

MicroRNA Function and Mechanism: Insights from Zebra Fish

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MicroRNAs (miRNAs) are small RNAs that bind to the 3'UTR of mRNAs. We are using zebra fish as a model system to study the developmental roles of miRNAs and to determine the mechanisms by which miRNAs regulate target mRNAs. We generated zebra fish embryos that lack the miRNA-processing enzyme Dicer. Mutant embryos are devoid of mature miRNAs and have morphogenesis defects, but differentiate multiple cell types. Injection of miR-430 miRNAs, a miRNA family expressed at the onset of zygotic transcription, rescues the early morphogenesis defects in *dicer* mutants. miR-430 accelerates the decay of hundreds of maternal mRNAs and induces the deadenylation of target mRNAs. These studies suggest that miRNAs are not obligatory components of all fate specification or signaling pathways but facilitate developmental transitions and induce the deadenylation and decay of hundreds of target mRNAs.

miRNAs are approximately 22-nucleotide small RNAs that act as posttranscriptional repressors by binding to the 3'UTR of target mRNAs (Lee et al. 1993; Reinhart et al. 2000; Bartel 2004; Kloosterman and Plasterk 2006). In animals, mature miRNAs are generated from a primary transcript (pri-miRNA) through sequential cleavage by nucleases belonging to the RNase III family. Initially, Drosha cleaves the pri-miRNA and excises a stem-loop precursor (pre-miRNA), which is then cleaved by Dicer into an RNA duplex (Bernstein et al. 2001; Grishok et al. 2001; Hutvagner et al. 2001; Ketting et al. 2001; Knight and Bass 2001). One strand of the duplex constitutes the mature miRNA, which is incorporated into a silencing complex (RISC) and guides it to target mRNAs (Hammond et al. 2000; Hutvagner and Zamore 2002; Khvorova et al. 2003; Schwarz et al. 2003). The 5' region of the miRNA (seed) is the main determinant of target recognition (Lai 2002; Lewis et al. 2003, 2005; Doench and Sharp 2004; Brennecke et al. 2005). Hundreds of miRNAs have been identified and thousands of targets have been predicted (Lagos-Quintana et al. 2001; Lau et al. 2001; Lee and Ambros 2001; Enright et al. 2003; Stark et al. 2003, 2005; Ambros 2004; Bartel 2004; Rehmsmeier et al. 2004; Berezikov et al. 2005; Farh et al. 2005; Krek et al. 2005; Lai 2005; Lewis et al. 2005; Miranda et al. 2006; Rajewsky 2006; Sood et al. 2006). However, the developmental roles of microRNAs are largely elusive (Alvarez-Garcia and Miska 2005; Kloosterman and Plasterk 2006), and it is still controversial how target mRNAs are repressed (Pillai 2005; Valencia-Sanchez et al. 2006). We have used the zebra fish embryo as a model system to investigate these issues.

ZEBRA FISH EMBRYOS THAT LACK MATURE miRNAs UNDERGO ABNORMAL MORPHOGENESIS

The global function of miRNAs has been unclear, and it is controversial whether miRNAs act as switches or modulators of biological processes (Bartel and Chen 2004). We therefore wished to create embryos that lack all

mature miRNAs (Fig. 1B). To this end, we used genetic and embryological manipulations to generate embryos that lacked all Dicer activity (Fig. 1B; Fig. 2) (Ciruna et al. 2002; Giraldez et al. 2005). Several lines of evidence indicated that these maternal-zygotic *dicer* mutants (*MZdicer*) lacked mature miRNAs. For example, northern analyses detected only pre-miRNAs, and reporter genes that contain miRNA target sites in their 3'UTRs were repressed in wild type but not in *MZdicer* mutants (Giraldez et al. 2005).

MZdicer mutant embryos had severe morphogenesis defects, including abnormal gastrulation movements, impaired brain ventricle formation, and somite defects, and died on day 5 of development (Giraldez et al. 2005). Interestingly, however, different cell fates were specified, including hematopoietic, muscle, and neuronal cell types (Fig. 3B). Dorsal-ventral and anterior-posterior patterning were normal, and the major signaling pathways active in the early embryo were not grossly misregulated, including signaling by Nodal, BMP, Wnt, FGF, Hedgehog, and Retinoic Acid. Most strikingly, germ cells devoid of Dicer activity developed, renewed, and generated oocytes and sperm without apparent defects. These results indicate that mature miRNAs are not obligatory regulators of cell-fate

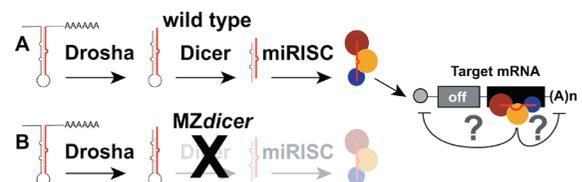


Figure 1. Maternal-zygotic *dicer* mutants do not process miRNAs. (A) Drosha cleaves the pri-miRNA and excises a stem-loop precursor. Dicer cleaves the hairpin to give rise to a miRNA:miRNA* duplex. One strand of this duplex is incorporated in a protein complex to form a miRNA-induced silencing complex (miRISC). The miRNA serves as a guide for this complex to the target mRNA. This leads to translational repression and accelerated mRNA degradation. (B) *MZdicer* mutants lack the miRNA processing enzyme Dicer and lack mature miRNAs.

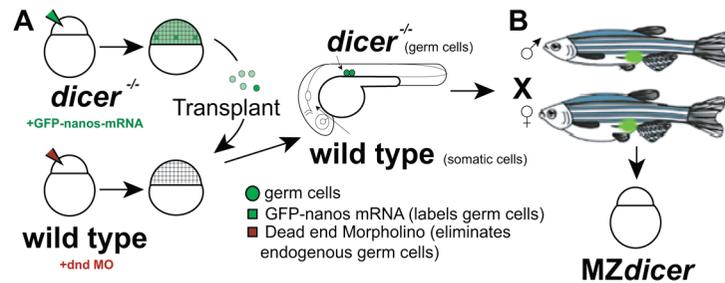


Figure 2. Germ-line replacement technique to generate MZ*dicer* mutants. (A) Schematic representation of the germ-line replacement technique used to generate maternal-zygotic *dicer* mutants. Depletion of host germ cells was accomplished by injection of a morpholino (Dead end morpholino; dnd MO) that blocks primordial germ cell development in host embryos, resulting in sterility. Donor germ cells labeled with GFP-nos-3'UTR were transplanted into these hosts during early embryogenesis (Ciruna et al. 2002). (B) Hosts containing *dicer/dicer* germ cells were raised to adulthood. These fish only harbored *dicer/dicer* PGCs. Intercrossing resulted in embryos that lacked both maternal and zygotic Dicer RNase III activity (MZ*dicer*). Additional fertile adults were generated using MZ*dicer* embryos as germ cell donors. These germ cells gave rise to mature and functional eggs and sperm, which generated embryos with a MZ*dicer* phenotype.

specification, differentiation, and signaling during early embryonic development in zebra fish. Instead, miRNA function appears to be required for the repression of mRNAs whose misexpression interferes with morphogenetic processes (Fig. 3B) (Giraldez et al. 2005).

THE miR-430 FAMILY REGULATES MORPHOGENESIS

In vitro generated miRNA duplexes were active upon injection into MZ*dicer* mutants (Fig. 3A), suggesting that Dicer is not required for the effector steps of miRNA function (Giraldez et al. 2005). Using this injection assay, we found that members of the miR-430 family rescued the early morphogenesis defects of MZ*dicer* mutants but were not able to suppress later phenotypes (Fig. 3B–D and Fig. 4) (Giraldez et al. 2005). miR-430 expression begins after the 500-cell stage, when the zygotic genome initiates transcription. Members of the miR-430 family are the major class of miRNAs during early embryogenesis and are expressed

ubiquitously throughout embryonic development in zebra fish and *Xenopus*. After mid-embryogenesis, many other miRNAs initiate their expression (Fig. 4A) (Chen et al. 2005; Giraldez et al. 2005; Watanabe et al. 2005). Their lack likely accounts for the later defects and lethality of miR-430-injected MZ*dicer* mutants. These results suggest that miR-430 regulates mRNAs whose overexpression affects morphogenesis.

miR-430 HAS HUNDREDS OF TARGET MRNAs

Reporter mRNAs with miR-430 target sites were efficiently degraded in wild type but not in MZ*dicer* mutants (Giraldez et al. 2006). These results led us to hypothesize that in vivo targets might also accumulate in the absence of miR-430 (Fig. 5A). We therefore performed expression profiling on wild-type embryos, MZ*dicer* mutants, and MZ*dicer* mutants injected with miR-430, leading to the identification of more than 700 mRNAs that accumulated in the absence of miR-430 (Giraldez et al. 2006). Strikingly, two-thirds of these

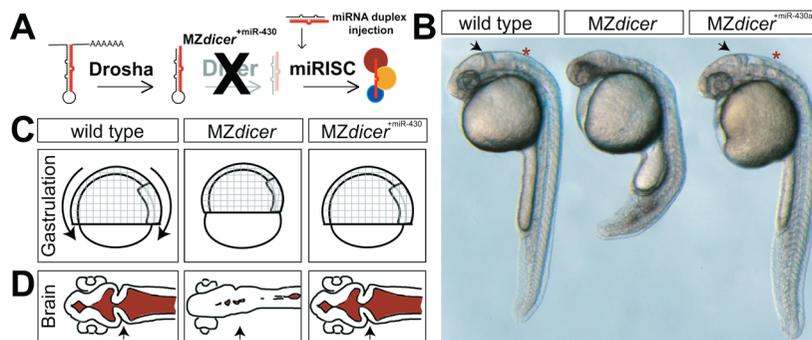


Figure 3. Abnormal morphogenesis in MZ*dicer* mutants. (A) Schematic representation showing that MZ*dicer* mutants lack mature miRNAs but injected processed miRNA duplexes reconstitute active miRISC. (B) Wild type (left), MZ*dicer* mutants (center) display morphogenesis defects in the retina, brain, trunk, and tail. MZ*dicer* injected at the one-cell stage with miR-430a+b duplex (right). Note the rescue of brain morphogenesis (asterisk), the midbrain-hindbrain boundary (arrow), and the trunk morphology. (C) Schematic representation shows the gastrulation defects in MZ*dicer* mutants with a slower epiboly compared to wild type. (D) Schematic representation shows a dorsal view of a 30-hr zebra fish brain in wild type, MZ*dicer*, and rescued embryos. The brain ventricles are labeled in red, and the arrow indicates the mid-hindbrain boundary.

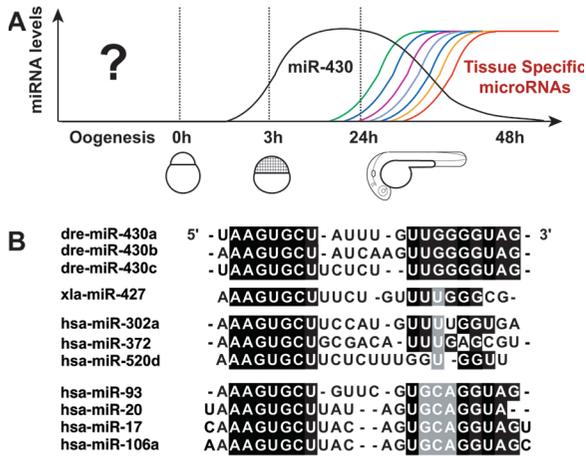


Figure 4. The miR-430 family of miRNAs. (A) Schematic representation showing the expression of miR-430 compared to other miRNAs in zebrafish embryos. MiR-430 starts to be expressed at the onset of zygotic transcription, is the predominant miRNA expressed during the first 18 hours of development, and decays by 48 hours postfertilization. Other miRNAs start to be expressed in a tissue-specific manner after ~18 hours postfertilization (Wienholds et al. 2005). It is currently unknown which miRNAs, if any, are expressed during oogenesis or before zygotic transcription. (B) Alignment of the zebrafish miR-430 family of miRNAs (*Danio rerio*, dre) with the *Xenopus laevis* miR-427 (Watanabe et al. 2005) and human miRNAs. miR-520 is a human miRNA belonging to a large miRNA cluster expressed in the placenta (Bentwich et al. 2005). miR-372 is a stem-cell miRNA that can cause germ cell tumors (Voorhoeve et al. 2006). The miR-17-20 family of miRNAs has been identified as a potential human oncogene (Ota et al. 2004; He et al. 2005).

mRNAs had 3'UTR sequences that were complementary to the miR-430 seed. Large-scale target validation using in vivo reporter assays revealed that the majority of putative targets was regulated by miR-430 in vivo (Fig. 5B). We estimate that miR-430 regulates at least 300 mRNAs. This number is likely an underestimate. First, the microarray contained only about 50% of all zebrafish genes. Second, expression profiling would not identify targets that are regulated only at the translational level. Hence, it is conceivable that miR-430 directly regulates more than 1000 different mRNAs (Giraldez et al. 2006).

miR-430 ACCELERATES THE CLEARANCE OF MATERNAL mRNAs

Strikingly, the large majority of miR-430 targets was maternally expressed and accumulated in the absence of miR-430 (Giraldez et al. 2006). Conversely, we found that 40% of maternal mRNAs had predicted miR-430 sites. These observations suggest that miR-430 is a key regulator of maternal mRNAs and accelerates their decay (Fig. 6). The aberrant accumulation of maternal mRNAs and their prolonged translation is therefore the likely cause of the morphogenesis defects observed in the absence of miR-430.

These results establish miR-430 as a key regulator of the maternal-to-zygotic transition. This is a universal transition in animal development when the embryo initiates the transcription of its genome (zygotic phase). The preceding stage of development is driven by maternally deposited mRNAs, and the genome is silent (maternal phase). The maternal-to-zygotic transition is still poorly understood, but it coincides with the patterning of the embryo and the

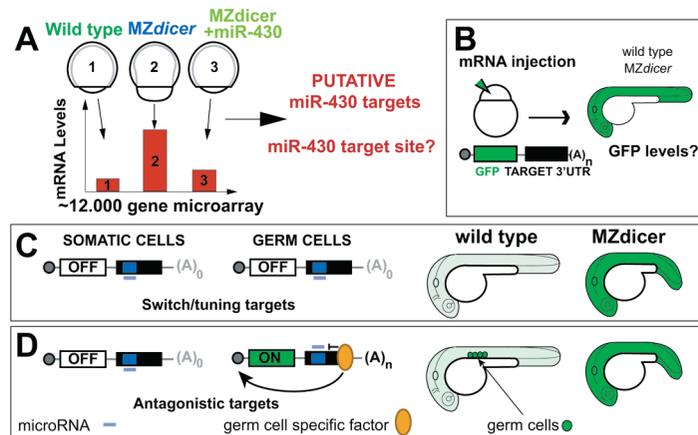


Figure 5. Regulation of miR-430 targets. (A) Schematic representation showing the strategy used to identify miR-430 targets in vivo using microarray analysis. Comparison of mRNA expression levels between MZdicer mutants and [MZdicer^{+miR-430} and wild type]. Putative targets must fulfill two criteria: (1) the mRNA must be up-regulated more than 1.5-fold in MZdicer compared to wild type and MZdicer^{+miR-430}; (2) it must contain a 6-mer sequence in the 3'UTR complementary to miR-430 seed. (B) A fraction of the putative targets were tested using injection of a GFP reporter mRNA with the 3'UTR of the target mRNA that is wild type or mutant for the miR-430 target sites. The levels of GFP expression in wild-type and MZdicer embryos were compared at 25–30 hours postfertilization. (C) Schematic representation showing that a target is validated when the GFP expression is repressed in wild-type but not in MZdicer embryos. In case of direct regulation, mutations in the miR-430 target site abolish repression in wild type. (D) Schematic representation showing that some targets are regulated by miR-430 in somatic cells but not germ cells despite the presence of miR-430 in both cell types. Model showing that a germ-cell-specific factor (orange) binds to the 3'UTR of antagonistic targets to inhibit miRNA function or enhance translation resulting in germ-cell-specific expression of the reporter mRNA.

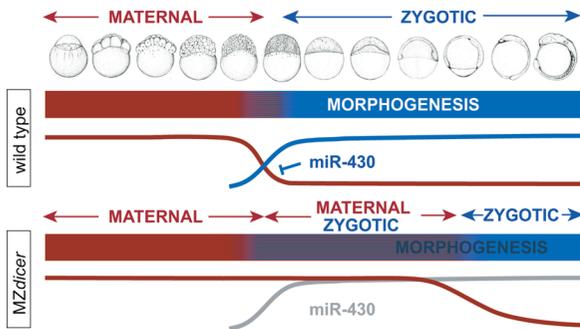


Figure 6. miR-430 accelerates the clearance of maternal mRNAs. Role of miR-430 in maternal-to-zygotic transition. In the presence of miR-430 (*blue*), a large set of maternal mRNAs (*red*) is posttranscriptionally regulated, allowing normal morphogenesis during zygotic stages. In the absence of miR-430 (*gray*), maternal mRNAs and their products accumulate and interfere with morphogenesis. (Reprinted, with permission, from Giraldez et al. 2006 [© AAAS].)

degradation of maternal mRNAs (Newport and Kirschner 1982a,b; Richter et al. 1990; Kane and Kimmel 1993; Richter 1999; Pelegri 2003; de Moor et al. 2005). Our results suggest that miR-430 transcription initiates at the maternal-to-zygotic transition to accelerate the degradation and repression of a large fraction of maternal mRNAs.

TWO CLASSES OF miR-430 TARGETS

Reporter assays using the 3'UTR of putative miR-430 target mRNAs revealed that the large majority of targets were uniformly repressed in all embryonic cells; i.e., in germ cells and all somatic cells (Fig. 5C). This observation is consistent with the ubiquitous expression of miR-430. Strikingly, however, at least two genes, *nanos* and

tudor-like, were more susceptible to miR-430 repression in somatic cells than in germ cells (Fig. 5D) (Mishima et al. 2006). Zebra fish germ cells are set aside during blastula stages and accumulate specific mRNAs and proteins (Yoon et al. 1997; Knaut et al. 2000; Kopranner et al. 2001; Raz 2004). We found that the 3'UTRs of *nanos* and *tudor-like* were repressed in somatic cells in the presence, but not in the absence, of miR-430. In contrast, expression in germ cells was maintained even when miR-430 was present (Fig. 5D). These results suggest that some 3'UTRs can be effective miRNA targets in one cell type but not another. Such 3'UTRs might contain activating or derepressing elements that counteract the effects of the miRNA, resulting in differential, tissue-specific regulation (Fig. 4D) (Mishima et al. 2006).

miRNAs INDUCE DEADENYLATION AND DECAY OF TARGET MRNAs

Previous studies had shown that miRNAs block the translation of target mRNAs and, in some cases, induced mRNA decay (Lee et al. 1993; Olsen and Ambros 1999; Reinhart et al. 2000; Bagga et al. 2005; Lim et al. 2005; Pillai 2005; Pillai et al. 2005). Since mRNA translation and stability depend on the poly(A) tail (Gallie 1991; de Moor et al. 2005), we tested the polyadenylation status of miR-430 targets (Fig. 7). We found that miR-430 induced the rapid deadenylation of target mRNAs (Giraldez et al. 2006; Mishima et al. 2006). Deadenylation was not an indirect effect of translational repression, because non-translatable targets were efficiently deadenylated in the presence, but not the absence, of miR-430 (Giraldez et al. 2006; Mishima et al. 2006). Many maternal mRNAs are deadenylated in the egg and polyadenylated upon fertilization (Slater et al. 1972; Slater et al. 1973; Wilt 1973; McGrew et al. 1989; Richter 1999; de Moor et al. 2005).

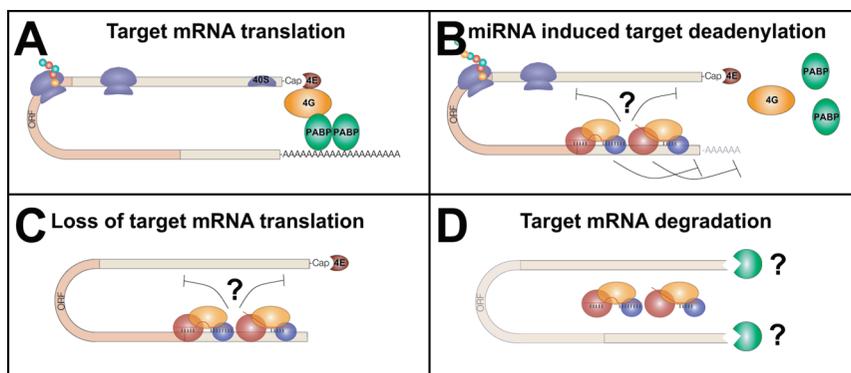


Figure 7. Model for the posttranscriptional regulation of mRNA targets by miRNAs. (A) Target mRNA translation: interaction between poly(A)-binding protein (PABP) on poly(A) tail with translation initiation factors eIF4G/eIF4E on Cap stimulates translation (Gallie 1991; Kapp and Lorsch 2004). (B) miRNA-induced target mRNA deadenylation: miRISC (Pillai 2005; Valencia-Sanchez et al. 2006) is recruited to the 3'UTR of target mRNA and accelerates deadenylation by a 3'-to-5' exonuclease (Richter 1999; de Moor et al. 2005; Giraldez et al. 2006; Vasudevan et al. 2006; Wu et al. 2006). (C) Loss of target mRNA translation: the interaction between PABP and eIF4G/eIF4E is disrupted, resulting in loss of translation initiation (Gallie 1991; Kapp and Lorsch 2004; Valencia-Sanchez et al. 2006). (D) Target mRNA degradation: Loss of poly(A) tail results in decapping and degradation of target mRNA (Rehwinkel et al. 2005). Steps 3 and 4 are likely to occur in P-bodies (Sheth and Parker 2003; Chen et al. 2005; Ding et al. 2005; Jakymiw et al. 2005; Liu et al. 2005; Pillai 2005; Pillai et al. 2005; Rehwinkel et al. 2005; Sen and Blau 2005). The suggested role for miRNAs in accelerating mRNA deadenylation would disrupt the interaction Cap-poly(A) tail and provide a substrate for exonucleases. This could explain how miRNAs cause translational repression and mRNA decay. (Reprinted, with permission, from Giraldez et al. 2006 [© AAAS].)

miR-430 seems to revert this process by inducing the deadenylation and decay of maternal mRNAs.

CONCLUSIONS

The powerful combination of genetics, embryology, molecular biology, and bioinformatics has helped us to clarify the functions and mechanisms of miRNAs during vertebrate embryogenesis. But how general are the lessons learned from miR-430 and zebra fish embryos?

miRNAs HAVE MODULATORY ROLES

Lack of miRNAs in *MZdicer* mutants leads to morphogenesis defects but does not lead to global disruption of fate specification or signaling. Our results therefore suggest that miRNAs have mainly modulatory roles. Indeed, miR-430 is the most abundant, if not the only, miRNA expressed during early zebra fish embryogenesis (Chen et al. 2005; Giraldez et al. 2005; Wienholds et al. 2005). Apparently, miRNAs are employed in a much more restricted fashion than are the dozens of transcription factors that specify embryonic cell fates (Schier and Talbot 2005). Similarly, loss of Dicer activity does not appear to affect zebra fish germ-cell development, suggesting that miRNAs play no or minor roles in the development of some cell types. Moreover, it is conceivable that no or few miRNAs are expressed in germ cells, despite or because of the presence of other small RNAs (Aravin et al. 2006; Girard et al. 2006; Grivna et al. 2006; Lau et al. 2006). Taken together, the results in zebra fish indicate that some cell types do not rely on microRNAs for specification and differentiation.

Studies in other systems partially support this view. For example, conditional ablation of mouse *dicer* in limbs or skin results in abnormal morphogenesis but does not lead to defects in cell-fate specification (Harfe et al. 2005; Andl et al. 2006; Yi et al. 2006). Similarly, loss of *Drosophila* miR-1, a muscle-specific miRNA, does not affect cell-fate specification, but morphogenesis during larval stages (Sokol and Ambros 2005). In contrast, studies in *Caenorhabditis elegans* have emphasized the role of the miRNAs *lin-4*, *let-7*, and *lsey-6* in cell-fate decisions (Lee et al. 1993; Wightman et al. 1993; Reinhart et al. 2000; Johnston and Hobert 2003) and miR-61 in cell signaling (Yoo and Greenwald 2005). However, it is possible that the *C. elegans* data are skewed, because these miRNAs were identified in specific screens for fate defects. Our results would predict that other *C. elegans* miRNAs have more subtle roles. Conversely, it will be important to determine whether the many tissue-specific miRNAs expressed at later stages of zebra fish development have roles in fate specification or tissue homeostasis and physiology (Stark et al. 2003, 2005; Xu et al. 2003; Poy et al. 2004; Boehm and Slack 2005; Farh et al. 2005; Krutzfeldt et al. 2005; Lim et al. 2005; Wienholds et al. 2005; Hornstein and Shomron 2006; Kloosterman and Plasterk 2006; Kloosterman et al. 2006; Sood et al. 2006; Teleman et al. 2006). Ultimately, it is likely that miRNAs will be found to have a wide range of functions, but it is striking that current evidence points to modulatory or highly redundant roles.

miRNAs FACILITATE DEVELOPMENTAL TRANSITIONS

miR-430 facilitates the maternal-to-zygotic transition. Importantly, this transition is not blocked in the absence of miR-430, and the embryo does not arrest in the maternal state. Rather, the zygotic state is initiated, but mRNAs from the maternal phase are not cleared efficiently and thus interfere with subsequent development. These observations led us to suggest that miRNAs might not serve as developmental switches, but simply remove mRNAs from previous developmental states. This “spring cleaning” function ensures proper transitions from states A to B to C, and thus avoids the formation of mixed states B/C (Fig. 8). This model contrasts with *C. elegans* studies which have shown that loss of *lin-4* and *let-7* leads to heterochronic phenotypes, in which the animal reiterates earlier cell-fate decisions instead of initiating the next developmental stage (Ambros 1989; Lee et al. 1993; Wightman et al. 1993; Reinhart et al. 2000; Alvarez-Garcia and Miska 2005). Instead of transitioning from A to B to C, certain lineages repeat the A to B transition and do not enter C. Despite these apparent differences, miR-430, *lin-4*, and *let-7* all act during developmental transitions, and it is possible that a specific phenotypic defect depends on the nature of the targets and the developmental context. For example, the accumulation of some targets might block the transition to the next stage, but in other systems the loss of the miRNA might cause a mixed state or delay the transition to a new state.

The role of miRNAs in developmental transitions has important implications for miRNA misregulation. For example, premature expression of a miRNA could preemptively block genes expressed at a later stage and thus

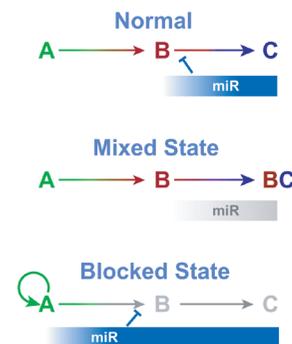


Figure 8. Model of miRNA function during development. Cell transitions from state A to state B to state C. *Normal:* miRNA is activated at the B-to-C transition and posttranscriptionally regulates mRNAs expressed in state B, thus sharpening and accelerating the transition from state B to state C (Rhoades et al. 2002; Giraldez et al. 2006). *Mixed State:* In the absence of the miRNA, gene products from state B accumulate, leading to a mixed state B/C. *Blocked State:* In disease states or upon experimental misexpression, the miRNA is expressed prematurely, resulting in the posttranscriptional regulation of genes normally expressed in state B. This prevents the transition from state A to state B, leading to the maintenance of state A. In the case of cancer, state A might correspond to a cancer stem cell (Beachy et al. 2004; He et al. 2005; Lu et al. 2005; Dalerba et al. 2006; Dews et al. 2006; Voorhoeve et al. 2006). (Reprinted, with permission, from Giraldez et al. 2006 [© AAAS].)

prevent developmental transitions (Fig. 8). This mechanism might explain how some miRNAs can function as oncogenes (He et al. 2005; Lu et al. 2005; Dews et al. 2006; Voorhoeve et al. 2006).

MiR-430 continues to be expressed during the first two days of zebra fish embryogenesis, and many of its targets are expressed during these stages (Giraldez et al. 2006). It is therefore likely that miR-430 does not only function in the maternal-to-zygotic transition, but also dampens the expression of zygotically expressed genes. Such a homeostatic role resembles the proposed roles of miRNAs in maintenance of tissue states or metabolic states (Stark et al. 2003, 2005; Xu et al. 2003; Poy et al. 2004; Boehm and Slack 2005; Farh et al. 2005; Krutzfeldt et al. 2005; Lim et al. 2005; Hornstein and Shomron 2006; Sood et al. 2006; Teleman et al. 2006).

miRNAs HAVE HUNDREDS OF IN VIVO TARGETS

miR-430 regulates hundreds of mRNAs. These in vivo observations support cell culture and bioinformatics studies which have suggested that miRNAs have dozens of targets (Enright et al. 2003; Stark et al. 2003, 2005; Kiriakidou et al. 2004; Poy et al. 2004; Rehmsmeier et al. 2004; Krek et al. 2005; Lewis et al. 2005; Lall et al. 2006; Miranda et al. 2006). In contrast, genetic studies in *C. elegans* have identified a few key targets whose up-regulation leads to the phenotypes caused by loss of specific miRNAs (Lee et al. 1993; Wightman et al. 1993; Reinhart et al. 2000; Slack et al. 2000; Abrahante et al. 2003; Johnston and Hobert 2003; Lin et al. 2003; Abbott et al. 2005; Boehm and Slack 2005; Grosshans et al. 2005; Johnson et al. 2005; Yoo and Greenwald 2005). These two observations are not necessarily contradictory. It is conceivable that miRNAs have the capacity to regulate hundreds of targets but that the up-regulation of only a small subset of targets has severe phenotypic consequences. Detailed functional analysis of target genes is necessary to determine which and how many targets contribute to a given phenotype.

SOME miRNA TARGETS ARE DIFFERENTIALLY REGULATED

Most miR-430 targets are uniformly susceptible to repression, but a small subset appears to be partially protected in germ cells. It is therefore conceivable that many mRNAs have acquired specific sequences that counteract the effect of miRNAs in specific tissues, times, or cell states. For example, *nanos* and *tudor-like* are miR-430 targets in somatic cells but are translated in germ cells (Mishima et al. 2006), and in tissue culture, repression of *cat-1* by miR-122 is relieved upon cellular stress (Bhattacharyya et al. 2006). Such “antagonistic targets” complement “anti-targets,” which have evolved to lack miRNA-binding sites (Bartel and Chen 2004; Farh et al. 2005; Stark et al. 2005; Sood et al. 2006). Hence, mRNAs can avoid miRNA regulation by either lacking a miRNA-binding site (anti-targets) or by evolving sites that counteract the miRNA’s effects (antagonistic targets). The advantage of the latter strategy is that target repression can

be regulated in a tissue-, stage-, or state-specific manner. It remains to be seen how common this mechanism is, but it might account for the observation that many predicted miRNA targets do not seem to be regulated by a given miRNA (Lewis et al. 2003; Stark et al. 2003, 2005; Giraldez et al. 2006; Miranda et al. 2006). These results also highlight the importance of in vivo validation of putative targets by testing the role of their full-length 3’UTRs.

miRNAs REGULATE TARGET mRNAs BY DEADENYLATION

miR-430 accelerates the deadenylation of mRNA targets (Giraldez et al. 2006; Mishima et al. 2006). Further support for a role for miRNAs in deadenylation has come from cell culture studies (Wu et al. 2006) and the identification of deadenylases involved in miRNA target degradation (Behm-Ansmant et al. 2006). In some cases, however, miRNAs seem to be able to repress translation in the absence of deadenylation (Humphreys et al. 2005; Wu et al. 2006) or mRNA degradation (Pillai 2005; Petersen et al. 2006). It is therefore possible that deadenylation is one of several mechanisms that contribute to target regulation. However, since deadenylation ultimately leads to both translational repression and mRNA decay, the two main effects of miRNA action, it is tempting to speculate that deadenylation is the trigger for miRNA-mediated repression.

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