sundaresan V. 2005. Computational prediction of miRNAs in *Arabidopsis thaliana*. Genome Res 15:78–91.

Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M. 1997. Genes involved in organ separation in Arabidopsis: An analysis of the cup-shaped cotyledon mutant. Plant Cell 9:841–857.

Alvarez JP, Pekker I, Goldshmidt A, Blum E, Amsellem Z, Eshed Y. 2006. Endogenous and synthetic micrornas Stimulate simultaneous, efficient, and localized regulation of multiple targets in diverse species. Plant Cell 18:1134–1151.

Alvarez-Garcia I, Miska EA. 2005. MicroRNA functions in animal development and human disease. Development 132:4653–4662.

Ambros V. 2001. microRNAs: Tiny regulators with great potential. Cell 107:823–826.

Ambros V. 2004. The functions of animal microRNAs. Nature 431:350–355.

Ambros V, Horvitz HR. 1987. The lin-14 locus of Caenorhabditis elegans controls the time of expression of specific postembryonic developmental events. Genes Dev 1:398–414.

Aukerman MJ, Sakai H. 2003. Regulation of flowering time and floral organ identity by a microRNA and its APETALA2-like target genes. Plant Cell 15:2730–2741.

Axtell MJ, Bartel DP. 2005. Antiquity of microRNAs and their targets in land plants. Plant Cell 17:1658–1673.

Axton M. 2004. Predicting miRNA targets. Nat Genet 36:1253–1253.

Babak T, Zhang W, Morris Q, Blencowe BJ, Hughes TR. 2004. Probing microRNAs with microarrays: Tissue specificity and functional inference. RNA 10:1813–1819.

Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, Pasquinelli AE. 2005. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 122:553–563.

Baldini A. 2004. DiGeorge syndrome: An update. Curr Opin Cardiol 19:201–204.

Bao N, Lye KW, Barton MK. 2004. MicroRNA binding sites in *Arabidopsis* class IIIHD-ZIP mRNAs are required for methylation of the template chromosome. Dev Cell 7:653–662.

Barad O, Meiri E, Avniel A, Aharonov R, Barzilai A, Bentwich I, Einav U, Glad S, Hurban P, Karov Y, Lobenhofer EK, Sharon E, Shiboleth YM, Shtutman M, Bentwich Z, Einat P. 2004. MicroRNA expression detected by oligonucleotide microarrays: System establishment and expression profiling in human tissues. Genome Res 14:2486–2494.

Bartel DP. 2004. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116:281–297.

Bartel B. 2005. MicroRNAs directing siRNA biogenesis. Nat Struct Mol Biol 12:569–571.

Baskerville S, Bartel DP. 2005. Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. RNA 11:241–247.

Bentwich I. 2005. Prediction and validation of microRNAs and their targets. FEBS Lett 579:5904–5910.

Biemar F, Zinzen R, Ronshaugen M, Sementchenko V, Manak JR, Levine MS. 2005. Spatial regulation of microRNA gene expression in the Drosophila embryo. Proc Natl Acad Sci U S A 102:15907–15911.

Bohnsack MT, Czaplinski K, Gorlich D. 2004. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 10:185–191.

Bollman KM, Aukerman MJ, Park MY, Hunter C, Berardini TZ, Poethig RS. 2003. HASTY, the *Arabidopsis* ortholog of exportin 5/MSN5, regulates phase change and morphogenesis. Development 130:1493–1504.

Bonnet E, Wuyts J, Rouze P, Van de Peer Y. 2004. Detection of 91 potential in plant conserved plant microRNAs in *Arabidopsis thaliana* and *Oryza sativa* identifies important target genes. Proc Natl Acad Sci U S A 101:11511–11516.

Boutet S, Vazquez F, Liu J, Beclin C, Fagard M, Gratias A, Morel JB, Crete P, Chen XM, Vaucheret H. 2003. *Arabidopsis* HEN1: A genetic link between endogenous miRNA controlling development and siRNA controlling transgene silencing and virus resistance. Curr Biol 13:843–848.

Bowman JL. 2004. Class III HD-Zip gene regulation, the golden fleece of ARGONAUTE activity? Bioessays 26:938–942.

Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM. 2003. bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. Cell 113:25–36.

Brennecke J, Stark A, Russell RB, Cohen SM. 2005. Principles of MicroRNA-target recognition. Plos Biol 3:404–418.

Brown JR, Sanseau P. 2005. A computational view of microRNAs and their targets. Drug Discov Today 10:595–601.

Burgler C, Macdonald PM. 2005. Prediction and verification of microRNA targets by MovingTargets, a highly adaptable prediction method. BMC Gen 6:88.

Cai XZ, Cullen BR. 2006. Transcriptional origin of Kaposi's sarcoma-associated herpesvirus MicroRNAs. J Virol 80:2234–2242.

Cai X, Hagedorn CH, Cullen BR. 2004. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA (New York, NY) 10:1957–1966.

Cai XZ, Lu SH, Zhang ZH, Gonzalez CM, Damania B, Cullen BR. 2005. Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. Proc Natl Acad Sci U S A 102:5570–5575.

Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, et al. 2002. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 99:15524–15529.

Calin GA, Sevignani C, Dan Dumitru C, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM. 2004. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 101:2999–3004.

Carrington JC, Ambros V. 2003. Role of microRNAs in plant and animal development. Science 301:336–338.

Chapman EJ, Prokhnevsky AI, Gopinath K, Dolja VV, Carrington JC. 2004. Viral RNA silencing suppressors inhibit the microRNA pathway at an intermediate step. Genes Dev 18:1179–1186.

Chen XM. 2004. A microRNA as a translational repressor of APETALA2 in *Arabidopsis* flower development. Science 303:2022–2025.

Chen J, Li WX, Xie DX, Peng JR, Ding SW. 2004. Viral virulence protein suppresses RNA silencing-mediated defense but upregulates the role of MicroRNA in host gene expression. Plant Cell 16:1302–1313.

Chen CF, Ridzon DA, Broomer AJ, Zhou ZH, Lee DH, Nguyen JT, Barbisin M, Xu NL, Mahuvakar VR, Andersen MR, Lao KQ, Livak KJ, Guegler KJ. 2005. Real-time quantification of microRNAs by stem-loop RT-PCR. Nucleic Acids Res 33:e179.

Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, Shiekhattar R. 2005. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature 436:740–744.

Cheng AM, Byrom MW, Shelton J, Ford LP. 2005. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acids Res 33:1290–1297.

Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. 2005. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 102:13944–13949.

Coller J, Parker R. 2004. Eukaryotic mRNA decapping. Annu Rev Biochem 73:861–890.

Cuellar TL, McManus MT. 2005. MicroRNAs and endocrine biology. J Endocrinol 187:327–332.

Denli AM, Tops BBJ, Plasterk RHA, Ketting RF, Hannon GJ. 2004. Processing of primary microRNAs by the Microprocessor complex. Nature 432:231–235.

Dezulian T, Remmert M, Palatnik JF, Weigel D, Huson DH. 2006. Identification of plant microRNA homologs. Bioinformatics 22:359–360.

Doench JG, Sharp PA. 2004. Specificity of microRNA target selection in translational repression. Genes Dev 18:504–511.

Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Dohner K, Bentz M, Lichter P. 2000. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 343:1910–1916.

Dsouza M, Larsen N, Overbeek R. 1997. Searching for patterns in genomic data. Trends Genet 13:497–498.

Eder M, Scherr M. 2005. MicroRNA and lung cancer. N Engl J Med 352:2446–2448.

El-Mounayri O, Triplett JW, Yates CW, Herring BP. 2005. Regulation of smooth muscle-specific gene expression by homeodomain proteins, Hoxa10 and Hoxb8. J Biol Chem 280:25854–25863.

Emery JF, Floyd SK, Alvarez J, Eshed Y, Hawker NP, Izhaki A, Baum SF, Bowman JL. 2003. Radial patterning of *Arabidopsis* shoots by class IIIHD-ZIP and KANADI genes. Cur Biol 13:1768–1774.

Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS. 2003. MicroRNA targets in *Drosophila*. Genome Biol 5:R1.

Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, Watts L, Booten SL, Graham M, McKay R, Subramaniam A, Propp S, Lollo BA, Freier S, Bennett CF, Bhanot S, Monia BP. 2006. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 3: 87–98.

Floyd SK, Bowman JL. 2004. Gene regulation: Ancient microRNA target sequences in plants. Nature 428:485–486.

Forstemann K, Tomari Y, Du TT, Vagin VV, Denli AM, Bratu DP, Klattenhoff C, Theurkauf WE, Zamore PD. 2005. Normal microRNA maturation and germ-line stem cell maintenance requires loquacious, a double-stranded RNA-binding domain protein. Plos Biol 3:1187–1201.

Fu HJ, Tie Y, Xu CW, Zhang ZY, Zhu J, Shi YX, Jiang H, Sun ZX, Zheng XF. 2005. Identification of human fetal liver miRNAs by a novel method. FEBS Lett 579:3849–3854.

Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, Hammond SM, Bartel DP, Schier AF. 2005. MicroRNAs regulate brain morphogenesis in zebrafish. Science 308:833–838.

Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ, Schier AF. 2006. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. Science 312:75–79.

Greer JM, Capecchi MR. 2002. Hoxb8 is required for normal grooming behavior in mice. Neuron 33:23–34.

Gregory RI, Shiekhattar R. 2005. MicroRNA biogenesis and cancer. Cancer Res 65:3509–3512.

Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R. 2004. The Microprocessor complex mediates the genesis of microRNAs. Nature 432:235–240.

Grey F, Antoniewicz A, Allen E, Saugstad J, McShea A, Carrington JC, Nelson J. 2005. Identification and characterization of human cytomegalovirus-encoded microRNAs. J Virol 79:12095–12099.

Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. 2006. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 34:D140–D144.

Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, Baillie DL, Fire A, Ruvkun G, Mello CC. 2001. Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. Cell 106:23–34.

Grun D, Wang Y-L, Langenberger D, Gunsalus KC, Rajewsky N. 2005. microRNA target predictions across seven *Drosophila* species and comparison to mammalian targets. PLoS Comput Biol 1:e13.

Guenther MG, Jenner RG, Chevalier B, Nakamura T, Croce CM, Canaani E, Young RA. 2005. Global and Hox-specific roles for the MLL1 methyltransferase. Proc Natl Acad Sci U S A 102:8603–8608.

Guo HS, Xie Q, Fei JF, Chua NH. 2005. MicroRNA directs mRNA cleavage of the transcription factor NAC1 to downregulate auxin signals for *Arabidopsis* lateral root development. Plant Cell 17:1376–1386.

Hammond SM. 2005. Dicing and slicing: The core machinery of the RNA interference pathway. FEBS Lett 579:5822–5829.

Han JJ, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. 2004a. The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev 18:3016–3027.

Han MH, Goud S, Song L, Fedoroff N. 2004b. The *Arabidopsis* double-stranded RNA-binding protein HYL1 plays a role in microRNA-mediated gene regulation. Proc Natl Acad Sci U S A 101:1093–1098.

Harfe BD, McManus MT, Mansfield JH, Hornstein E, Tabin CJ. 2005. The RNaseIII enzyme Dicer is required for morphogenesis but not patterning of the vertebrate limb. Proc Natl Acad Sci U S A 102:10898–10903.

Hariharan M, Scaria V, Pillai B, Brahmachari SK. 2005. Targets for human encoded microRNAs in HIV genes. Biochem Biophys Res Commun 337:1214–1218.

Hatfield SD, Shcherbata HR, Fischer KA, Nakahara K, Carthew RW, Ruohola-Baker H. 2005. Stem cell division is regulated by the microRNA pathway. Nature 435:974–978.

Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, Yatabe Y, Kawahara K, Sekido Y, Takahashi T. 2005. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res 65:9628–9632.

Hibara K, Takada S, Tasaka M. 2003. CUC1 gene activates the expression of SAM-related genes to induce adventitious shoot formation. Plant J 36:687–696.

Hornstein E, Mansfield JH, Yekta S, Hu JKH, Harfe BD, McManus MT, Baskerville S, Bartel DP, Tabin CJ. 2005. The microRNA miR-196 acts upstream of Hoxb8 and Shh in limb development. Nature 438:671–674.

Houbaviy HB, Murray MF, Sharp PA. 2003. Embryonic stem cell-specific microRNAs. Dev Cell 5:351–358.

Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD. 2001. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 293:834–838.

Hutvagner G, Simard MJ, Mello CC, Zamore PD. 2004. Sequence-specific inhibition of small RNA function. PLOS Biol 2:465–475.

Jacobson A, Peltz SW. 1996. Interrelationships of the pathways of mRNA decay and translation in eukaryotic cells. Annu Rev Biochem 65:693–739.

Jiang JM, Lee EJ, Schmittgen TD. 2006. Increased expression of microRNA-155 in Epstein-Barr virus transformed lymphoblastoid cell lines. Genes Chromosomes Cancer 45:103–106.

Jing Q, Huang S, Guth S, Zarubin T, Motoyama A, Chen JM, Di Padova F, Lin SC, Gram H, Han JH. 2005. Involvement of MicroRNA in AU-rich element-mediated mRNA instability. Cell 120:623–634.

John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. 2004. Human MicroRNA targets. Plos Biol 2:1862–1879.

Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. 2005. RAS is regulated by the *let-7* microRNA family. Cell 120:635–647.

Johnston RJ, Hobert O. 2003. A microRNA controlling left/right neuronal asymmetry in *Caenorhabditis elegans*. Nature 426:845–849.

Jones-Rhoades MW, Bartel DP. 2004. Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. Mol Cell 14:787–799.

Juarez M, Timmermans M. 2004. MiRNAs specify dorsoventral polarity during leaf development. Dev Biol 271:551–552.

Juarez MT, Kui JS, Thomas J, Heller BA, Timmermans MCP. 2004. microRNA-mediated repression of rolled leaf1 specifies maize leaf polarity. Nature 428:84–88.

Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM, Rajewsky K. 2005. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. Genes Dev 19:489–501.

Karp X, Ambros V. 2005. Encountering microRNAs in cell fate signaling. Science 310:1288–1289.

Karube Y, Tanaka H, Osada H, Tomida S, Tatematsu Y, Yanagisawa K, Yatabe Y, Takamizawa J, Miyoshi S, Mitsudomi T, Takahashi T. 2005. Reduced expression of Dicer associated with poor prognosis in lung cancer patients. Cancer Sci 96:111–115.

Kasschau KD, Xie ZX, Allen E, Llave C, Chapman EJ, Krizan KA, Carrington JC. 2003. P1/HC-Pro, a viral suppressor of RNA silencing, interferes with Arabidopsis development and miRNA function. Dev Cell 4:205–217.

Kawasaki H, Taira K. 2005. Identification of target genes of microRNAs in retinoic acid-induced neuronal differentiation. Pure Appl Chem 77:313–318.

Ketting RF, Fischer SEJ, Bernstein E, Sijen T, Hannon GJ, Plasterk RHA. 2001. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*. Genes Dev 15:2654–2659.

Khvorova A, Reynolds A, Jayasena SD. 2003. Functional siRNAs and rniRNAs exhibit strand bias. Cell 115:209–216.

Kim J, Jung JH, Reyes JL, Kim YS, Kim SY, Chung KS, Kim JA, Lee M, Lee Y, Kim VN, Chua NH, Park CM. 2005. microRNA-directed cleavage of ATHB15 mRNA regulates vascular development in Arabidopsis inflorescence stems. Plant J 42:84–94.

Kiriakidou M, Nelson PT, Kouranov A, Fitziev P, Bouyioukos C, Mourelatos Z, Hatzigeorgiou A. 2004. A combined computational-experimental approach predicts human microRNA targets. Genes Dev 18:1165–1178.

Kloosterman WP, Wienholds E, Ketting RF, Plasterk RHA. 2004. Substrate requirements for let-7 function in the developing zebrafish embryo. Nucleic Acids Res 32:6284–6291.

Ko JH, Prassinos C, Han KH. 2006. Developmental and seasonal expression of PtaHB1, a Populus gene encoding a class IIIHD-Zip protein, is closely associated with secondary growth and inversely correlated with the level of microRNA (miR166). New Phytologist 169:469–478.

Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, MacMenamin P, da Piedade I, Gunsalus KC, Stoffel M, Rajewsky N. 2005. Combinatorial microRNA target predictions. Nature Genet 37:495–500.

Krichevsky AM, King KS, Donahue CP, Khrapko K, Kosik KS. 2003. A microRNA array reveals extensive regulation of microRNAs during brain development. RNA 9:1274–1281.

Kurihara Y, Watanabe Y. 2004. *Arabidopsis* micro-RNA biogenesis through Dicer-like 1 protein functions. Proc Natl Acad Sci U S A 101:12753–12758.

Kurihara Y, Takashi Y, Watanabe Y. 2006. The interaction between DCL1 and HYL1 is important for efficient and precise processing of pri-miRNA in plant microRNA biogenesis. RNA 12:206–212.

Kwon C, Han Z, Olson EN, Srivastava D. 2005. MicroRNA1 influences cardiac differentiation in Drosophila and regulates notch signaling. Proc Natl Acad Sci USA 102:18986–18991.

Labourier E, Shingara J, Wolf I, Jeffers K, Brown D. 2004. MicroRNAs as potential diagnostic markers of disease. J Mol Diag 6:433–433.

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

Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. 2002. Identification of tissue-specific microRNAs from mouse. Curr Biol 12:735–739.

Lai EC. 2004. Predicting and validating microRNA targets. Genome Biol 5:115.

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

Lancman JJ, Caruccio NC, Harfe BD, Pasquinelli AE, Schageman JJ, Pertsemlidis A, Fallon JF. 2005. Analysis of the regulation of lin-41 during chick and mouse limb development. Dev Dyn 234:948–960.

Landthaler M, Yalcin A, Tuschl T. 2004. The human DiGeorge syndrome critical region gene 8 and its D-melanogaster homolog are required for miRNA biogenesis. Curr Biol 14:2162–2167.

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.

Laufs P, Peaucelle A, Morin H, Traas J. 2004. MicroRNA regulation of the CUC genes is required for boundary size control in Arabidopsis meristems. Development 131:4311–4322.

Lauter N, Kampani A, Carlson S, Goebel M, Moose SP. 2005. microRNA172 down-regulates glossy15 to promote vegetative phase change in maize. Proc Natl Acad Sci U S A 102:9412–9417.

Lecellier CH, Dunoyer P, Arar K, Lehmann-Che J, Eyquem S, Himber C, Saib A, Voinnet O. 2005. A cellular MicroRNA mediates antiviral defense in human cells. Science 308:557–560.

Lechman ER, Hope KJ, Saiz FJS, Takenaka K, Croce CM, Minden MD, Dick JE. 2005. MicroRNA expression profiling in sorted AML subpopulations: A possible role for miR-155/BIC in stem cell maintenance and leukemogenesis. Blood 106:140A–140A.

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.

Lee Y, Kim M, Han JJ, Yeom KH, Lee S, Baek SH, Kim VN. 2004. MicroRNA genes are transcribed by RNA polymerase II. EMBO J 23:4051–4060.

Lee YS, Kim HK, Chung S, Kim K-S, Dutta A. 2005. Depletion of human microRNA miR-125b reveals that it is critical for the proliferation of differentiated cells but not for the down-regulation of putative targets during differentiation. J Biol Chem 280:16635–16641.

Legendre M, Lambert A, Gautheret D. 2005. Profile-based detection of microRNA precursors in animal genomes. Bioinformatics 21:841–845.

Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. 2003. Prediction of mammalian microRNA targets. Cell 115:787–798.

Lewis BP, Burge CB, Bartel DP. 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120:15–20.

Li X, Zhang YZ. 2005. Computational detection of microRNAs targeting transcription factor genes in *Arabidopsis thaliana*. Comput Biol Chem 29: 360–367.

Liang RQ, Li W, Li Y, Tan CY, Li JX, Jin YX, Ruan KC. 2005. An oligonucleotide microarray for microRNA expression analysis based on labeling RNA with quantum dot and nanogold probe. Nucleic Acids Res 33:e17.

Lim LP, Glasner ME, Yekta S, Burge CB, Bartel DP. 2003a. Vertebrate microRNA genes. Science 299:1540–1540.

Lim LP, Lau NC, Weinstein EG, Abdelhakim A, Yekta S, Rhoades MW, Burge CB, Bartel DP. 2003b. The microRNAs of *Caenorhabditis elegans*. Genes Dev 17:991–1008.

Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. 2005. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433:769–773.

Liu CG, Calin GA, Meloon B, Gamliel N, Sevignani C, Ferracin M, Dumitru CD, Shimizu M, Zupo S, Dono M, Alder H, Bullrich F, Negrini M, Croce CM. 2004. An oligonucleotide microchip for genome-wide microRNA profiling in human and mouse tissues. Proc Natl Acad Sci U S A 101:9740–9744.

Liu B, Li PC, Li X, Liu CY, Cao SY, Chu CC, Cao XF. 2005. Loss of function of OsDCL1 affects microRNA accumulation and causes developmental defects in rice. Plant Physiol 139:296–305.

Llave C. 2004. MicroRNAs: More than a role in plant development? Mol Plant Pathol 5:361–366.

Llave C, Xie ZX, Kasschau KD, Carrington JC. 2002. Cleavage of Scarecrow-like mRNA targets directed by a class of *Arabidopsis* miRNA. Science 297:2053–2056.

Lohmann JU, Weigel D. 2002. Building beauty: The genetic control of floral patterning. Dev Cell 2:135–142.

Lu C, Tej SS, Luo SJ, Haudenschild CD, Meyers BC, Green PJ. 2005a. Elucidation of the small RNA component of the transcriptome. Science 309: 1567–1569.

Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebet BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. 2005b. MicroRNA expression profiles classify human cancers. Nature 435:834–838.

Lu SF, Sun YH, Shi R, Clark C, Li LG, Chiang VL. 2005c. Novel and mechanical stress-responsive microRNAs in *Populus trichocarpa* that are absent from *Arabidopsis*. Plant Cell 17:2186–2203.

Lugli G, Larson J, Martone ME, Jones Y, Smalheiser NR. 2005. Dicer and eIF2c are enriched at postsynaptic densities in adult mouse brain and are modified by neuronal activity in a calpain-dependent manner. J Neurochem 94:896–905.

Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. 2004. Nuclear export of microRNA precursors. Science 303:95–98.

Maatouk DM, McManus MT, Harfe BD. 2005. MicroRNA regulation of murine limb development. Dev Biol 283:698–698.

Mahajan S, Tuteja N. 2005. Cold, salinity and drought stresses: An overview. Arch Biochem Biophys 444:139–158.

Mallory AC, Dugas DV, Bartel DP, Bartel B. 2004a. MicroRNA regulation of NAC-domain targets is required for proper formation and separation of adjacent embryonic, vegetative, and floral organs. Curr Biol 14:1035–1046.

Mallory AC, Reinhart BJ, Jones-Rhoades MW, Tang GL, Zamore PD, Barton MK, Bartel DP. 2004b. MicroRNA control of PHABULOSA in leaf development: Importance of pairing to the microRNA 5′ region. EMBO J 23:3356–3364.

Mallory AC, Bartel DP, Bartel B. 2005. MicroRNA-directed regulation of *Arabidopsis* AUXIN RESPONSE FACTOR17 is essential for proper development and modulates expression of early auxin response genes. Plant Cell 17:1360–1375.

Mansfield JH, Harfe BD, Nissen R, Obenauer J, Srineel J, Chaudhuri A, Farzan-Kashani R, Zuker M, Pasquinelli AE, Ruvkun G, Sharp PA, Tabin CJ, McManus MT. 2004. MicroRNA-responsive 'sensor' transgenes uncover Hox-like and other developmentally regulated patterns of vertebrate microRNA expression. Nature Genet 36:1079–1083.

McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK. 2001. Role of PHABULOSA and PHAVOLUTA in determining radial patterning in shoots. Nature 411:709–713.

Meister G, Landthaler M, Dorsett Y, Tuschl T. 2004a. Sequence-specific inhibition of microRNA- and siRNA-induced RNA silencing. RNA 10:544–550.

Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G, Tuschl T. 2004b. Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. Mol Cell 15:185–197.

Millar AA, Waterhouse PM. 2005. Plant and animal microRNAs: Similarities and differences. Funct Integr Genomics 5:129–135.

Miska EA, Alvarez-Saavedra E, Townsend M, Yoshii A, Sestan N, Rakic P, Constantine-Paton M, Horvitz HR. 2004. Microarray analysis of microRNA expression in the developing mammalian brain. Genome Biol 5:R68.

Morris JP, 4th, McManus MT. 2005. Slowing down the Ras lane: MiRNAs as tumor suppressors? Sci STKE 2005:e41.

Moss EG, Lee RC, Ambros V. 1997. The cold shock domain protein LIN-28 controls developmental timing in *C. elegans* and is regulated by the lin-4 RNA. Cell 88:637–646.

Murchison EP, Partridge JF, Tam OH, Cheloufi S, Hannon GJ. 2005. Characterization of Dicer-deficient murine embryonic stem cells. Proc Natl Acad Sci U S A 102:12135–12140.

Naguibneva I, Ameyar-Zazoua M, Polesskaya A, Ait-Si-Ali S, Groisman R, Souidi M, Cuvellier S, Harel-Bellan A. 2006. The microRNA miR-181 targets the homeobox protein Hox-A11 during mammalian myoblast differentiation. Nat Cell Biol 8:278–284.

Nakahara K, Kim K, Sciulli C, Dowd SR, Minden JS, Carthew RW. 2005. Targets of microRNA regulation in the Drosophila oocyte proteorne. Proc Natl Acad Sci U S A 102:12023–12028.

Nakashima K, Yamaguchi-Shinozaki K. 2006. Regulons involved in osmotic stress-responsive and cold stress-responsive gene expression in plants. Physiologia Plantarum 126:62–71.

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JDG. 2006. A Plant miRNA Contributes to Antibacterial Resistance by Repressing Auxin Signaling. Science 312:436–439.

Nelson PT, Mourelatos Z. 2005. Particular micornas (miRNAs) help to discriminate brain tumor from normal brain tissue. J Neuropathol Exp Neurol 64:439–439.

Nelson PT, Baldwin DA, Oberholtzer JC, Mourelatos Z. 2004a. A microarray-based method for studying micro-RNA (miRNA) expression. J Neuropathol Exp Neurol 63:520–520.

Nelson PT, Baldwin DA, Scearce LM, Oberholtzer JC, Tobias JW, Mourelatos Z. 2004b. Microarray-based, high-throughput gene expression profiling of microRNAs. Nature Methods 1:155–161.

Nelson PT, Baldwin DA, Kloosterman WP, Kauppinen S, Plasterk RHA, Mourelatos Z. 2006. RAKE and LNA-ISH reveal microRNA expression and localization in archival human brain. RNA 12:187–191.

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

Ota A, Tagawa H, Karnan S, Tsuzuki S, Karpas A, Kira S, Yoshida Y, Seto M. 2004. Identification and characterization of a novel gene, C13orf25, as a target for 13q31-q32 amplification in malignant lymphoma. Cancer Res 64:3087–3095.

Palatnik JF, Allen E, Wu XL, Schommer C, Schwab R, Carrington JC, Weigel D. 2003. Control of leaf morphogenesis by microRNAs. Nature 425:257–263.

Papp I, Mette MF, Aufsatz W, Daxinger L, Schauer SE, Ray A, van der Winden J, Matzke M, Matzke AJM. 2003. Evidence for nuclear processing of plant micro RNA and short interfering RNA precursors. Plant Physiol 132:1382–1390.

Park W, Li JJ, Song RT, Messing J, Chen XM. 2002. CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana*. Curr Biol 12:1484–1495.

Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS. 2005. Nuclear processing and export of microRNAs in *Arabidopsis*. Proc Natl Acad Sci USA 102:3691–3696.

Pasquinelli AE, Ruvkun G. 2002. Control of developmental timing by microRNAs and their targets. Annu Rev Cell Dev Biol 18:495–513.

Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E, Ruvkun G. 2000.

Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 408:86–89.
Pfeffer S, Zavolan M, Grasser FA, Chien MC, Russo JJ, Ju JY, John B, Enright AJ, Marks D, Sander C, Tuschl T. 2004. Identification of virus-encoded microRNAs. Science 304:734–736.
Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grasser FA, van Dyk LF, Ho CK, Shuman S, Chien MC, Russo JJ, Ju JY, Randall G, Lindenbach BD, Rice CM, Simon V, Ho DD, Zavolan M, Tuschl T. 2005. Identification of microRNAs of the herpesvirus family. Nature Methods 2:269–276.
Plisson C, Drucker M, Blanc S, German-Retana S, Le Gall O , Thomas D, Bron P. 2003. Structural characterization of HC-Pro, a plant virus multifunctional protein. J Biol Chem 278:23753–23761.
Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma XS, MacDonald PE, Pfeffer B, Tuschl T, Rajewsky N, Rorsman P, Stoffel M. 2004. A pancreatic islet-specific microRNA regulates insulin secretion. Nature 432:226–230.
Rajewsky N, Socci ND. 2004. Computational identification of microRNA targets. Dev Biol 267:529–535.
Raymond CK, Roberts BS, Garrett-Engele P, Lim LP, Johnson JM. 2005. Simple, quantitative primer-extension PCR assay for direct monitoring of microRNAs and short-interfering RNAs. RNA 11:1737–1744.
Rehmsmeier M, Steffen P, Hochsmann M, Giegerich R. 2004. Fast and effective prediction of microRNA/target duplexes. RNA—-A Pub RNA Soc 10:1507–1517.
Reilly CE. 2002. Disruption of Hoxb8 gene leads to obsessive grooming behaviour. J Neurol 249:499–501.
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.
Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP. 2002. MicroRNAs in plants. Genes Dev 16:1616–1626.
Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP. 2002. Prediction of plant microRNA targets. Cell 110:513–520.
Robins H, Li Y, Padgett RW. 2005. Incorporating structure to predict microRNA targets. Proc Natl Acad Sci U S A 102:4006–4009.
Rogaev EI. 2005. Small RNAs in human brain development and disorders. Biochemistry-Moscow 70:1404–1407.
Rogelj B, Giese KP. 2004. Expression and function of brain specific small RNAs. Rev Neurosci 15:185–198.
Rowan A. 2005. Development—MicroRNAs and brain morphogenesis. Nature Rev Neurosci 6:499–499.
Saetrom O, Snove O, Saetrom P. 2005. Weighted sequence motifs as an improved seeding step in microRNA target prediction algorithms. RNA—A Pub RNA Soc 11:995–1003.
Saxena S, Jonsson ZO, Dutta A. 2003. Small RNAs with imperfect match to endogenous mRNA repress translation—Implications for off-target activity of small inhibitory RNA in mammalian cells. J Biol Chem 278:44312–44319.
Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, Greenberg ME. 2006. A brain-specific microRNA regulates dendritic spine development. Nature 439:283–289.
Schubert C. 2005. MicroRNAs manage the heart. Nature Med 11:714–714.
Schuetz S, Sarnow P. 2006. Interaction of viruses with the mammalian RNA interference pathway. Virology 344:151–157.
Schulman BRM, Esquela-Kerscher A, Slack FJ. 2005. Reciprocal expression of lin-41 and the microRNAs let-7 and mir-125 during mouse embryogenesis. Dev Dyn 234:1046–1054.
Schwab R, Palatnik JF, Riester M, Schommer C, Schmid M, Weigel D. 2005. Specific effects of MicroRNAs on the plant transcriptome. Dev Cell 8:517–527.
Schwab R, Ossowski S, Riester M, Warthmann N, Weigel D. 2006. Highly specific gene silencing by artificial microRNAs in *Arabidopsis*. Plant Cell 18: In press.
Schwarz DS, Hutvagner G, Du T, Xu ZS, Aronin N, Zamore PD. 2003. Asymmetry in the assembly of the RNAi enzyme complex. Cell 115:199–208.
Sempere LF, Freemantle S, Pitha-Rowe I, Moss E, Dmitrovsky E, Ambros V. 2004. Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol 5:R13.
Shi R, Chiang VL. 2005. Facile means for quantifying microRNA expression by real-time PCR. Biotechniques 39:519–525.
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.
Smalheiser NR. 2003. EST analyses predict the existence of a population of chimeric microRNA precursor-mRNA transcripts expressed in normal human and mouse tissues. Genome Biol 4:403.
Sorin C, Bussell JD, Camus I, Ljung K, Kowalczyk M, Geiss G, McKhann H, Garcion C, Vaucheret H, Sandberg G, Bellini C. 2005. Auxin and light control of adventitious rooting in Arabidopsis require ARGONAUTE1. Plant Cell 17: 1343–1359.
Stark A, Brennecke J, Russell RB, Cohen SM. 2003. Identification of Drosophila MicroRNA targets. Plos Biol 1:397–409.
Suh MR, Lee Y, Kim JY, Kim SK, Moon SH, Lee JY, Cha KY, Chung HM, Yoon HS, Moon SY, Kim VN, Kim KS. 2004. Human embryonic stem cells express a unique set of microRNAs. Dev Biol 270:488–498.
Sullivan CS, Ganem D. 2005a. MicroRNAs and viral infection. Mol Cell 20:3–7.
Sullivan CS, Ganem D. 2005b. A virus-encoded inhibitor that blocks RNA interference in mammalian cells. J Virol 79:7371–7379.
Sullivan CS, Grundhoff AT, Tevethia S, Pipas JM, Ganem D. 2005. SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells. Nature 435:682–686.
Sunkar R, Zhu JK. 2004. Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*. Plant Cell 16:2001–2019.
Sunkar R, Girke T, Jain PK, Zhu JK. 2005. Cloning and characterization of MicroRNAs from rice. Plant Cell 17:1397–1411.
Takada S, Hibara K, Ishida T, Tasaka M. 2001. The CUP-SHAPED COTYLEDON1 gene of Arabidopsis regulates shoot apical meristem formation. Development 128:1127–1135.
Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, Mitsudomi T, Takahashi T. 2004. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res 64:3753–3756.
Thomson JM, Parker J, Perou CM, Hammond SM. 2004. A custom microarray platform for analysis of microRNA gene expression. Nature Methods 1:47–53.
Tijsterman M, Plasterk RHA. 2004. Dicers at RISC; the mechanism of RNAi. Cell 117:1–3.
Valliyodan B, Nguyen HT. 2006. Understanding regulatory networks and engineering for enhanced drought tolerance in plants. Curr Opin Plant Biol 9:189–195.
van den Akker E, Reijnen M, Korving J, Brouwer A, Meijlink F, Deschamps J. 1999. Targeted inactivation of Hoxb8 affects survival of a spinal ganglion and causes aberrant limb reflexes. Mech Dev 89:103–114.
Vazquez F, Gasciolli V, Crete P, Vaucheret H. 2004. The nuclear dsRNA binding protein HYL1 is required for MicroRNA accumulation and plant development, but not posttranscriptional transgene silencing. Curr Biol 14:346–351.
Vella MC, Reinert K, Slack FJ. 2004. Architecture of a validated MicroRNA: Target interaction. Chem Biol 11:1619–1623.
Vinocur B, Altman A. 2005. Recent advances in engineering plant tolerance to abiotic stress: Achievements and limitations. Curr Opin Biotechnol 16:123–132.
Wang XJ, Reyes JL, Chua NH, Gaasterland T. 2004. Prediction and identification of *Arabidopsis thaliana* microRNAs and their mRNA targets. Genome Biol 5:R65.
Wang JW, Wang LJ, Mao YB, Cai WJ, Xue HW, Chen XY. 2005a. Control of root cap formation by microRNA-targeted auxin response factors in *Arabidopsis*. Plant Cell 17:2204–2216.
Wang XW, Zhang J, Gu J, He T, Zhang XG, Li YD. 2005b. MicroRNA identification based on sequence and structure alignment. Bioinformatics 21:3610–3614.
Weber MJ. 2005. New human and mouse microRNA genes found by homology search. FEBS J 272:59–73.
Wienholds E, Plasterk RHA. 2005. MicroRNA function in animal development. FEBS Letts 579:5911–5922.
Wienholds E, Koudijs MJ, van Eeden FJM, Cuppen E, Plasterk RHA. 2003. The microRNA-producing enzyme Dicer1 is essential for zebrafish development. Nature Genet 35:217–218.
Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E, de Bruijn E, Horvitz HR, Kauppinen S, Plasterk RHA. 2005. MicroRNA expression in zebrafish embryonic development. Science 309:310–311.
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.
Williams L, Carles CC, Osmont KS, Fletcher JC. 2005a. A database analysis method identifies an endogenous trans-acting short-interfering RNA that targets the Arabidopsis ARF2, ARF3, and ARF4 genes. Proc Natl Acad Sci USA 102:9703–9708.
Williams L, Grigg SP, Xie MT, Christensen S, Fletcher JC. 2005b. Regulation of Arabidopsis shoot apical meristem and lateral organ formation by microRNA miR166g and its AtHD-ZIP target genes. Development 132:3657–3668.
Wu LG, Belasco JG. 2005. Micro-RNA regulation of the mammalian lin-28 gene during neuronal differentiation of embryonal carcinoma cells. Mol Cell Biol 25:9198–9208.
Wu LG, Fan JH, Belasco JG. 2006. MicroRNAs direct rapid deadenylation of mRNA. Proc Natl Acad Sci U S A 103:4034–4039.
Xie XH, Lu J, Kulbokas EJ, Golub TR, Mootha V, Lindblad-Toh K, Lander ES, Kellis M. 2005. Systematic discovery of regulatory motifs in human promoters and 3′ UTRs by comparison of several mammals. Nature 434:338–345.
Xu PZ, Vernooy SY, Guo M, Hay BA. 2003. The Drosophila MicroRNA mir-14 suppresses cell death and is required for normal fat metabolism. Curr Biol 13:790–795.
Yamaguchi T, Blumwald E. 2005. Developing salt-tolerant crop plants: Challenges and opportunities. Trends Plant Sci 10:615–620.
Yang WJ, Yang DD, Na SQ, Sandusky GE, Zhang Q, Zhao GS. 2005. Dicer is required for embryonic angiogenesis during mouse development. J Biol Chem 280:9330–9335.
Yang JH, Han SJ, Yoon EK, Lee WS. 2006. Evidence of an auxin signal pathway, microRNA167-ARF8-GH3, and its response to exogenous auxin in cultured rice cells. Nucleic Acids Res 34:1892–1899.
Yekta S, Shih IH, Bartel DP. 2004. MicroRNA-directed cleavage of HOXB8 mRNA. Science 304:594–596.
Yi R, Qin Y, Macara IG, Cullen BR. 2003. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 17:3011–3016.
Yoon SR, De Micheli G. 2005. Prediction of regulatory modules comprising microRNAs and target genes. Bioinformatics 21:93–100.
Yu B, Yang ZY, Li JJ, Minakhina S, Yang MC, Padgett RW, Steward R, Chen XM. 2005. Methylation as a crucial step in plant microRNA biogenesis. Science 307:932–935.
Zeng Y, Wagner EJ, Cullen BR. 2002. Both natural and designed micro RNAs technique can inhibit the expression of cognate mRNAs when expressed in human cells. Mol Cell 9:1327–1333.
Zhang YJ. 2005. MiRU: An automated plant miRNA target prediction server. Nucleic Acids Res 33:W701–W704.
Zhang BH, Liu F, Yao CB, Wang KB. 2000. Recent progress in cotton biotechnology and genetic engineering in China. Curr Sci 79:37–44.
Zhang HD, Kolb FA, Jaskiewicz L, Westhof E, Filipowicz W. 2004. Single processing center models for human dicer and bacterial RNase III. Cell 118:57–68.
Zhang BH, Pan XP, Wang QL, Cobb GP, Anderson TA. 2005. Identification and characterization of new plant microRNAs using EST analysis. Cell Res 15:336–360.
Zhang BH, Pan XP, Anderson TA. 2006a. Identification of 188 conserved maize microRNAs and their targets. FEBS Lett 580:3753–3762.
Zhang BH, Pan XP, Anderson TA. 2006b. MicroRNA: A new player in stem cells. J Cell Physiol 209:266–269.
Zhang BH, Pan XP, Cannon CH, Cobb GP, Anderson TA. 2006c. Conservation and divergence of plant microRNA genes. Plant J 46:243–259.
Zhang BH, Pan XP, Cobb GP, Anderson TA. 2006d. Plant microRNA: A small regulatory molecule with big impact. Dev Biol 289:3–16.

Zhang BH, Pan XP, Cox SB, Cobb GP, Anderson TA. 2006e. Evidence that miRNAs are different from other RNAs. Cell Mol Life Sci 63:246–254.
Zhang BH, Pan XP, Cobb GP, Anderson TA. 2006f. MicroRNAs as oncogenes and tumor suppressors. Dev Bio , in press.
Zhao Y, Samal E, Srivastava D. 2005a. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature 436:214–220.
Zhao Y, Samal E, Srivastava D. 2005b. Serum, response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Circulation 112:U107–U107.
Zhong RQ, Ye ZH. 2004. Amphivasal vascular bundle 1, a gain-of-function mutation of the IFL1/REV gene, is associated with alterations in the polarity of leaves, stems and carpels. Plant Cell Physiol 45:369–385.
Zhu JK. 2001. Plant salt tolerance. Trends Plant Sci 6:66–71.