

Why mouse oocytes and early embryos ignore miRNAs?

Petr Svoboda

Institute of Molecular Genetics AS CR; Videnska, Czech Republic

Small RNA molecules regulating gene expression received a status of omnipresent master regulators of eukaryotic lives with almost supernatural powers. Mammals hold at least three mechanisms employing small RNA molecules for regulating gene expression. One of these mechanisms, the microRNA (miRNA) pathway, currently involves over a thousand of genome-encoded different miRNAs that are claimed to extend their control over more than a half of a genome. Here, I discuss how and why mouse oocytes and early embryos ignore the regulatory power of miRNAs, adding another surprising feature to the field of small RNAs.

Introduction

Commonly known as RNA silencing, eukaryotic regulation of gene expression by small (20–30 nucleotides long) RNAs involves sequence-specific mRNA cleavage, repression of mRNA translation, transcriptional silencing, heterochromatinization or even DNA deletion (reviewed in ref. 1). RNA silencing is initiated by processing different RNA substrates into short RNA molecules, which are loaded on an effector ribonucleoprotein complex. Targeted nucleic acids are recognized upon basepairing with guiding small RNAs and are regulated based upon the type of effector complexes. Three mammalian RNA silencing pathways were identified and their mechanisms extensively explored: microRNA (miRNA), RNA interference (RNAi) and PIWI-associated RNA (piRNA) pathways.

Mammalian miRNA and RNAi Pathways

Mammalian miRNA and RNAi pathways share common factors, including production of miRNAs and small interfering RNAs (siRNAs) in the RNAi pathway by the same RNase III Dicer and loading these small RNAs on the same set of Argonaute proteins (reviewed in ref. 2). The mammalian Argonaute family consists of eight members. Four of them (Piwi subfamily) are expressed in germ cells and function in the piRNA pathway^{3–6} while the other four (Ago subfamily, AGO1 through AGO4) are expressed ubiquitously and bind miRNAs and siRNAs.⁷ Predicted hybrids between mammalian miRNAs and their cognate mRNAs typically contain bulges and mismatches⁸ and cause translational repression, which requires also function of a translational repressor TNRC6 (GW182).^{9,10} AGO2 is also the “slicer”—it can mediate sequence-specific endonucleolytic cleavage of a target mRNA in the middle of the perfect basepairing between a small RNA and its cognate mRNA.⁷ This sequence-specific cleavage is a hallmark of RNAi¹¹ but it is also found among miRNAs binding with perfect complementarity like miR-196 interaction with *HoxB8* mRNA.¹² Thus, unlike in invertebrates,^{13–16} the association of translational repression with miRNAs and sequence-specific cleavage of mRNA with RNAi is loose in mammals and is not underlied by separated molecular mechanisms. Notably, miRNAs can also cause substantial mRNA degradation without extensive base pairing to their targets,^{17,18} likely because of

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Correspondence to: Petr Svoboda;
Email: svobodap@img.cas.cz

the relocation of translationally repressed mRNA into P-bodies.^{19,20} P-bodies are discrete cytoplasmic foci containing a number of RNA binding proteins, translational repressors and mRNA degrading enzymes involved in decapping, deadenylation, and the 5' to 3' degradation, which are centers of RNA metabolism (reviewed in ref. 21).

Mammalian RNAi and miRNA pathways mostly differ in biological roles and in origins and sequence definitions of small RNAs. miRNAs have defined sequences, are genome-encoded and inhibit normal protein-coding genes. Dicer cleavage of miRNA precursors is preceded by nuclear processing of primary transcripts by the microprocessor complex containing DGCR8 and Drosha proteins (reviewed in ref. 2). Each cell produces a specific set of miRNAs and many hundreds of mammalian miRNAs were identified.²² The highest estimates claim that miRNAs directly target over 60% of mammalian genes.²³ miRNAs are viewed as a “buffering system” for gene expression, which is important during processes involving global changes of gene expression, such as differentiation.²⁴

In the context of mammals, the term RNAi is usually used for hijacking the miRNA pathway with artificial complementary small RNAs. The endogenous RNAi pathway is initiated by long double-stranded RNA (dsRNA), which is processed by Dicer, hence producing a pool of siRNAs with different sequences. Natural RNAi targets mobile, viral or other aberrant and potentially harmful sequences and it is thought to act as a sort of innate immunity (reviewed in ref. 25). In mammals, the endogenous RNAi was unambiguously found only in mouse oocytes and embryonic stem cells (ESCs), where deep sequencing identified endogenous siRNAs (endo-siRNAs) produced from retrotransposons and, unexpectedly, from processed pseudogenes.^{5,6,26} This suggests that, in addition to the protective role, RNAi in mouse oocytes regulates expression of protein-coding genes. Unlike in invertebrates,²⁷⁻²⁹ mammals do not employ dsRNA-derived siRNAs to combat viral infections as siRNAs were not found in a number of virus-infected mammalian cells.³⁰

Studies of RNA Silencing in Mouse Oocytes

The first report of mammalian RNA silencing came from mouse oocytes where microinjected dsRNA induced sequence-specific posttranscriptional silencing.^{31,32} RNAi was not expected in mammals because long dsRNA had been a known inducer of a complex sequence-independent response, commonly known as the interferon response (reviewed in ref. 33). Serendipitously, this response to long dsRNA is apparently suppressed in the oocyte.³⁴ Mammalian oocytes, however, were left behind the rapidly evolving field, so interpretations of RNA silencing studies in mouse oocytes were based on concepts developed in somatic cells where miRNAs dominated and the endogenous RNAi was absent.

Cloning of small RNAs from mouse oocytes revealed three classes of small RNAs—piRNAs, miRNAs and endo-siRNAs, which is unique among mammalian cells.^{5,6} However, piRNAs are nonessential for the mouse female germline.^{3,4,35} In contrast, the loss of Dicer during oocyte growth caused defects in meiotic spindle formation and infertility.^{36,37} It seemed most plausible that the loss of maternal miRNAs was the underlying cause but direct evidence was missing. However, it seemed unlikely that the loss of endo-siRNAs would cause a spindle defect while mature miRNAs were readily detected in the wild-type oocyte.^{36,37}

Ineffective miRNAs in Oocytes and Early Embryos

Recent data question the activity and the role of maternal miRNAs and change the view of RNA silencing in mouse oocytes and early embryos.³⁸⁻⁴⁰ A surprising study of the maternal loss of *Dgcr8*, which is involved in biogenesis of canonical miRNA, found that *Dgcr8*-null oocytes develop and ovulate normally, can be fertilized, and embryos develop into viable mice.⁴⁰ Although the loss of maternal miRNAs slightly reduced developmental competence, it became clear that oocytes have an impressive tolerance to the loss of miRNAs, which extends into the early

development because *Dgcr8*-null oocytes fertilized with *Dgcr8*-null sperm develop as far as the blastocyst stage.⁴⁰

In parallel, a confusing absence of P-bodies in oocytes was discovered. Using immunofluorescence or visualizing mRNAs targeted to P-bodies, they were not found in fully-grown mouse oocytes.^{38,39} While P-bodies were present in small postnatal oocytes, they disappeared as oocytes grew in size and appeared again at the morula and blastocyst stages³⁸ (Fig. 1). Because formation of P-bodies is thought to be a consequence of miRNA activity,⁴¹ these data suggested that the miRNA pathway might be ineffective.

To further explore the activity of miRNAs, luciferase-based reporters were used, which served previously to distinguish ability of endogenous miRNAs to induce RNAi-like cleavage and translational repression.⁴² Reporters measuring activity of endogenous Let-7 and miR-30 showed that ability of miRNAs to repress translation is strongly reduced during oocyte growth and maturation while RNAi-like cleavage was less affected. Interestingly, the less abundant miR-30 apparently retained more silencing activity suggesting that additional mechanism(s) reduce Let-7 activity in the oocyte.

Thus, mouse oocytes express a fair amount of matured miRNAs,^{5,6,37} which are loaded on the effector complex containing AGO2 as evidenced by down-regulation of reporters carrying a perfectly complementary miRNA binding site.³⁹ At the same time, translational repression, the typical effect of mammalian miRNAs, is ineffective and miRNAs are not required for oocyte-to-zygote transition. This is consistent with disappearance of P-bodies at the beginning of oocyte growth, particularly with the loss of co-localization between GW182 and AGO2,³⁸ which might reflect the loss of interaction between GW182 and AGO2. This could explain the relief of translational repression since GW182 is required for the repression.⁴³ Expression level is probably not a limiting factor although it cannot be fully excluded. The loss of interaction could also be caused by a secondary modification or a factor binding AGO2 or GW182, thus preventing their interaction.

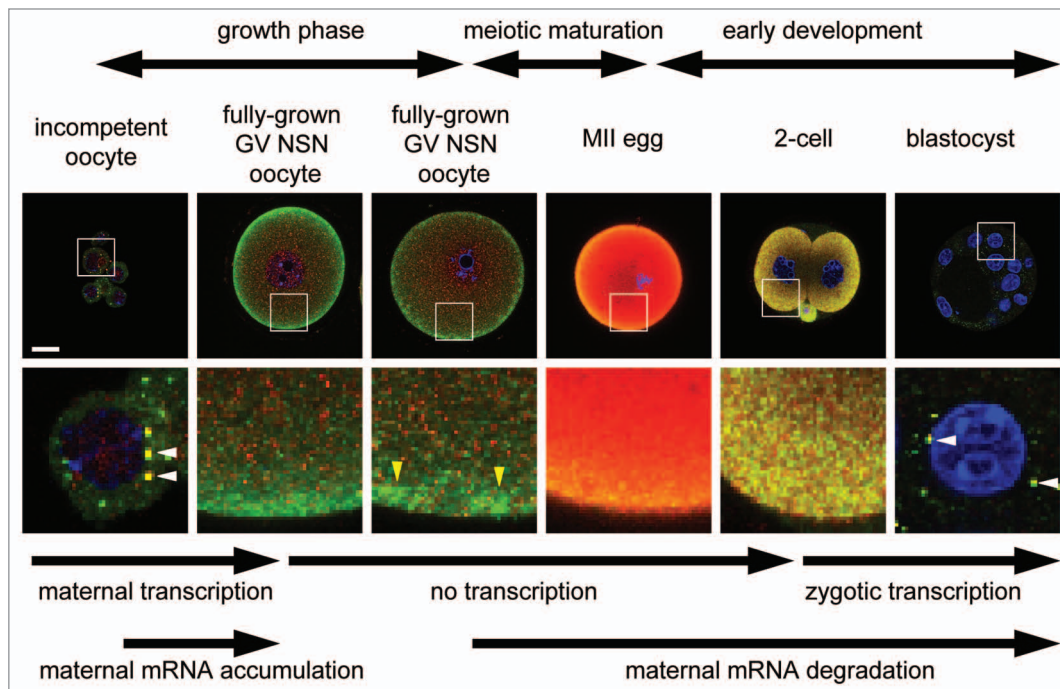


Figure 1. Overview of oocyte-to-zygote transition showing expression of GW182 and DCP1a. Confocal images of immunostained oocytes show GW182 (green), DCP1A (red) and DNA (blue). Co-localization of GW182 and DCP1A yields yellow color. Images were obtained with the same microscope settings and antibody dilutions. Details of the immunofluorescent analysis of different stages are described elsewhere.³⁸ P-bodies are indicated by white horizontal arrowheads. Yellow vertical arrowheads denote subcortical ribonucleoprotein aggregates that store untranslated maternal mRNAs. Note the absence of co-localization in fully-grown germinal vesicle (GV) intact oocytes. Fully-grown oocytes are further classified based on chromatin structure as non-surrounded nucleolus (NSN) and surrounded nucleolus (SN) stage where the later has the highest developmental competence. Apparent co-localization of GW182 and DCP1A in MII eggs and at the 2-cell stage is probably an artifact caused by high abundance of DCP1A. Scale bar = 20 μ m.

Testing these scenarios, however, is difficult as biochemical approaches are severely limited and the absence of transcription in fully-grown oocyte prevents using plasmid reporters.

Causes and Consequences of Ineffective miRNAs

Regardless of understanding the mechanistical basis of downregulation of the miRNA pathway, there is another question: Why is the miRNA pathway turned off during the largest genome reprogramming event in the mammalian life cycle? Kinetics and the precise time of suppression and reactivation of miRNA-mediated translational repression are unknown but data are consistent with a model where the miRNA pathway becomes progressively inhibited at the very beginning of oocyte growth and becomes functional at or just after the 8-cell stage. During this time, miRNAs are produced in a fair amount and loaded on AGO proteins, but then they idle and do not block translation. We

do not know yet whether there is any benefit of idling miRNAs. When the mechanism of miRNA inhibition is understood, one could test if aberrantly reactivated miRNAs in the oocyte and the early embryos would cause any phenotype. At the moment, we can only speculate that the relief of miRNA repression contributes to oocyte-to-zygote transition by facilitating the switch between maternal miRNAs (especially the Let-7 family) and zygotic miRNAs (particularly the miR-290 family). The oocyte can be seen as a differentiated cell undergoing reprogramming to produce pluripotent cells of the early embryo. Zygotic genome activation (ZGA), the initial phase of reprogramming and establishment of the pluripotent transcriptional circuit, is controlled by maternally provided transcription factors. Maternal miRNAs might represent a problem for accumulation of transcription factors (or their mRNAs) that will establish pluripotency in the embryo, so inhibition of maternal miRNAs might be a solution to this problem. This hypothesis

is consistent with data from ESCs where the opposing role of Let-7 and miR-290 families was studied.⁴⁴ There is likely an apparently multilayered suppression of Let-7, as evidenced by weaker repression mediated by Let-7 miRNAs than by less abundant miR-30 miRNAs.³⁹ The multilayer suppression is further supported by high maternal mRNA level of *Lin28*, a Let-7 biogenesis repressor.^{45,46} Because mature Let-7 is highly abundant in the oocyte³⁷ it is likely that LIN28A-mediated repression prevents Let-7 expression during ZGA.

Another reason for relief of translational repression by miRNAs might be associated with the endogenous RNAi pathway, which was found in oocytes and ES cells^{5,6,26} and is apparently present throughout the preimplantation development.³² Dicer and Dgcr8 maternal knockouts imply that the RNAi pathway is likely essential for oocyte development. It almost looks like there is a temporary shift from miRNA to RNAi pathway in terms of silencing activity while miRNAs

are still being produced and loaded on effector complexes. To maintain RNAi functional and block maternal miRNAs, two strategies are available: blocking nuclear processing or relieving translational repression. The first strategy is an equivalent of the *Dgcr8* knock-out. The reason why we do not see suppression of miRNA biogenesis might be that there is still some minimal role of canonical miRNAs, which is underlying reduced developmental capacity of *Dgcr8*-null oocytes, hence this solution might have been selected against. Idling miRNA seem to be a waste of resources, but it is a simple solution to disengage maternal miRNAs and engage zygotic miRNAs, a gear-shift principle applied on the oocyte-to-zygote transition.

Summary and Outlook

Experimental data show that maternal and early zygotic miRNAs are not involved in oocyte-to-zygote transitions. This surprising discovery changed the view of RNA silencing in mammals and raised a number of questions. The mammalian RNAi pathway apparently acquired a role in regulation of endogenous genes, which needs to be described in more detail. Although the evidence, including a recent *Ago2* maternal knock-out,⁴⁷ points toward the “slicer” activity being essential for oocyte development, it needs to be verified by “slicer” deficient *Ago2* mutant. The exact cause of the meiotic spindle defect also remains to be determined. The most interesting problem is probably the mechanism of inhibition of miRNA function in the oocyte. What would happen if miRNAs are forced to function? Finally, understanding suppression of the miRNA pathway by uncoupling translational repression from the effector complex will also open new possibilities in manipulating miRNA pathway in different areas, including cancer and stem cell research.

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