

microPrimer: the biogenesis and function of microRNA

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Summary

Discovered in nematodes in 1993, microRNAs (miRNAs) are non-coding RNAs that are related to small interfering RNAs (siRNAs), the small RNAs that guide RNA interference (RNAi). miRNAs sculpt gene expression profiles during plant and animal development. In fact, miRNAs may regulate as many as one-third of human

genes. miRNAs are found only in plants and animals, and in the viruses that infect them. miRNAs function very much like siRNAs, but these two types of small RNAs can be distinguished by their distinct pathways for maturation and by the logic by which they regulate gene expression.

microHistory

The first miRNA, *lin-4*, was identified in 1993 in a genetic screen for mutants that disrupt the timing of post-embryonic development in *Caenorhabditis elegans* (Lee et al., 1993). Cloning of the locus revealed that *lin-4* produces a 22-nucleotide non-coding RNA, rather than a protein-coding mRNA (Lee et al., 1993). *lin-4* represses the expression of *lin-14*, which encodes a nuclear protein (Lee et al., 1993; Wightman et al., 1993) whose concentration must be reduced for worms to progress from their first larval stage to the second (Rougvie, 2005). The negative regulation of *lin-14* by *lin-4* requires partial complementarity between *lin-4* and sites in the 3'-untranslated region (UTR) of *lin-14* mRNA (Ha et al., 1996; Olsen and Ambros, 1999). It was not until 2000 that a second miRNA, *let-7*, was discovered, again in worms (Reinhart et al., 2000). *let-7* functions in a manner similar to *lin-4*, repressing the expression of the *lin-41* and *hbl-1* mRNAs by binding to their 3' UTRs (Reinhart et al., 2000; Slack et al., 2000; Lin et al., 2003; Vella et al., 2004). *let-7* is conserved throughout metazoans (Pasquinelli et al., 2000), and the discovery of *let-7* (Reinhart et al., 2000), together with the subsequent large-scale searches for additional miRNAs, established miRNAs as a new and large class of ribo-regulators (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001), and fueled speculation that tiny RNAs are a major feature of the gene regulatory networks of animals. Now more than 1600 miRNAs have been identified in plants, animals and viruses (Lai et al., 2003; Lim et al., 2003a; Lim et al., 2003b). The human genome alone may contain 800-1000 miRNAs, a large portion of which may be specific to primates (Bentwich et al., 2005; Berezikow et al., 2005; Xie et al., 2005).

microMaturation

miRNAs are transcribed by RNA polymerase II as primary miRNAs (pri-miRNAs), which range from hundreds to thousands of nucleotides in length (Cai et al., 2004; Lee et al., 2004; Parizotto et al., 2004). Most miRNAs are transcribed from regions of the genome that are distinct from previously annotated protein-coding sequences (Fig. 1). Some miRNA-

encoding loci reside well apart from other miRNAs, suggesting that they form their own transcription units; others are clustered and share similar expression patterns, implying that they are transcribed as polycistronic transcripts (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee et al., 2002; Reinhart et al., 2002). About half of the known mammalian miRNAs are within the introns of protein-coding genes, or within either the introns or exons of non-coding RNAs, rather than in their own unique transcription units (Rodriguez et al., 2004). Intronic miRNAs usually lie in the same orientation as, and are coordinately expressed with, the pre-mRNA in which they reside; that is, they share a single primary transcript (Rodriguez et al., 2004; Baskerville and Bartel, 2005). A very few miRNAs reside in the untranslated regions of protein-coding mRNAs; it is likely that these transcripts can make either the miRNA or the protein, but not both, from a single molecule of mRNA (Cullen, 2004).

Animal microMaturation

In animals, two processing steps yield mature miRNAs (Fig. 2A). Each step is catalyzed by a ribonuclease III (RNase III) endonuclease together with a double-stranded RNA-binding domain (dsRBD) protein partner. First, Drosha, a nuclear RNase III, cleaves the flanks of pri-miRNA to liberate an ~70-nucleotide stem loop, the precursor miRNA (pre-miRNA) (Lee et al., 2002; Lee et al., 2003; Denli et al., 2004; Gregory et al., 2004; Han et al., 2004; Landthaler et al., 2004). The efficient processing of pri-miRNA by Drosha requires: a large terminal loop (≥ 10 nucleotides) in the hairpin; a stem region that is about one helical turn longer than the slightly more than two helical turns of the stem of the resulting pre-miRNA; and 5' and 3' single-stranded RNA extensions at the base of the future pre-miRNA (Lee et al., 2003; Zeng and Cullen, 2003; Zeng and Cullen, 2005; Zeng et al., 2005). Accurate and efficient pri-miRNA processing by Drosha requires a dsRBD protein, known as Pasha in *Drosophila*, Pash-1 in *C. elegans* and DGCR8 in mammals (Denli et al., 2004; Gregory et al., 2004; Han et al., 2004; Landthaler et al., 2004). The resulting pre-miRNA have 5' phosphate and 3' hydroxy termini, and two- or three-nucleotide 3' single-stranded overhanging ends, all of which

are characteristics of RNase III cleavage of dsRNA. Thus, Drosha cleavage defines either the 5' or the 3' end of the mature miRNA. (The mature miRNA resides in the 5' arm of some pre-miRNA and in the 3' arm in others.) The pre-miRNA is then exported from nucleus to cytoplasm by Exportin 5/RanGTP, which specifically recognizes the characteristic end structure of pre-miRNAs (Yi et al., 2003; Bohnsack et al., 2004; Lund et al., 2004; Zeng and Cullen, 2004).

In the cytoplasm, a second RNase III, Dicer, together with its dsRBD protein partner, Loquacious (Loqs) in *Drosophila* or the *trans-activator RNA (tar)*-binding protein (TRBP) in humans, makes a pair of cuts that defines the other end of the mature miRNA, liberating an ~21-nucleotide RNA duplex (Bernstein et al., 2001; Grishok et al., 2001; Hutvagner et al., 2001; Ketting et al., 2001; Chendrimada et al., 2005; Forstemann et al., 2005; Jiang et al., 2005; Saito et al., 2005). This RNA duplex has essentially the same structure as a double-stranded siRNA, except that the mature miRNA is only partially paired to the miRNA* – the small RNA that resides on the side of the pre-miRNA stem opposite the

miRNA – because the stems of pre-miRNAs are imperfectly double stranded. From the miRNA/miRNA* duplex, one strand, the miRNA, preferentially enters the protein complex that represses target gene expression, the RNA-induced silencing complex (RISC), whereas the other strand, the miRNA* strand, is degraded. The choice of strand relies on the local thermodynamic stability of the miRNA/miRNA* duplex – the strand whose 5' end is less stably paired is loaded into the RISC (Khvorova et al., 2003; Schwarz et al., 2003). This thermodynamic difference arises, in part, because miRNAs tend to begin with uracil, and, in part, because miRNA/miRNA* duplexes contain mismatches and bulges that favor the miRNA strand being loaded into the RISC.

Plant microMaturation

miRNA maturation in plants differs from the pathway in animals because plants lack a Drosha homolog (Fig. 2B). Instead, the RNase III enzyme DICER-LIKE 1 (DCL1), which is homologous to animal Dicer, is required for miRNA maturation (Park et al., 2002; Reinhart et al., 2002; Papp et al., 2003; Xie et al., 2004). In plants, DCL1 is localized in the nucleus and can make both the first pair of cuts made by Drosha and the second pair of cuts made by animal Dicer. As for animal Dicer, a dsRNA-binding domain protein partner, HYL1, has been implicated in DCL1 function in plant miRNA maturation (Papp et al., 2003; Vazquez et al., 2004a). The resulting miRNA/miRNA* duplex is exported from the nucleus by HASTY (HST), the plant ortholog of Exportin 5, and completes its assembly into the RISC in the cytoplasm (Peragine et al., 2004; Park et al., 2005). Unlike animal miRNAs, which end with free 2', 3' hydroxyl groups, plant miRNAs have a methyl group on the ribose of the last nucleotide. The terminal methyl group is added by the *S*-adenosyl methionine (SAM)-dependent methyltransferase HEN1, and the modification of the miRNA by HEN1 either protects the miRNA from further modification or degradation, or may facilitate its assembly into the RISC (Boutet et al., 2003; Yu et al., 2005). In plants, RNA-dependent RNA polymerases may use small RNAs as primers to synthesize double-stranded RNA from aberrant single-stranded transcripts, raising the possibility that the terminal methoxy modification on miRNA serves to prevent miRNA from acting as primers.

The RISC directs gene silencing

The RISC carries out small RNA-directed gene silencing in both the miRNA and the RNAi pathways in plants and animals (Hammond et al., 2000; Hammond et al., 2001; Hutvagner and Zamore, 2002; Zeng et al., 2002; Doench et al., 2003). When the small RNA guide in the RISC pairs extensively to a target mRNA, the RISC functions as an endonuclease, cleaving the mRNA between the target nucleotides paired to bases 10 and 11 of the miRNA or siRNA. The core component of every RISC is a member of the Argonaute (Ago) protein family, whose members all contain a central PAZ domain (named after the family member proteins Piwi, Argonaute and Zwiille) and a carboxy terminal PIWI domain. Structural studies show that the PIWI domain binds to small RNAs at their 5' end, whereas the PAZ domain binds to the 3' end of single-stranded RNAs (Song et al., 2003; Yan et al., 2003; Lingel et al., 2004; Parker et al., 2004; Ma et al., 2005; Parker et al., 2005).

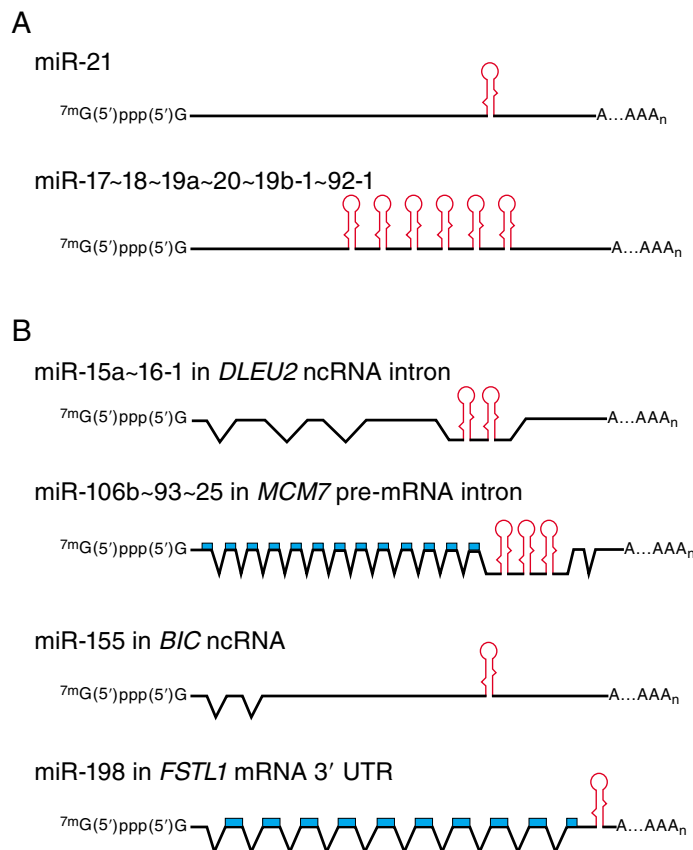


Fig. 1. The structure of human pri-miRNAs. (A) Two examples of miRNAs with their own transcription units, such as miR-21 and the polycistronic miR-17~92-1 cluster (Cai et al., 2004; He et al., 2005). (B) miRNAs that are transcribed with other genes. miR-15a~16-1 resides in the intron of a non-coding RNA (ncRNA) (Calin et al., 2004) and miR-106b~93~25 lies in the intron of a protein-coding RNA (Rodriguez et al., 2004). miR-155 is found in the exon of a ncRNA (Eis et al., 2005), whereas miR-198 is in the exon of a protein-coding mRNA (Cullen, 2004). DLEU2, deleted in lymphocytic leukemia 2; MCM7, minichromosome maintenance deficient 7; BIC, B-cell integration cluster; FSTL1, follistatin-like 1.

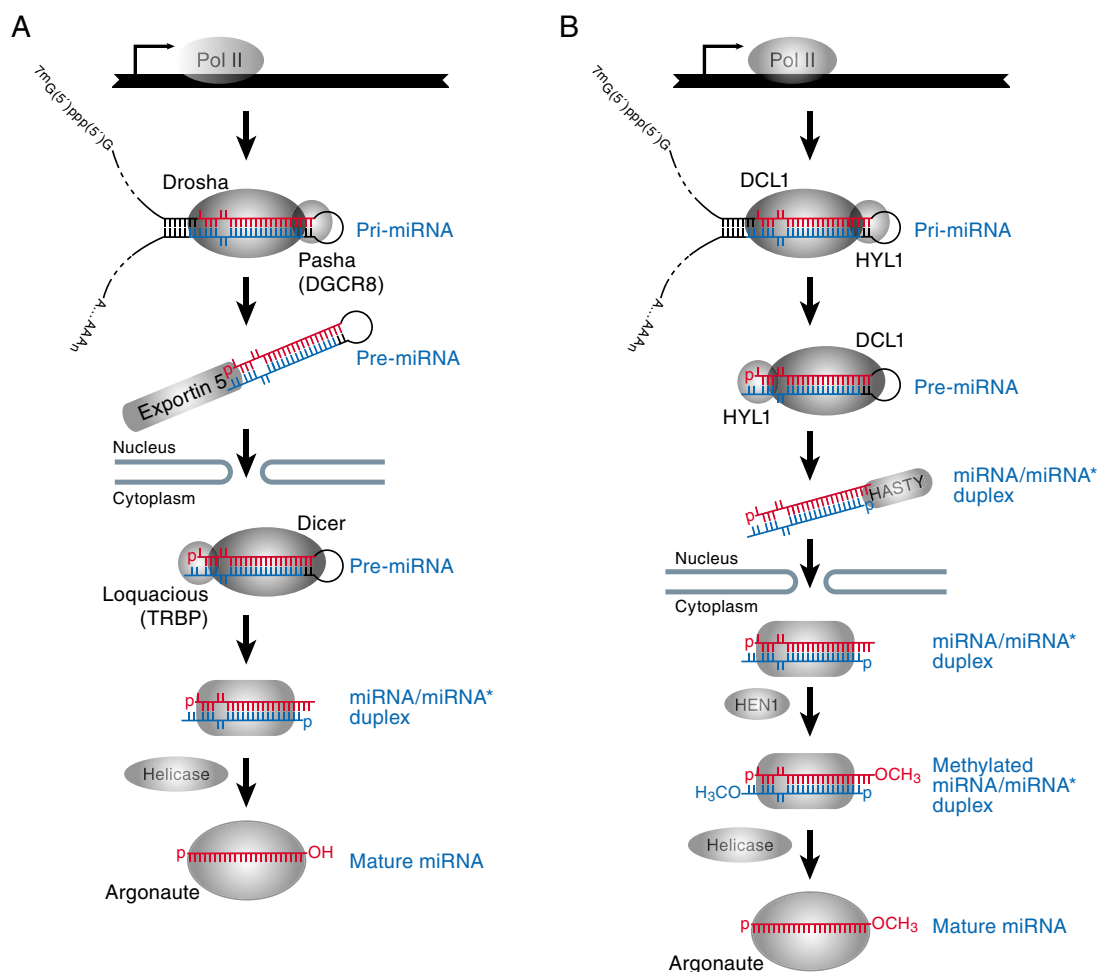


Fig. 2. The miRNA biogenesis pathway. (A) Animal and (B) plant miRNA biogenesis. Mature miRNAs are indicated in red, whereas the miRNA* strands are in blue.

Moreover, the three-dimensional structure of the PIWI domain closely resembles that of RNase H, the enzyme that cleaves the RNA strand of an RNA-DNA hybrid (Song et al., 2004; Nowotny et al., 2005), and both structural and biochemical studies have confirmed that Argonaute is the target-cleaving endonuclease of the RISC (Liu et al., 2004; Rand et al., 2004; Song et al., 2004; Bamberger and Baulcombe, 2005; Qi et al., 2005; Rivas et al., 2005). Notably, a subpopulation of Argonaute proteins do not retain all the amino acids that are crucial for RISC catalytic activity, and thus cannot cleave a target RNA even when the small RNA guide is sufficiently complementary to the target (Liu et al., 2004; Meister et al., 2004b; Rivas et al., 2005). The RISC-associated proteins include the putative RNA-binding protein VIG (Vasa intronic gene), the Fragile-X related protein in *Drosophila*, the exonuclease Tudor-SN, and several putative helicases (Caudy et al., 2002; Hutvagner and Zamore, 2002; Ishizuka et al., 2002; Mourelatos et al., 2002; Caudy et al., 2003). The molecular function of these proteins in RNA silencing is not known.

microMechanism

miRNAs regulate their target genes via two main mechanisms: target mRNA cleavage and 'translational repression'.

In plants, most miRNAs have perfect or near perfect complementarity to their mRNA targets (Rhoades et al., 2002). Upon binding to their mRNA targets, the miRNA-containing RISCs function as endonucleases, cleaving the mRNA (Llave et al., 2002; Tang et al., 2003). Single miRNA-binding motifs are found both in the coding regions, such as the miR-166-targeting site in the *PHABULOSA* mRNA, and in the untranslated regions of miRNA-regulated plant mRNAs, such as the miR-156-targeting site in the *SPL4* mRNA, albeit mainly in coding sequences, perhaps because cleavage here most strongly inactivates translation of the mRNA into functional protein (Rhoades et al., 2002). At least eight animal miRNAs, miR-127, miR-136, miR-196, miR-431, miR-433-3p, miR-433-5p, miR-434-3p and miR-434-5, and two viral miRNAs, miR-BART2 and SVmiRNA, also act to cleave their targets (Mansfield et al., 2004; Pfeffer et al., 2004; Yekta et al., 2004; Davis et al., 2005; Sullivan et al., 2005).

In contrast to plant miRNAs, the complementarity between animal miRNAs and their targets is usually restricted to the 5' region (nucleotides 2-8 or 2-7) of the miRNA, i.e. to the 3' region of the target site (Lewis et al., 2003; Lai, 2004; Brennecke et al., 2005; Lewis et al., 2005; Xie et al., 2005). This 5' miRNA region has been called the 'seed region' to describe its disproportionate contribution to target-RNA

binding. Because the seed region of a miRNA is so short, miRNAs are predicted to regulate surprisingly large numbers of genes; the complete complement of human miRNAs may regulate as many as one-third of the human protein-coding genes (Lewis et al., 2005; Xie et al., 2005)! In the absence of extensive complementarity between the miRNA and the target, binding of the RISC blocks translation of the target mRNA into protein, rather than catalyzing its cleavage into two pieces (Olsen and Ambros, 1999). Recent results suggest that regulation by miRNAs can direct target mRNA degradation through a pathway that is distinct from small RNA-directed endonucleolytic cleavage (Bagga et al., 2005; Lim et al., 2005). In human cells, the core component of the RISCs, Argonaute proteins, together with mRNAs that are targeted for silencing by miRNAs are concentrated in cytoplasmic foci called Processing bodies (P-bodies) (Liu et al., 2005; Pillai et al., 2005; Sen and Blau, 2005). (P-bodies are also known as cytoplasmic bodies or GW-bodies.) miRNAs may initially block translational initiation, causing the miRNA-programmed RISC and the target mRNA to be re-localized to the P-body (Pillai et al., 2005). In *C. elegans*, a P-body protein, AIN-1, interacts with the miRNA-programmed RISC component ALG-1, an Argonaute protein, and is sufficient to localize ALG-1 to the P-body (Ding et al., 2005). Thus, P-body-mediated miRNA-directed regulation may be a general mechanism among animals.

microFunctions

miRNAs function in a broad range of biological processes in plants and animals (Kidner and Martienssen, 2005; Alvarez-Garcia and Miska, 2005). The first insight into their function came from phenotypic studies of mutations that disrupt core components of the miRNA pathway. *dicer* mutants show diverse developmental defects, including abnormal embryogenesis in *Arabidopsis*, delayed germ-line stem-cell (GSC) division in *Drosophila*, germ-line defects in *C. elegans*, abnormal embryonic morphogenesis in zebrafish and stem-cell differentiation defects in mice (Knight and Bass, 2001; Park et al., 2002; Bernstein et al., 2003; Wienholds et al., 2003; Giraldez et al., 2005; Hatfield et al., 2005). Similarly, the disruption of Argonaute function causes widespread developmental defects, such as defective stem-cell maintenance and failure to form axillary meristem in an *Arabidopsis* mutant for *PINHEAD/ZWILLE* (*PNH/ZLL*) or *ARGONAUTE 1* (*AGO1*), a stem-cell self-renewal defect in *Drosophila piwi* mutants, and defective early development in *C. elegans alg-1* and *alg-2* mutants (Bohmert et al., 1998; Cox et al., 1998; Moussian et al., 1998; Grishok et al., 2001). *Arabidopsis* plants mutant for *ZIPPY* (*ZIP*), an Argonaute gene, and *HASTY* (*HST*), which encodes the miRNA export receptor, exhibit a precocious vegetative phenotype and produce abnormal flowers (Peragine et al., 2004). Overall, these phenotypes suggest that at least a subset of miRNAs play important roles in early development.

Target prediction

The functional characterization of miRNAs relies largely on the identification of their regulatory targets. In plants, because miRNAs are almost perfectly complementary to their targets, target prediction is straightforward (Rhoades et al., 2002), and automated plant miRNA target prediction can now be

performed online (Zhang, 2005). At least half of the predicted plant miRNA targets are transcription factors, although transcription factors represent only 6% of *Arabidopsis* protein-coding genes (Riechmann et al., 2000; Rhoades et al., 2002; Jones-Rhoades et al., 2004). Typically, many members of a family of related transcription factors are coordinately repressed by a single miRNA. These miRNA-regulated transcription factors control developmental patterning, cell proliferation, and environmental and hormonal responses (Kidner and Martienssen, 2005). *DCL1* and *AGO1* themselves are also miRNA targets, suggesting a negative-feedback mechanism in which miRNAs tune their own expression (Rhoades et al., 2002a; Xie et al., 2003; Vaucheret et al., 2004).

The bioinformatic prediction of animal miRNA targets is more complex because animal miRNAs display only modest complementarity to their targets. Different algorithms have been developed to predict animal miRNA targets, using at least some of the following criteria: (1) perfect or nearly perfect pairing of the 'seed region' at the 5' end of the miRNAs and the 3' UTR of the target mRNA; (2) putative miRNA-binding site conservation between closely related species; (3) multiple miRNA-binding sites in a single target; and (4) lack of a strong secondary structure at the miRNA-binding site on the target (Enright et al., 2003; Lewis et al., 2003; Stark et al., 2003; Kiriakidou et al., 2004; Rajewsky and Socci, 2004; Brennecke et al., 2005; Krek et al., 2005; Lewis et al., 2005; Zhao et al., 2005). The computational prediction of animal miRNA targets suggests that the logic of miRNA regulation differs between animals and plants (see Box 1). In animals, miRNA has been proposed to fine-tune the expression of hundreds of genes, but to dramatically downregulate the expression of a much smaller number of transcripts (Bartel, 2004); such dramatic downregulation of transcript levels appears to be widespread for plant miRNAs. Moreover, animal miRNAs, but perhaps not plant miRNAs, may act combinatorially, with several miRNAs binding a single transcript. Thus, one miRNA might be expressed early in development, reducing the steady-state level of protein synthesis from a targeted mRNA by just a bit, a tuning function. The subsequent expression of additional miRNAs targeting the same mRNA would lower its expression still further (Bartel, 2004). Cell culture experiments suggest that when multiple miRNAs bind the same target, they act cooperatively, reducing mRNA translation by more than the sum of their individual effects (Doench et al., 2003).

miRNA-target relationships can also be identified by beginning with a target mRNA and searching for one or more regulatory miRNAs. In one case, the earlier finding that GY-box, Brd-box and K-box motifs in the 3' UTR of Notch mRNAs mediate their post-transcriptional repression helped to identify three families of *Drosophila* miRNAs that are direct regulators of Notch target genes (Lai and Posakony, 1997; Lai et al., 1998; Lai, 2002; Stark et al., 2003; Lai et al., 2005). Another example is the proposal that miR-16 in *Drosophila* plays a role in AU-rich element (ARE)-mediated mRNA degradation (Jing et al., 2005). Because depletion by RNAi of key RNA silencing proteins – Dcr-1, Ago-1 and Ago-2 – inhibited the rapid mRNA decay normally triggered by AREs, the authors broke with 'microOrthodoxy' and proposed that the ARE is a potential miRNA target site, perhaps binding miR-16 in a unconventional mode that does not require seed-sequence pairing.

microProfiling

miRNA profiling has also been used to identify miRNAs with potentially important developmental roles. The rationale is that if a miRNA is highly expressed in a tissue or cell type or at a specific developmental stage, it may reasonably play a regulatory role in specifying tissue or cell identity, or in regulating developmental timing. miRNA expression can be profiled by the cloning and sequencing miRNAs from specific tissues or developmental states (a labor-intensive method that has the benefit of uncovering new miRNAs), or by microarray analysis (a more high-throughput method that can only reveal the expression of known miRNAs). For example, *miR-181*, which is highly expressed in mouse bone marrow B-lymphoid cells, but not in T-cells, was found to promote hematopoietic differentiation towards the B-cell lineage (Chen et al., 2004). *miR-375*, an evolutionarily conserved, pancreatic islet-specific miRNA identified by small RNA cloning from glucose-responsive murine pancreatic cell lines, suppresses glucose-induced insulin secretion by repressing *myotrophin (Mtpn)* expression (Poy et al., 2004). miRNA expression profiling has also identified a zebrafish miRNA that regulates brain morphogenesis, *miR-430*, whose expression peaks 4 hours after fertilization (when most fish miRNAs are first expressed) and decreases after 24 hours (Chen et al., 2005; Giraldez et al., 2005), and also *miR-1*, a mouse miRNA whose expression is confined to cardiac and skeletal muscle precursor cells, and

which may control the balance between differentiation and proliferation during cardiogenesis by regulating the expression of *Hand2* mRNA (Wienholds et al., 2005; Zhao et al., 2005).

To date, hundreds of miRNAs have been identified in different organisms, which makes it possible to study their function individually by suppressing their expression in cells. However, genetic depletion, i.e. making miRNA mutants, is labor intensive. 2'-O-methyl antisense oligonucleotides complementary to endogenous miRNAs provide an alternative to genetic mutation. These antisense oligonucleotides transiently block miRNA function (Hutvagner et al., 2004; Meister et al., 2004a). Thus, injecting into worms a 2'-O-methyl oligonucleotide that binds *let-7* recapitulates the *let-7* mutant phenotype (Hutvagner et al., 2004). In early syncytial *Drosophila* embryos, where injection of oligonucleotides is straightforward, a panel of 2'-O-methyl oligonucleotides was used to reveal the embryonic loss-of-function phenotypes of 46 miRNAs (Leaman et al., 2005). This study suggests that miRNAs specifically regulate a broad range of developmental events. In another study, Lecellier et al. used antisense locked-nucleic acid (LNA) oligonucleotides, nucleic acid molecules that are modified to dramatically increase their binding affinities, to block miR-32 in cultured human cells, a miRNA proposed to mediate innate anti-viral defense (Lecellier et al., 2005). RNAi itself has also been used to block miRNA expression in cultured cells, but its broad utility is not yet established (Jing et al., 2005; Lee et al., 2005).

Human viruses also express their own miRNAs (Pfeffer et al., 2004; Cai et al., 2005; Pfeffer et al., 2005; Sullivan et al., 2005). Viral miRNAs are proposed to regulate both viral and host gene expression (Pfeffer et al., 2004; Cai et al., 2005), but only viral mRNA targets have been experimentally validated (Pfeffer et al., 2004). Recently, Simian Virus 40-encoded miRNAs have been identified. These viral miRNAs accumulate at late times in infection, and target early viral RNAs for cleavage and reduce viral susceptibility to cytotoxic T cells (Sullivan et al., 2005). Whether viral miRNAs always mediate the cleavage of viral mRNAs or whether they can also act more like animal miRNAs to 'tune' gene expression remains unknown.

microPrognostication

A dozen years after their discovery, miRNAs represent a large class of regulators of gene expression that control a broad range of physiological and developmental processes in plants and animals. An immediate challenge is to tabulate the functions of each and every miRNA. For this, improved computational and experimental methods for the identification of miRNA targets will be essential. These efforts will no doubt be informed by our expanding knowledge of the mechanism by which miRNAs recognize and regulate their targets. The regulation of miRNA expression and maturation remains largely unknown, but many laboratories have begun to map where miRNAs are expressed and what factors regulate their transcription. Perhaps the most important goal in understanding miRNAs will be to describe how miRNAs function as a network, for it is in studying the coordinate action of multiple miRNAs on a single mRNA target that we are likely to reach a deeper understanding of the logic of these small but powerful ribo-regulators.

Box 1. MicroRNAs and siRNAs: the logic of small RNAs

Small interfering RNAs (siRNAs) are a class of small RNA guides that are distinct from miRNAs (Tomari and Zamore, 2005). These two classes of RNA cannot be distinguished by either their chemical composition or their function. Both are produced by Dicer-mediated cleavage of longer, double-stranded RNA precursors. Consequently, both miRNAs and siRNAs are ~21 nucleotides in length, with 5'-phosphate and 3'-hydroxy termini. siRNAs and miRNAs are functionally interchangeable: both can direct target mRNA cleavage or translational repression, depending on the degree of complementarity between the small RNA and its target. Nonetheless, the two classes of small RNAs can be distinguished both by their biogenesis pathways and by the logic by which they regulate target genes, especially in animals. miRNAs are processed from small hairpin transcripts, called pre-miRNAs, which are embedded in much longer primary transcripts, the pri-miRNA. Each miRNA corresponds to ~21 nucleotides of one arm of the pre-miRNA stem. The resulting miRNA may regulate tens or hundreds of target RNAs, whose only common features are the short sequences that are complementary to as few as six or seven bases of the miRNA. siRNAs, by contrast, derive from endogenous or exogenous long double-stranded RNAs, typically comprising hundreds or thousands of base pairs. Such precursor double-stranded RNAs typically yield many siRNAs from both strands. The regulatory targets of siRNAs are usually highly homologous to the trigger double-stranded RNA itself, i.e. the trigger and target genes are paralogs. In some cases, such as with heterochromatic siRNAs, which initiate heterochromatin assembly, the trigger and target genes are one-and-the-same. A notable exception is trans-acting siRNAs (tasiRNAs) in plants, in which only a few of the many siRNAs generated from the long, double-stranded RNA trigger appear to correspond to regulated target RNAs (Allen et al., 2005; Peragine et al., 2004; Vazquez et al., 2004b).

References

- Allen, E., Xie, Z., Gustafson, A. M. and Carrington, J. C. (2005). microRNA-directed phasing during trans-acting siRNA biogenesis in plants. *Cell* **121**, 207-221.
- Alvarez-Garcia, I. and Miska, E. A. (2005). MicroRNA function: animal development and human disease. *Development* **132**, 4653-4662.
- Bagga, S., Bracht, J., Hunter, S., Massirer, K., Holtz, J., Eachus, R. and Pasquinelli, A. E. (2005). Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. *Cell* **122**, 553-563.
- Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**, 281-297.
- Baskerville, S. and Bartel, D. P. (2005). Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. *RNA* **11**, 241-247.
- Baumberger, N. and Baulcombe, D. C. (2005). Arabidopsis ARGONAUTE1 is an RNA Slicer that selectively recruits microRNAs and short interfering RNAs. *Proc. Natl. Acad. Sci. USA* **102**, 11928-11933.
- Bentwich, I., Avniel, A., Karov, Y., Aharonov, R., Gilad, S., Barad, O., Barzilai, A., Einat, P., Einav, U., Meiri, E. et al. (2005). Identification of hundreds of conserved and nonconserved human microRNAs. *Nat. Genet.* **37**, 766-770.
- Berezikov, E., Guryev, V., van de Belt, J., Wienholds, E., Plasterk, R. H. and Cuppen, E. (2005). Phylogenetic shadowing and computational identification of human microRNA genes. *Cell* **120**, 21-24.
- Bernstein, E., Caudy, A. A., Hammond, S. M. and Hannon, G. J. (2001). Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* **409**, 363-366.
- Bernstein, E., Kim, S. Y., Carmell, M. A., Murchison, E. P., Alcorn, H., Li, M. Z., Mills, A. A., Elledge, S. J., Anderson, K. V. and Hannon, G. J. (2003). Dicer is essential for mouse development. *Nat. Genet.* **35**, 215-217.
- Bohmert, K., Camus, I., Bellini, C., Bouchez, D., Caboche, M. and Benning, C. (1998). AGO1 defines a novel locus of Arabidopsis controlling leaf development. *EMBO J.* **17**, 170-180.
- Bohsack, M. T., Czaplinski, K. and Gorlich, D. (2004). Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* **10**, 185-191.
- Boutet, S., Vazquez, F., Liu, J., Beclin, C., Fagard, M., Gratias, A., Morel, J. B., Crete, P., Chen, X. and 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.
- Brennecke, J., Stark, A., Russell, R. B. and Cohen, S. M. (2005). Principles of microRNA-target recognition. *PLoS Biol.* **3**, E85.
- Cai, X., Hagedorn, C. H. and Cullen, B. R. (2004). Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA* **10**, 1957-1966.
- Cai, X., Lu, S., Zhang, Z., Gonzalez, C. M., Damania, B. and Cullen, B. R. (2005). Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. *Proc. Natl. Acad. Sci. USA* **102**, 5570-5575.
- Calin, G. A., Sevignani, C., Dumitru, C. D., Hyslop, T., Noch, E., Yendamuri, S., Shimizu, M., Rattan, S., Bullrich, F., Negrini, M. et al. (2004). Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc. Natl. Acad. Sci. USA* **101**, 2999-3004.
- Caudy, A. A., Myers, M., Hannon, G. J. and Hammond, S. M. (2002). Fragile X-related protein and VIG associate with the RNA interference machinery. *Genes Dev.* **16**, 2491-2496.
- Caudy, A. A., Ketting, R. F., Hammond, S. M., Denli, A. M., Bathoorn, A. M., Tops, B. B., Silva, J. M., Myers, M. M., Hannon, G. J. and Plasterk, R. H. (2003). A micrococcal nuclease homologue in RNAi effector complexes. *Nature* **425**, 411-414.
- Chen, C. Z., Li, L., Lodish, H. F. and Bartel, D. P. (2004). MicroRNAs modulate hematopoietic lineage differentiation. *Science* **303**, 83-86.
- Chen, P. Y., Manning, H., Slanchev, K., Chien, M., Russo, J. J., Ju, J., Sheridan, R., John, B., Marks, D. S., Gaidatzis, D. et al. (2005). The developmental miRNA profiles of zebrafish as determined by small RNA cloning. *Genes Dev.* **19**, 1288-1293.
- Chendrimada, T. P., Gregory, R. I., Kumaraswamy, E., Norman, J., Cooch, N., Nishikura, K. and Shiekhattar, R. (2005). TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* **436**, 740-744.
- Cox, D. N., Chao, A., Baker, J., Chang, L., Qiao, D. and Lin, H. (1998). A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal. *Genes Dev.* **12**, 3715-3727.
- Cullen, B. R. (2004). Transcription and processing of human microRNA precursors. *Mol. Cell* **16**, 861-865.
- Davis, E., Caiment, F., Tordoir, X., Cavaille, J., Ferguson-Smith, A., Cockett, N., Georges, M. and Charlier, C. (2005). RNAi-mediated allelic trans-interaction at the imprinted Rtl1/Peg11 locus. *Curr. Biol.* **15**, 743-749.
- Denli, A. M., Tops, B. B., Plasterk, R. H., Ketting, R. F. and Hannon, G. J. (2004). Processing of primary microRNAs by the Microprocessor complex. *Nature* **432**, 231-235.
- Ding, L., Spencer, A., Morita, K. and Han, M. (2005). The developmental timing regulator AIN-1 interacts with miRISCs and may target the Argonaute protein ALG-1 to cytoplasmic P Bodies in *C. elegans*. *Mol. Cell* **19**, 437-447.
- Doench, J. G., Petersen, C. P. and Sharp, P. A. (2003). siRNAs can function as miRNAs. *Genes Dev.* **17**, 438-442.
- Eis, P. S., Tam, W., Sun, L., Chadburn, A., Li, Z., Gomez, M. F., Lund, E. and Dahlberg, J. E. (2005). Accumulation of miR-155 and BIC RNA in human B cell lymphomas. *Proc. Natl. Acad. Sci. USA* **102**, 3627-3632.
- Enright, A. J., John, B., Gaul, U., Tuschl, T., Sander, C. and Marks, D. S. (2003). MicroRNA targets in *Drosophila*. *Genome Biol.* **5**, R1.
- Forstemann, K., Tomari, Y., Du, T., Vagin, V. V., Denli, A. M., Bratu, D. P., Klattenhoff, C., Theurkauf, W. E. and Zamore, P. D. (2005). Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein. *PLoS Biol.* **3**, E236.
- Giraldez, A. J., Cinalli, R. M., Glasner, M. E., Enright, A. J., Thomson, J. M., Baskerville, S., Hammond, S. M., Bartel, D. P. and Schier, A. F. (2005). MicroRNAs regulate brain morphogenesis in zebrafish. *Science* **308**, 833-838.
- Gregory, R. I., Yan, K. P., Amuthan, G., Chendrimada, T., Doratota, B., Cooch, N. and Shiekhattar, R. (2004). The Microprocessor complex mediates the genesis of microRNAs. *Nature* **432**, 235-240.
- Grishok, A., Pasquinelli, A. E., Conte, D., Li, N., Parrish, S., Ha, I., Baillye, D. L., Fire, A., Ruvkun, G. and Mello, C. C. (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.
- Ha, I., Wightman, B. and Ruvkun, G. (1996). A bulged lin-4/lin-14 RNA duplex is sufficient for *Caenorhabditis elegans* lin-14 temporal gradient formation. *Genes Dev.* **10**, 3041-3050.
- Hammond, S. M., Bernstein, E., Beach, D. and Hannon, G. J. (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* **404**, 293-296.
- Hammond, S. M., Boettcher, S., Caudy, A. A., Kobayashi, R. and Hannon, G. J. (2001). Argonaute2, a link between genetic and biochemical analyses of RNAi. *Science* **293**, 1146-1150.
- Han, J., Lee, Y., Yeom, K. H., Kim, Y. K., Jin, H. and Kim, V. N. (2004). The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev.* **18**, 3016-3027.
- Hatfield, S. D., Scherbata, H. R., Fischer, K. A., Nakahara, K., Carthew, R. W. and Ruohola-Baker, H. (2005). Stem cell division is regulated by the microRNA pathway. *Nature* **435**, 974-978.
- He, L., Thomson, J. M., Hemann, M. T., Hernandez-Monge, E., Mu, D., Goodson, S., Powers, S., Cordon-Cardo, C., Lowe, S. W., Hannon, G. J. et al. (2005). A microRNA polycistron as a potential human oncogene. *Nature* **435**, 828-833.
- Hutvagner, G. and Zamore, P. D. (2002). A microRNA in a multiple-turnover RNAi enzyme complex. *Science* **297**, 2056-2060.
- Hutvagner, G., McLachlan, J., Pasquinelli, A. E., Balint, E., Tuschl, T. and Zamore, P. D. (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, M. J., Mello, C. C. and Zamore, P. D. (2004). Sequence-specific inhibition of small RNA function. *PLoS Biol.* **2**, E98.
- Ishizuka, A., Siomi, M. C. and Siomi, H. (2002). A *Drosophila* fragile X protein interacts with components of RNAi and ribosomal proteins. *Genes Dev.* **16**, 2497-2508.
- Jiang, F., Ye, X., Liu, X., Fincher, L., McKearin, D. and Liu, Q. (2005). Dicer-1 and R3D1-L catalyze microRNA maturation in *Drosophila*. *Genes Dev.* **19**, 1674-1679.
- Jing, Q., Huang, S., Guth, S., Zarubin, T., Motoyama, A., Chen, J., Di Padova, F., Lin, S. C., Gram, H. and Han, J. (2005). Involvement of microRNA in AU-rich element-mediated mRNA instability. *Cell* **120**, 623-634.
- Jones-Rhoades, M. W. and Bartel, D. P. (2004). Computational identification

- of plant microRNAs and their targets, including a stress-induced miRNA. *Mol. Cell* **14**, 787-799.
- Ketting, R. F., Fischer, S. E., Bernstein, E., Sijen, T., Hannon, G. J. and Plasterk, R. H.** (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. and Jayasena, S. D.** (2003). Functional siRNAs and miRNAs exhibit strand bias. *Cell* **115**, 209-216.
- Kidner, C. A. and Martienssen, R. A.** (2005). The developmental role of microRNA in plants. *Curr. Opin. Plant Biol.* **8**, 38-44.
- Kiriakidou, M., Nelson, P. T., Kouranov, A., Fitziev, P., Bouyioukos, C., Mourelatos, Z. and Hatzigeorgiou, A.** (2004). A combined computational-experimental approach predicts human microRNA targets. *Genes Dev.* **18**, 1165-1178.
- Knight, S. W. and Bass, B. L.** (2001). A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in *Caenorhabditis elegans*. *Science* **293**, 2269-2271.
- Krek, A., Grun, D., Poy, M. N., Wolf, R., Rosenberg, L., Epstein, E. J., MacMenamin, P., da Piedade, I., Gunsalus, K. C., Stoffel, M. et al.** (2005). Combinatorial microRNA target predictions. *Nat. Genet.* **37**, 495-500.
- Lagos-Quintana, M., Rauhut, R., Lendeckel, W. and Tuschl, T.** (2001). Identification of novel genes coding for small expressed RNAs. *Science* **294**, 853-858.
- Lai, E. C.** (2002). Micro RNAs are complementary to 3' UTR sequence motifs that mediate negative post-transcriptional regulation. *Nat. Genet.* **30**, 363-364.
- Lai, E. C.** (2004). Predicting and validating microRNA targets. *Genome Biol.* **5**, 115.
- Lai, E. C. and Posakony, J. W.** (1997). The Bearded box, a novel 3' UTR sequence motif, mediates negative post-transcriptional regulation of Bearded and Enhancer of split Complex gene expression. *Development* **124**, 4847-4856.
- Lai, E. C., Burks, C. and Posakony, J. W.** (1998). The K box, a conserved 3' UTR sequence motif, negatively regulates accumulation of enhancer of split complex transcripts. *Development* **125**, 4077-4088.
- Lai, E. C., Tomancak, P., Williams, R. W. and Rubin, G. M.** (2003). Computational identification of *Drosophila* microRNA genes. *Genome Biol.* **4**, R42.
- Lai, E. C., Tam, B. and Rubin, G. M.** (2005). Pervasive regulation of *Drosophila* Notch target genes by GY-box-, Brd-box-, and K-box-class microRNAs. *Genes Dev.* **19**, 1067-1080.
- Landthaler, M., Yalcin, A. and 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, N. C., Lim, L. P., Weinstein, E. G. and Bartel, D. P.** (2001). An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* **294**, 858-862.
- Leaman, D., Chen, P. Y., Fak, J., Yalcin, A., Pearce, M., Unnerstall, U., Marks, D. S., Sander, C., Tuschl, T. and Gaul, U.** (2005). Antisense-mediated depletion reveals essential and specific functions of microRNAs in *Drosophila* development. *Cell* **121**, 1097-1108.
- Lecellier, C. H., Dunoyer, P., Arar, K., Lehmann-Che, J., Eyquem, S., Himber, C., Saib, A. and Voinnet, O.** (2005). A cellular microRNA mediates antiviral defense in human cells. *Science* **308**, 557-560.
- Lee, R. C. and Ambros, V.** (2001). An extensive class of small RNAs in *Caenorhabditis elegans*. *Science* **294**, 862-864.
- Lee, R. C., Feinbaum, R. L. and 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., Jeon, K., Lee, J. T., Kim, S. and Kim, V. N.** (2002). MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J.* **21**, 4663-4670.
- Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Radmark, O., Kim, S. et al.** (2003). The nuclear RNase III Droscha initiates microRNA processing. *Nature* **425**, 415-419.
- Lee, Y., Kim, M., Han, J., Yeom, K. H., Lee, S., Baek, S. H. and Kim, V. N.** (2004). MicroRNA genes are transcribed by RNA polymerase II. *EMBO J.* **23**, 4051-4060.
- Lee, Y. S., Kim, H. K., Chung, S., Kim, K. S. and Dutta, A.** (2005). Depletion of human micro-RNA 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.
- Lewis, B. P., Shih, I. H., Jones-Rhoades, M. W., Bartel, D. P. and Burge, C. B.** (2003). Prediction of mammalian microRNA targets. *Cell* **115**, 787-798.
- Lewis, B. P., Burge, C. B. and Bartel, D. P.** (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* **120**, 15-20.
- Lim, L. P., Glasner, M. E., Yekta, S., Burge, C. B. and Bartel, D. P.** (2003a). Vertebrate microRNA genes. *Science* **299**, 1540.
- Lim, L. P., Lau, N. C., Weinstein, E. G., Abdelhakim, A., Yekta, S., Rhoades, M. W., Burge, C. B. and Bartel, D. P.** (2003b). The microRNAs of *Caenorhabditis elegans*. *Genes Dev.* **17**, 991-1008.
- Lim, L. P., Lau, N. C., Garrett-Engle, P., Grimson, A., Schelter, J. M., Castle, J., Bartel, D. P., Linsley, P. S. and Johnson, J. M.** (2005). Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* **433**, 769-773.
- Lin, S. Y., Johnson, S. M., Abraham, M., Vella, M. C., Pasquinelli, A., Gamberi, C., Gottlieb, E. and Slack, F. J.** (2003). The *C. elegans* hunchback homolog, *hbl-1*, controls temporal patterning and is a probable microRNA target. *Dev. Cell* **4**, 639-650.
- Lingel, A., Simon, B., Izaurralde, E. and Sattler, M.** (2004). Nucleic acid 3'-end recognition by the Argonaute2 PAZ domain. *Nat. Struct. Mol. Biol.* **11**, 576-577.
- Liu, J., Carmell, M. A., Rivas, F. V., Marsden, C. G., Thomson, J. M., Song, J. J., Hammond, S. M., Joshua-Tor, L. and Hannon, G. J.** (2004). Argonaute2 is the catalytic engine of mammalian RNAi. *Science* **305**, 1437-1441.
- Liu, J., Valencia-Sanchez, M. A., Hannon, G. J. and Parker, R.** (2005). MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat. Cell Biol.* **7**, 719-723.
- Llave, C., Xie, Z., Kasschau, K. D. and Carrington, J. C.** (2002). Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. *Science* **297**, 2053-2056.
- Lund, E., Guttinger, S., Calado, A., Dahlberg, J. E. and Kutay, U.** (2004). Nuclear export of microRNA precursors. *Science* **303**, 95-98.
- Ma, J. B., Yuan, Y. R., Meister, G., Pei, Y., Tuschl, T. and Patel, D. J.** (2005). Structural basis for 5'-end-specific recognition of guide RNA by the *A. fulgidus* Piwi protein. *Nature* **434**, 666-670.
- Mansfield, J. H., Harfe, B. D., Nissen, R., Obenaus, J., Srineel, J., Chaudhuri, A., Farzan-Kashani, R., Zuker, M., Pasquinelli, A. E., Ruvkun, G. et al.** (2004). MicroRNA-responsive 'sensor' transgenes uncover Hox-like and other developmentally regulated patterns of vertebrate microRNA expression. *Nat. Genet.* **36**, 1079-1083.
- Meister, G., Landthaler, M., Dorsett, Y. and 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. and Tuschl, T.** (2004b). Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol. Cell* **15**, 185-197.
- Mourelatos, Z., Dostie, J., Paushkin, S., Sharma, A., Charroux, B., Abel, L., Rappilber, J., Mann, M. and Dreyfuss, G.** (2002). miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. *Genes Dev.* **16**, 720-728.
- Moussian, B., Schoof, H., Haecker, A., Jurgens, G. and Laux, T.** (1998). Role of the ZWILLE gene in the regulation of central shoot meristem cell fate during Arabidopsis embryogenesis. *EMBO J.* **17**, 1799-1809.
- Nowotny, M., Gaidamakov, S. A., Crouch, R. J. and Yang, W.** (2005). Crystal structures of RNase H Bound to an RNA/DNA hybrid: substrate specificity and metal-dependent catalysis. *Cell* **121**, 1005-1016.
- Olsen, P. H. and Ambros, V.** (1999). The *lin-4* regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. *Dev. Biol.* **216**, 671-680.
- Papp, I., Mette, M. F., Aufsatz, W., Daxinger, L., Schauer, S. E., Ray, A., van der Winden, J., Matzke, M. and Matzke, A. J.** (2003). Evidence for nuclear processing of plant micro RNA and short interfering RNA precursors. *Plant Physiol.* **132**, 1382-1390.
- Parizotto, E. A., Dunoyer, P., Rahm, N., Himber, C. and Voinnet, O.** (2004). In vivo investigation of the transcription, processing, endonucleolytic activity, and functional relevance of the spatial distribution of a plant miRNA. *Genes Dev.* **18**, 2237-2242.
- Park, M. Y., Wu, G., Gonzalez-Sulser, A., Vaucheret, H. and Poethig, R. S.** (2005). Nuclear processing and export of microRNAs in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **102**, 3691-3696.
- Park, W., Li, J., Song, R., Messing, J. and Chen, X.** (2002). CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana*. *Curr. Biol.* **12**, 1484-1495.

- Parker, J. S., Roe, S. M. and Barford, D. (2004). Crystal structure of a PIWI protein suggests mechanisms for siRNA recognition and slicer activity. *EMBO J.* **23**, 4727-4737.
- Parker, J. S., Roe, S. M. and Barford, D. (2005). Structural insights into mRNA recognition from a PIWI domain-siRNA guide complex. *Nature* **434**, 663-666.
- Pasquinelli, A. E., Reinhart, B. J., Slack, F., Martindale, M. Q., Kuroda, M. I., Maller, B., Hayward, D. C., Ball, E. E., Degnan, B., Muller, P. et al. (2000). Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* **408**, 86-89.
- Peragine, A., Yoshikawa, M., Wu, G., Albrecht, H. L. and Poethig, R. S. (2004). SGS3 and SGS2/SDE1/RDR6 are required for juvenile development and the production of trans-acting siRNAs in Arabidopsis. *Genes Dev.* **18**, 2368-2379.
- Pfeffer, S., Zavolan, M., Grasser, F. A., Chien, M., Russo, J. J., Ju, J., John, B., Enright, A. J., Marks, D., Sander, C. et al. (2004). Identification of virus-encoded microRNAs. *Science* **304**, 734-736.
- Pfeffer, S., Sewer, A., Lagos-Quintana, M., Sheridan, R., Sander, C., Grasser, F. A., van Dyk, L. F., Ho, C. K., Shuman, S., Chien, M. et al. (2005). Identification of microRNAs of the herpesvirus family. *Nat. Methods* **2**, 269-276.
- Pillai, R. S., Bhattacharyya, S. N., Artus, C. G., Zoller, T., Cougot, N., Basyuk, E., Bertrand, E. and Filipowicz, W. (2005). Inhibition of translational initiation by let-7 microRNA in human cells. *Science* **309**, 1573-1576.
- Poy, M. N., Eliasson, L., Krutzfeldt, J., Kuwajima, S., Ma, X., Macdonald, P. E., Pfeffer, S., Tuschl, T., Rajewsky, N., Rorsman, P. et al. (2004). A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* **432**, 226-230.
- Qi, Y., Denli, A. M. and Hannon, G. J. (2005). Biochemical Specialization within Arabidopsis RNA Silencing Pathways. *Mol. Cell* **19**, 421-428.
- Rajewsky, N. and Succi, N. D. (2004). Computational identification of microRNA targets. *Dev. Biol.* **267**, 529-535.
- Rand, T. A., Ginalski, K., Grishin, N. V. and Wang, X. (2004). Biochemical identification of Argonaute 2 as the sole protein required for RNA-induced silencing complex activity. *Proc. Natl. Acad. Sci. USA* **101**, 14385-14389.
- Reinhart, B. J., Slack, F. J., Basson, M., Pasquinelli, A. E., Bettinger, J. C., Rougvie, A. E., Horvitz, H. R. and Ruvkun, G. (2000). The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* **403**, 901-906.
- Reinhart, B. J., Weinstein, E. G., Rhoades, M. W., Bartel, B. and Bartel, D. P. (2002). MicroRNAs in plants. *Genes Dev.* **16**, 1616-1626.
- Rhoades, M. W., Reinhart, B. J., Lim, L. P., Burge, C. B., Bartel, B. and Bartel, D. P. (2002). Prediction of plant microRNA targets. *Cell* **110**, 513-520.
- Riechmann, J. L., Heard, J., Martin, G., Reuber, L., Jiang, C., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O. J., Samaha, R. R. et al. (2000). Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science* **290**, 2105-2110.
- Rivas, F. V., Tolia, N. H., Song, J. J., Aragon, J. P., Liu, J., Hannon, G. J. and Joshua-Tor, L. (2005). Purified Argonaute2 and an siRNA form recombinant human RISC. *Nat. Struct. Mol. Biol.* **12**, 340-349.
- Rodriguez, A., Griffiths-Jones, S., Ashurst, J. L. and Bradley, A. (2004). Identification of mammalian microRNA host genes and transcription units. *Genome Res.* **14**, 1902-1910.
- Rougvie, A. D. (2005). Keeping time with microRNAs. *Development*, **132**, 3787-3798.
- Saito, K., Ishizuka, A., Siomi, H. and Siomi, M. C. (2005). Processing of pre-microRNAs by the dicer-1-loquacious complex in *Drosophila* cells. *PLoS Biol.* **3**, E235.
- Schwarz, D. S., Hutvagner, G., Du, T., Xu, Z., Aronin, N. and Zamore, P. D. (2003). Asymmetry in the assembly of the RNAi enzyme complex. *Cell* **115**, 199-208.
- Sen, G. L. and Blau, H. M. (2005). Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nat. Cell Biol.* **7**, 633-636.
- Slack, F. J., Basson, M., Liu, Z., Ambros, V., Horvitz, H. R. and 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.
- Song, J. J., Liu, J., Tolia, N. H., Schneiderman, J., Smith, S. K., Martienssen, R. A., Hannon, G. J. and Joshua-Tor, L. (2003). The crystal structure of the Argonaute2 PAZ domain reveals an RNA binding motif in RNAi effector complexes. *Nat. Struct. Mol. Biol.* **10**, 1026-1032.
- Song, J. J., Smith, S. K., Hannon, G. J. and Joshua-Tor, L. (2004). Crystal structure of Argonaute and its implications for RISC slicer activity. *Science* **305**, 1434-1437.
- Stark, A., Brennecke, J., Russell, R. B. and Cohen, S. M. (2003). Identification of *Drosophila* MicroRNA targets. *PLoS Biol.* **1**, E60.
- Sullivan, C. S., Grundhoff, A. T., Tevethia, S., Pipas, J. M. and Ganem, D. (2005). SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells. *Nature* **435**, 682-686.
- Tang, G., Reinhart, B. J., Bartel, D. P. and Zamore, P. D. (2003). A biochemical framework for RNA silencing in plants. *Genes Dev.* **17**, 49-63.
- Tomari, Y. and Zamore, P. D. (2005). Perspective: machines for RNAi. *Genes Dev.* **19**, 517-529.
- Vaucheret, H., Vazquez, F., Crete, P. and Bartel, D. P. (2004). The action of ARGONAUTE1 in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development. *Genes Dev.* **18**, 1187-1197.
- Vazquez, F., Gascioli, V., Crete, P. and Vaucheret, H. (2004a). The nuclear dsRNA binding protein HYL1 is required for microRNA accumulation and plant development, but not posttranscriptional transgene silencing. *Curr. Biol.* **14**, 346-351.
- Vazquez, F., Vaucheret, H., Rajagopalan, R., Lepers, C., Gascioli, V., Mallory, A. C., Hilbert, J. L., Bartel, D. P. and Crete, P. (2004b). Endogenous trans-acting siRNAs regulate the accumulation of Arabidopsis mRNAs. *Mol. Cell* **16**, 69-79.
- Vella, M. C., Choi, E. Y., Lin, S. Y., Reinert, K. and Slack, F. J. (2004). The *C. elegans* microRNA let-7 binds to imperfect let-7 complementary sites from the lin-41 3'UTR. *Genes Dev.* **18**, 132-137.
- Wienholds, E., Koudijs, M. J., van Eeden, F. J., Cuppen, E. and Plasterk, R. H. (2003). The microRNA-producing enzyme Dicer1 is essential for zebrafish development. *Nat. Genet.* **35**, 217-218.
- Wienholds, E., Kloosterman, W. P., Miska, E., Alvarez-Saavedra, E., Berezikov, E., de Bruijn, E., Horvitz, R. H., Kauppinen, S. and Plasterk, R. H. (2005). MicroRNA expression in zebrafish embryonic development. *Science* **309**, 310-311.
- Wightman, B., Ha, I. and 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.
- Xie, X., Lu, J., Kulbokas, E. J., Golub, T. R., Mootha, V., Lindblad-Toh, K., Lander, E. S. and Kellis, M. (2005). Systematic discovery of regulatory motifs in human promoters and 3' UTRs by comparison of several mammals. *Nature* **434**, 338-345.
- Xie, Z., Kasschau, K. D. and Carrington, J. C. (2003). Negative feedback regulation of Dicer-Like1 in Arabidopsis by microRNA-guided mRNA degradation. *Curr. Biol.* **13**, 784-789.
- Xie, Z., Johansen, L. K., Gustafson, A. M., Kasschau, K. D., Lellis, A. D., Zilberman, D., Jacobsen, S. E. and Carrington, J. C. (2004). Genetic and functional diversification of small RNA pathways in plants. *PLoS Biol.* **2**, E104.
- Yan, K. S., Yan, S., Farooq, A., Han, A., Zeng, L. and Zhou, M. M. (2003). Structure and conserved RNA binding of the PAZ domain. *Nature* **426**, 468-474.
- Yekta, S., Shih, I. H. and Bartel, D. P. (2004). MicroRNA-directed cleavage of HOXB8 mRNA. *Science* **304**, 594-596.
- Yi, R., Qin, Y., Macara, I. G. and Cullen, B. R. (2003). Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev.* **17**, 3011-3016.
- Yu, B., Yang, Z., Li, J., Minakhina, S., Yang, M., Padgett, R. W., Steward, R. and Chen, X. (2005). Methylation as a crucial step in plant microRNA biogenesis. *Science* **307**, 932-935.
- Zeng, Y. and Cullen, B. R. (2003). Sequence requirements for micro RNA processing and function in human cells. *RNA* **9**, 112-123.
- Zeng, Y. and Cullen, B. R. (2004). Structural requirements for pre-microRNA binding and nuclear export by Exportin 5. *Nucleic Acids Res.* **32**, 4776-4785.
- Zeng, Y. and Cullen, B. R. (2005). Efficient processing of primary microRNA hairpins by Drosha requires flanking non-structured RNA sequences. *J. Biol. Chem.* **280**, 27595-27603.
- Zeng, Y., Wagner, E. J. and Cullen, B. R. (2002). Both natural and designed micro RNAs can inhibit the expression of cognate mRNAs when expressed in human cells. *Mol. Cell* **9**, 1327-1333.
- Zeng, Y., Yi, R. and Cullen, B. R. (2005). Recognition and cleavage of primary microRNA precursors by the nuclear processing enzyme Drosha. *EMBO J.* **24**, 138-148.
- Zhang, Y. (2005). miRU: an automated plant miRNA target prediction server. *Nucleic Acids Res.* **33**, W701-W704.
- Zhao, Y., Samal, E. and Srivastava, D. (2005). Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature* **436**, 214-220.