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microRNAs, the cell's Nepenthe: clearing the past during the maternal-to-zygotic transition and cellular reprogramming

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The maternal-to-zygotic transition (MZT) is a universal step in animal development characterized by two major events: activation of zygotic transcription and degradation of maternally provided mRNAs. How zygotic gene products instruct the degradation of maternal messages remains a long-standing question in biology. MicroRNAs (miRNAs) have recently emerged as widespread regulators of gene expression. miRNAs control temporal and spatial gene expression by both accelerating the decay of mRNAs from previous developmental stages and modulating the levels of actively transcribed genes. In this review, I discuss recent studies of the roles of miRNAs during the maternal-to-zygotic transition and cellular reprogramming, where they reshape transcriptional landscapes to facilitate the establishment of novel cellular states.

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Introduction

The earliest stages of embryonic development depend on maternal instructions loaded into the oocyte in the form of mRNA and proteins [1,2,3•]. This maternal program is ultimately responsible for the activation of the zygotic genome. Within hours of fertilization, a large fraction of maternally deposited mRNAs is eliminated via two cooperative, yet distinct, programs [3•,4]. First, maternally encoded products initiate the destruction of maternal mRNAs. In *Drosophila*, the maternally provided RNA-binding protein Smaug is responsible for the deadenylation and clearance of the majority of unstable transcripts following egg activation [5,6•]. In *Xenopus*, maternal mRNAs possessing an embryonic deadenylation element (EDEN) within their 3′UTRs are targeted for deadenylation and translational silencing by the maternally provided EDEN-binding protein [7].

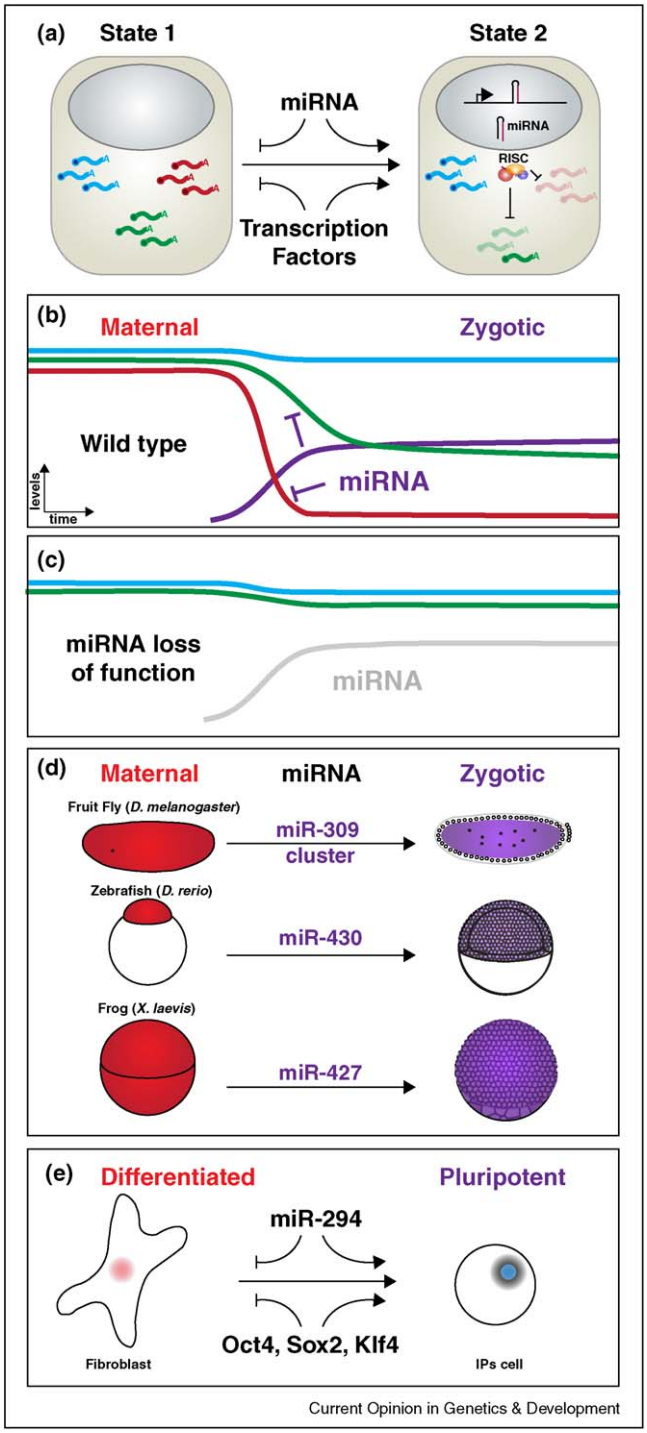
In addition to these maternal factors, which have been thoroughly reviewed elsewhere [2,3•,7], a second degradation program is initiated by, and is dependent on, zygotic transcription. In particular, zygotically expressed miRNAs have been shown to dramatically enhance the efficiency of maternal mRNA clearance in zebrafish [8•], *Xenopus* [9] and *Drosophila* [10•]. miRNAs are small ~22 nt RNAs that regulate gene expression post-transcriptionally [11–13]. Mature miRNAs are generated from longer primary transcripts through sequential cleavage by the RNaseIII enzymes, Drosha and Dicer. The mature miRNA, once incorporated into a silencing complex (miRISC), guides the miRISC to target mRNAs, resulting in their deadenylation, repression, and decay [8•,14–17] reviewed in [13,18,19]. Functional analyses have shown that miRNAs shape gene expression within multiple developmental contexts (reviewed in [20]). miRNAs have been shown to control temporal gene expression by downregulating mRNAs transcribed during previous developmental stages [8•,21–23]. On the other hand, miRNAs can shape spatial expression of a given gene, by modulating the levels of actively transcribed genes in a specific domain [24–27]. In the case of the maternal–zygotic transition, the removal of pre-existing mRNAs prevents their interference with zygotic development [8•,10•]. For example, by 'wiping the slate clean', the zygotic counterpart of a maternally provided ubiquitous mRNA can be expressed in a restricted pattern [28,29,30•]. In addition, the post-transcriptional nature of miRNA-mediated regulation provides an ideal mechanism to precisely modulate mRNA dosage of zygotic [31] and pre-existing maternal mRNAs deposited in the oocyte.

In this review, we highlight recent contributions to the molecular regulation of the maternal-to-zygotic transition by miRNAs, and how they function in development to clear maternal mRNAs, facilitate tissue-specific expression of maternal-ubiquitously provided mRNAs and improve cellular reprogramming by erasing the cell's transcriptional history.

miRNAs clear maternal mRNAs during the maternal-to-zygotic transition in vertebrates

Activation of zygotic transcription is intimately linked to the degradation of maternal messages [4,32–35] (reviewed in [3•]). Indeed, inhibition of zygotic transcription results in the stabilization of a large fraction of

Figure 1



MicroRNAs clear the cell's transcriptional landscape during developmental transitions. **(a)** Diagram of a cell in two different states. The expression of a miRNA (hairpin) in the second state leads to the clearance of some transcripts (red) and the partial downregulation of other targets (green). **(b,c)** Schematic representation of levels for different mRNAs and a miRNA in the maternal-to-zygotic transition in the presence **(b)** and absence **(c)** of a miRNA. The different curves represent the degradation profiles of maternal (red, blue) and maternal–zygotic (green) transcripts that are regulated by the miRNA during the maternal-to-zygotic transition or during reprogramming, resulting in a rapid decay of the mRNA levels. Targeting of the green transcript by the miRNA allows the cell to regulate the steady-state levels of this mRNA. **(d)** Diagram representing known examples of miRNAs that regulate the clearance of maternal transcripts during the maternal-to-zygotic transition in different organisms. **(e)** Reprogramming of somatic cells to a pluripotent state. Introducing the mouse ortholog of miR-430 (miR-294) into differentiated cells together with Oct4 Sox2, and Klf4 enhances the reprogramming efficiency.

maternal mRNAs [32]. Yet, the factors responsible for this selective and active degradation have remained mostly elusive. The orthologous miRNAs, miR-430 and miR-427, are abundantly expressed during the maternal-to-zygotic transition in zebrafish [36,37] and *Xenopus* [38,39], respectively. In the fish, transcriptional profiling of maternal–zygotic *dicer* mutants (MZ*dicer*) and *in vivo* validation experiments have identified more than 200 miR-430 target mRNAs *in vivo* [8•]. These targets are strongly enriched for maternally provided mRNAs (~4-fold). In addition, analysis of the maternal mRNA population (in which ~70% of all zebrafish genes are represented) reveals a ~4-fold enrichment for the presence of miR-430 complementary sites when compared to mRNAs that are strictly zygotic. Further, loss of miR-430 slows the decay of several hundred maternal mRNAs [8•] (Figure 1). miR-430-mediated mRNA degradation is achieved through the accelerated deadenylation of target transcripts, and has provided an entry point for understanding the molecular mechanisms behind miRNA-mediated target mRNA turnover.

These above findings support the hypothesis that miR-430 accelerates the deadenylation and decay of several hundred maternally loaded mRNAs. A similar scenario has been observed for the *Xenopus* miR-430 ortholog, miR-427 [9]. miR-427 is highly transcribed by RNA Pol II before general zygotic genome activation [38,39]. As a result, cells accumulate high levels of miR-427, with ~10^9 copies of mature miRNA/embryo [9]. Lund *et al.* provide evidence that miR-427 directly accelerates the deadenylation of maternally deposited cyclin B2 and A1 mRNAs. Although additional targets need to be validated, 3′UTR sequence analysis in *Xenopus* has identified conserved miR-427 target sites in eight additional genes. The miR-427 target list is likely to increase once the genome-wide functions of miR-427 are examined. It is important to note that multiple mammalian orthologs of miR-430 (miR-295 in mice, and miR-302, miR-372, miR-516-520 in humans and primates) are expressed during early embryogenesis and could potentially regulate the clearance of maternal transcripts in mammals. Despite the sequence homology among the miR-430 orthologs and their important roles during maternal clearance (Zebrafish and Xenopus) and stem cell maintenance (mouse and human), little is known about the upstream factors that activate their expression during development.

A recent study has shown that the stem cell factors Oct4 and Sox2 bind the promoter of miR-302 and activate its expression in ES cells [40]. Since these factors are also expressed in the early embryo, and at least Oct4 is maternally provided, it is tantalizing to speculate that the same factors might drive zygotic activation of miR-430/427 to initiate the degradation of the maternal mRNAs.

On the basis of the occurrence of complementary target sites, miR-430 has the potential to regulate up to 40% of all maternal messages present in the early fish embryo [8•]. However, most of these putative targets await experimental validation [8•]. The rules governing miRNA-target regulation are not yet fully understood [13]. The mere presence of a putative miRNA target site does not guarantee miRNA-mediated regulation. On the other hand, many *bona fide* targets that are primarily regulated at the level of translation will be missed in expression profiling studies. For many targets, it is likely that much finer-grained analyses will be required, especially in cases where the miRNA functions together with other RNA-binding proteins such as Smaug or Pumillio to confer an additional layer of repression [3•].

The role of miRNAs in the maternal-to-zygotic transition in *Drosophila*

How do other animals that lack miR-430 orthologs remove maternally deposited messages? The clearance of maternally provided mRNAs has been studied using chromosomal deletions to eliminate the confounding effect of transcription of the zygotic contribution of that gene. In contrast to that observed for miR-430 in the fish, De Renzis and colleagues did not find any predominant miRNA target site sequence that was enriched within the unstable maternal mRNA pool. This might be explained by the potential diluting effects of analyzing each miRNA separately [28]. Interestingly, the generation of a miRNA cluster knockout in *Drosophila* revealed that zygotic expression of the miR-309 cluster, which encodes miR-3, miR-4, miR-5, miR-6, and miR-309, directs the degradation of a subset of maternal mRNAs at the MZT [10•]. Loss of the miR-309 clusters results in the stabilization of a large set of maternal mRNAs that are rapidly degraded during the MZT. Interestingly, a comparison of the occurrence of all putative miRNA target sites versus those specific to the miR-309 cluster, suggests that maternal genes are enriched for, and zygotic genes are depleted of, miR-309 cluster target sites in their 3′UTRs. This finding suggests that the expression of the miR-309 cluster (zygotically provided) tends to be temporally anticorrelated with the expression of its targets (maternally provided) [10•].

The above findings clearly implicate a family of miRNAs in the clearance of maternal mRNAs in the fly [10•]. But how is this miRNA cluster activated in the first place to ensure the timely degradation of the maternal mRNAs? A recent study has identified a maternally deposited transcription factor, Zelda, is required for the activation of the miR-309 cluster in addition to other zygotic genes [41•]. Interestingly, there is a link between miR-309 activation and Smaug-mediated degradation of maternally deposited mRNAs. Smaug is a conserved RNA-binding protein required for the destruction of maternal mRNAs during the MZT in *Drosophila*. Intriguingly, roughly 85% of the 410 maternal mRNAs upregulated in the absence of the miR-309 cluster are also stabilized in s*maug* mutants. This result is likely due to reduced miR-309 expression in *smaug* mutant embryos, suggesting that maternal mRNA clearance (Smaug-mediated) is required for high-level zygotic activation, including the miR-309 cluster, which in turn leads to further destabilization of a subset of maternal mRNAs [6•].

Loading of the miRNA processing machinery in the egg allows timely regulation of maternal mRNAs

miRNAs must be processed into their mature forms to mediate repression. Interestingly, the miRNA processing machinery, including Dicer and Drosha, appears to be maternally supplied in frog, fish, and fly embryos. As a result, embryos are 'primed' for rapid processing once zygotic miRNAs are transcribed at the MZT. This triggers the deadenylation and clearance of a large fraction of the maternal mRNAs with the appropriate timing and decay rate. Consistent with this hypothesis, the lethality observed from maternal loss of zebrafish *dicer* cannot be rescued by zygotically provided dicer (inherited from the male). The time required to transcribe and translate zygotic Dicer results in an insurmountable delay in the processing of miR-430 and subsequent maternal clearance. However, direct injection of processed miR-430 into these embryos rescues the lethal phenotype (M*dicer*$^{-/-}$, *Zdicer*$^{-/+}$) to make viable adults, suggesting that miR-430 is the only miRNA that requires the maternal miRNA processing machinery for survival (Giraldez, unpublished results). Furthermore, in *Xenopus*, providing miR-427 before endogenous miR-427 is transcribed, causes the premature deadenylation of its targets [9]. These results indicate that the miRNA processing and effector machinery are maternally provided, thereby allowing the timely repression, deadenylation and decay of miRNA targets at the onset of zygotic transcription.

MicroRNAs regulate steady-state mRNA levels

miRNAs not only function to clear the target mRNAs expressed in a previous developmental state. A fraction of the target mRNAs is also zygotically transcribed. In this case, miR-430-mediated regulation firstly, tunes down the activation of the zygotic mRNA and secondly, modulates the steady-state mRNA levels for these transcripts (Figure 1). Indeed, more than 70% of the vertebrate mRNAs are thought to be under miRNA-mediated

regulation. This might reflect a role for miRNAs to control the rate of target mRNA decay and maintain mRNA homeostasis [8•,27,42].

A common theme for miRNA function, clear your past

The finding that unrelated miRNA clusters (miR-309 in drosophila and miR-430/427 in Zebrafish/Xenopus) have taken on similar functions during MZT illustrates a striking example of convergent evolution. In both cases, the expression of the miRNA serves to clear the cell of previously expressed transcripts, thereby setting the stage for subsequent developmental stages (Figure 1). In many ways, the functions of miR-309 and miR-430 during MZT are reminiscent of those observed for the founding miRNAs lin-4 and let-7 in *C. elegans*. Both lin-4 and let-7 clear and repress previously expressed transcripts and facilitate progression to the following developmental stage [21,22,43,44]. In hindsight, the use of miRNAs in shaping the temporal dynamics underlying developmental transitions provides a versatile system with multiple advantages for the embryo. Maternally deposited mRNAs can only be regulated post-transcriptionally and as such, miRNAs can easily regulate mRNAs that have been previously generated. In addition, the small 'seed' size of miRNA target sites (6–8-nt long) allows for a miRNA to simultaneously control a large number of unrelated transcripts. Simply by acquiring few mutations in their 3′UTRs during evolution, genes can gain and lose target sites, with no impact on their coding sequences. The level of regulation can be modified by changing the extent of complementarity between the target and the miRNA as well as the number of complementary sites. Additional specificity can be conferred by RNA-binding proteins, discussed below, which modulate miRNA-mediated regulation in cell-type specific manner. Taken together, this inherent versatility allows the same miRNA to both remove previously transcribed mRNAs that are no longer needed and precisely regulate steady-state levels of those that are still being transcribed.

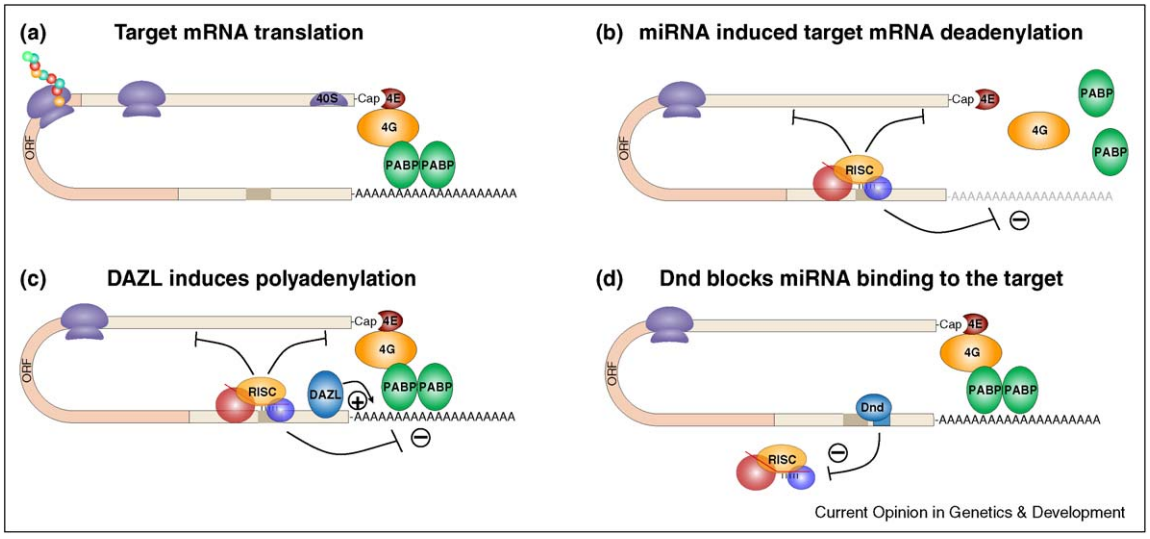
Antagonizing miRNA function during maternal clearance shapes gene expression

Recent studies have provided interesting insights into how RNA-binding proteins can modulate miRNA-mediated regulation depending on cell type or cellular state (Figure 2). In the germ line, the RNA-binding proteins, Deadend [45•] and Dazl [30•], protect some maternal mRNAs from the clearing effects of miR-430 [29]. These germ cell-specific factors counteract the effects of miR-430 on *nanos1* and Tudor-domain-containing-protein 7 (*Tdrd-7*) mRNA through two different mechanisms. The Deadend-binding site in the *nanos1* 3′UTR overlaps with the miR-430 site, such that interaction with Deadend provides steric protection from miR-430 [45•]. In contrast, Dazl antagonizes the activity of miR-430 by inducing mRNA polyadenylation when bound to the Tdrd-7 3′UTR [30•]. In both cases, the modulation of miRNA activity facilitates tissue-specific expression of ubiquitously provided mRNAs.

MicroRNAs clear the cell's history during cellular reprogramming

The maternal-to-zygotic transition is in some ways analogous to the process of cellular reprogramming. The nucleus of a differentiated cell, when exposed to the cytoplasm of the fertilized egg can be reprogrammed to a totipotent state [46,47]. In both cases, during the maternal-to-zygotic transition and during cellular reprogramming, the cell's history is erased to facilitate the establishment of novel cellular states by specific transcription factors (zygotic state or pluripotency). Intriguingly, the mammalian orthologs of miR-430 (miR-294 in mice and miR-302/-372 in humans) are abundantly expressed in embryonic stem cells and embryonic tissues [48,49]. While it is tempting to speculate that these miRNAs might play important roles in the clearance of the maternal transcripts in mammals, several lines of evidence suggest that these miRNAs contribute to pluripotency and have important roles in cellular reprogramming. Recent studies have shown that defined transcription factors can reprogram differentiated cells to adopt pluripotency (induced pluripotent stem cells: iPS cells). These factors include Oct4, Klf4, Sox2, and c-myc [50]. While only Oct4 appears to be critical [51], all of these factors increase the reprogramming efficiency. Interestingly, co-introduction of a miRNA (miR-294) with Oct4, Sox2, and Klf4 in differentiated fibroblasts dramatically enhances the reprogramming efficiency 10-fold compared to the three factors alone [52] (Figure 1). What could make the miR-294 family such an efficient reprogramming factor? ES cells defective in miRNA processing show defects in proliferation, differentiation, and self-renewal [53]. Interestingly, Blelloch and colleagues have shown that replenishing *dicer* mutant ES cells with miR-430/302/294 family members rescues proliferation defects [53]. However, promoting cell proliferation in differentiated cells only modestly increases the reprogramming efficiency, suggesting that there are additional functions of the miR-294/302 family other than accelerating proliferation. Because miRNAs shape gene expression in both spatial and temporal dimensions, they make ideal candidates to clear the cells' transcriptional memory. Indeed, it has been shown that many miRNAs tend to be expressed in an anticorrelative pattern with their targets [24,26,27,42,54]. Genes required for a specific cellular state tend to avoid strong repression by coexpressed miRNAs. For example, miRNAs expressed in embryonic stem cells will typically not target essential stem cell factors. In this scenario, introducing these ES cell miRNAs (miR-294) into differentiated cells is likely helping to erase the transcriptional landscape of the differentiated cell. This creates a clear slate where the specific reprogramming transcription factors can return the cell to a pluripotent state.

Figure 2



RNA-binding proteins modulate miRNA-mediated repression of maternal mRNAs in germ cells. Model for the post-transcriptional regulation of mRNA targets by miRNAs. **(a)** Target mRNA translation: interaction between poly(A) binding proteins (PABPs) on poly(A) tail with translation initiation factors eIF4G/eIF4E on Cap stimulates translation. **(b)** miRNA-mediated target mRNA deadenylation: the miRNA induced silencing complex (miRISC) is recruited to the 3′UTR of target mRNA and accelerates deadenylation. **(c)** Binding of the DAZL to the 3′UTR of the target (*Tdrd7*) antagonizes miRNA-mediated repression by promoting polyadenylation. **(d)** Binding of Dead end (Dnd) to the 3′UTR of *nanos1* blocks the binding of the miRISC to the target, and antagonizes miRNA-mediated repression of *nanos1* in germ cells.

Many miRNAs have a tissue-specific expression in the embryo [55] and shape the gene expression during differentiation [24,26,27,42]. As a consequence, miRNAs not only help cells to forget their past transcriptional history during reprogramming, but can also redirect their path during differentiation. For example, providing miR-145 into multipotent neural crest stem cells can influence their downstream differentiation path to vascular smooth muscle fate [56], suggesting that expression of specific miRNA can favor differentiation into specific fates. miRNAs might stabilize not only a cell fate, but also the differentiated state per se. In contrast to stem cell specific miRNAs, other miRNAs such as let-7 inhibit pluripotency once the cell becomes committed to a specific fate therefore stabilizing this decision [57•]. Conversely, loss-of-let7 has been associated with cancer [58], a state where the cell might return to an embryonic state by stimulating proliferation and de-repressing the pluripotent state.

Future outlook

Despite the wide variety of biological contexts where miRNAs function, a common theme emerges, whereby miRNAs shape spatial and temporal gene expression: firstly, modulate the levels of actively transcribed genes and secondly, accelerate the clearance of previously transcribed messages. However, miRNAs correspond only to a small fraction of the noncoding genome and, as such, represent just the tip of the noncoding iceberg. Future studies will be needed to shed light on the functions of additional small and long noncoding RNAs during the maternal-to-zygotic transition, in DNA integrity surveillance, and epigenetic regulation. Additional proteins are likely to modulate miRNA function in cells and the transcription factors responsible for their expression are largely unknown. RNA-binding proteins can antagonize or potentiate miRNA activity. The accessibility of the miRNA to the target mRNA could be regulated by modifying the secondary structure of the RNA, blocking the target site, or altering the polyadenylation site to change the length of the 3′UTR to include or exclude specific miRNA target sites. These modifiers can modulate the temporal and spatial activity of miRNAs providing a highly versatile system to regulate gene expression during embryogenesis. Although some miRNAs are ubiquitously expressed, the majority show restricted spatial expression within particular tissues and organs. Future studies will provide important insights into the use of miRNAs during cellular reprogramming to tailor the differentiation path of cells to specific fates *in vivo*.

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