The role of miRNAs and endogenous siRNAs in maternal-to-zygotic reprogramming and the establishment of pluripotency

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RNA silencing is a complex of mechanisms that regulate gene expression through small RNA molecules. The microRNA (miRNA) pathway is the most common of these in mammals. Genome-encoded miRNAs suppress translation in a sequence-specific manner and facilitate shifts in gene expression during developmental transitions. Here, we discuss the role of miRNAs in oocyte-to-zygote transition and in the control of pluripotency. Existing data suggest a common principle involving miRNAs in defining pluripotent and differentiated cells. RNA silencing pathways also rapidly evolve, resulting in many unique features of RNA silencing in different taxonomic groups. This is exemplified in the mouse model of oocyte-to-zygote transition, in which the endogenous RNA interference pathway has acquired a novel role in regulating protein-coding genes, while the miRNA pathway has become transiently suppressed.

Keywords: pluripotency; RNAi; oocyte; Dicer; Dgcr8; miR-290

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See Glossary for abbreviations used in this article.

Introduction

Pluripotency is the ability of cells to differentiate into any fetal or adult cell type. Pluripotency is formed during early development and self-renewing pluripotent cells can be maintained in culture under specific conditions. Pluripotency in mammals is most often studied in embryonic stem cells (ESCs), which are derived from the inner cell mass of the blastocyst (Smith, 2001), and induced pluripotent stem cells (iPSCs), which are formed after reprogramming gene expression in somatic cells with specific pluripotency factors (Takahashi & Yamanaka, 2006). The OCT4 (POU5F1), SOX2 and NANOG transcription factors form the core of a network responsible for the transcriptional control of ESC renewal and pluripotency (Boyer *et al*, 2005; Loh *et al*, 2006). Microarray data indicate that a similar transcription factor network also forms in a stepwise manner upon mouse zygotic genome activation (ZGA; Zeng *et al*, 2004; Zeng & Schultz, 2005), which starts around 1.0 dpc during the early two-cell stage (the blastocyst, which is the source of ESCs, forms at 3.5 dpc). Thus,

reprogramming of an oocyte into pluripotent blastomeres of an early embryo—here referred to as oocyte-to-zygote transition (OZT)—is a physiological analogy of the experimental reprogramming of somatic cells into iPSCs. In fact, the cytoplasm of an enucleated oocyte can induce pluripotency in the nuclei of somatic cells during nuclear transfer (reviewed in Gurdon & Melton, 2008), which is the basis of mammalian cloning (Wilmut *et al*, 1997). As there is no mRNA synthesis between the end of the mouse oocyte growth phase and the first zygotic cleavage, post-transcriptional mechanisms are essential for the natural formation of pluripotency.

Here, we review the role of mammalian miRNA and RNAi during OZT and in the control of pluripotency. MicroRNA and RNAi pathways use small RNA molecules as sequence-specific guides for translational repression and/or mRNA degradation (reviewed in Chapman & Carrington, 2007). Briefly, siRNAs are produced from long dsRNA by cleavage with RNase III Dicer. MicroRNAs originate as long primary transcripts with local hairpin structures, that are cleaved by the Microprocessor complex composed of DGCR8 and RNase III Drosha. MicroRNAs are produced from small hairpin precursors upon cleavage by Dicer. Small interfering RNAs and miRNAs are loaded on Argonaute (AGO) proteins, which mediate silencing effects (reviewed in Kim *et al*, 2009).

Animal miRNAs typically base-pair imperfectly with the 3'-UTR of target mRNAs and induce translational repression and/or mRNA degradation. MicroRNAs are the dominant class of short RNAs in mammalian cells—more than a thousand miRNAs with defined sequences have been identified and implicated in the regulation of many cellular processes (Kloosterman & Plasterk, 2006). It has been estimated that miRNAs could directly target more than half of all mammalian genes (Friedman *et al*, 2009).

Target mRNA recognition involves the nearly perfect hybridization of the miRNA 5'-end, which nucleates the miRNA-mRNA interaction (Brennecke *et al*, 2005; Lai, 2002). Perfect base-pairing of a miRNA or siRNA results in the AGO2-mediated sequence-specific endonucleolytic cleavage of the target in the middle of the base-pairing sequence (Liu *et al*, 2004; Meister *et al*, 2004). However, miRNAs can induce substantial mRNA degradation even in the absence of extensive base-pairing to their targets (Bagga *et al*, 2005; Lim *et al*, 2005). This is a likely consequence of translational repression and the relocation of repressed mRNAs to cytoplasmic foci known as P-bodies (reviewed in Eulalio *et al*, 2007a). P-bodies

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Fig 2 | Roles of the miR-290 cluster in undifferentiated mouse embryonic stem cells. Red frames highlight two important components of control of gene expression in ESCs: the core pluripotency transcription factor network (ESC core transcriptional circuit), which also controls expression of the second component, ESC miRNAs. The miR-290 cluster dominates miRNA function in ESCs and regulates ~250 primary targets, some of which are shown here. Murine miR-290 have a role in several processes including cell-cycle regulation, nodal signalling and transcriptional regulation. The miR-290 cluster indirectly regulates *de novo* DNA methylation by targeting RBL2, a transcriptional repressor acting on *Dnmt3* genes. MicroRNAs—including miR-145 and others—also contribute to suppression of the ESC core transcriptional circuit during differentiation. The scheme and listed miR-290 targets are based on published data (Marson *et al*, 2008; Melton *et al*, 2010; Sinkkonen *et al*, 2008; Tay *et al*, 2008; Xu *et al*, 2009). ESC, embryonic stem cell.

Two approaches have been taken to address the specific roles of miRNAs in mouse ESCs. Sinkkonen *et al* undertook a bioinformatic analysis of the transcriptome of *Dcr1*^{-/-} ESCs (Sinkkonen *et al*, 2008), while Wang *et al* screened rescue effects of individual miRNAs in *Dgcr8*^{-/-} ESCs (Wang *et al*, 2007). *Dcr1*^{-/-} analysis has revealed that the most significantly enriched motifs in the 3'UTRs of transcripts upregulated in *Dcr1*^{-/-} ESCs are sequences complementary to AAGUGC motifs. This implicates a dominant function for EEmiRC miRNAs among ESC miRNAs. Subsequent analysis has identified around 250 primary targets of EEmiRC miRNAs in ESCs (Sinkkonen *et al*, 2008), and *Rbl2* (*p130*) and *Cdkn1a* (*p21*) were verified as primary targets of EEmiRC miRNAs (Fig 2). RBL2 is a transcriptional repressor that suppresses expression of *de novo* DNA methyltransferases. Thus, in the absence of EEmiRC miRNAs, elevated levels of RBL2 reduce levels of DNMT3A and DNMT3B. This causes DNA methylation defects during differentiation, whereby the *Oct4* promoter does not accumulate *de novo* DNA methylation (Benetti *et al*, 2008; Sinkkonen *et al*, 2008). Defective *de novo* DNA methylation probably contributes to differentiation defects in ESCs, similarly to a *Dnmt3a* and *Dnmt3b* double knockout (Chen *et al*, 2003).

Glossary

DAZAP2	DAZ (deleted in azoospermia)-associated protein 2
DGCR8	Di George Critical Region 8
DCR	DICER, an RNase III family endoribonuclease
DNMT	<i>de novo</i> DNA methyltransferase
dpc	days post-coitum
dsRNA	double-stranded RNA
EB	embryoid bodies
EEmiRC	early embryonic microRNA cluster
ELAVL2	(HuB) A+U-rich sequences binding translational repressor
ESCs	embryonic stem cells
iPSCs	induced pluripotent stem cells
ESCC	ES cell-specific cell cycle-regulating miRNAs
miRNA	microRNA, a small RNA encoded by the genome
OZT	oocyte-to-zygote transition
OCT4	octamer 4, also known as POU class 5 homeobox
oncomiRs	miRNAs with oncogenic potential
piRNA	Piwi-interacting RNA
RBL2	retinoblastoma-like protein 2
RNAi	RNA interference, one of the RNA silencing pathways that uses short RNAs generated from long dsRNA to induce sequence-specific cleavage of cognate mRNAs
RT-PCR	reverse transcription PCR
siRNA	small interfering RNA
SOX2	(sex determining region Y)-Box 2 transcription factor
UTR	untranslated region
ZGA	zygotic genome activation

However, differentiation defects are not rescued by EEmiRC miRNAs (Wang *et al*, 2008), suggesting that the cause of differentiation defects includes the loss of other ESC miRNAs or perhaps miRNAs that appear and function during differentiation, such as miR-145 (Xu *et al*, 2009).

Studies of *Dcr1*^{-/-} and *Dgcr8*^{-/-} have also revealed that cell-cycle progression in ESCs is under the control of miRNAs. Sinkkonen *et al* reported that EEmiRC miRNAs regulate *p21* and that transfection of EEmiRC miRNAs partly rescues growth defects in *Dcr1*^{-/-} cells (Sinkkonen *et al*, 2008). Wang *et al* subsequently showed, in *Dgcr8*^{-/-} cells, that several ESC miRNAs designated ESCC contribute to the rescue of proliferation by the regulation of CDK2–cyclin E-dependent control of G1–S progression (Wang *et al*, 2008). ESCC miRNAs include EEmiRC miRNAs, members of the miR-17-92 cluster and an unrelated miR-223. MicroRNA-mediated control of proliferation also involves cyclin D1, which is targeted by miR-302 in human ESCs (Card *et al*, 2008).

Interestingly, despite the massive transcriptome remodeling in *Dcr1*^{-/-} ESCs, the loss of pluripotency is not accompanied by notable changes in the expression of the core pluripotency circuit factors (Sinkkonen *et al*, 2008). This implies that ESC miRNAs, the transcription of which is largely controlled by core pluripotency factors (Marson *et al*, 2008), do not form a feedback loop to maintain the core pluripotency factors and that the core pluripotency network is insufficient to maintain pluripotency in the absence of miRNAs. In addition to their role in the maintenance of pluripotency, EEmiRC miRNAs can also contribute to the establishment of pluripotency during the formation of iPSCs (Judson *et al*, 2009). miR291-3p, miR-294 and miR-295 increase the efficiency of reprogramming by pluripotency factors *Oct4*, *Sox2* and *Klf4*. Thus, AAGUGC miRNAs dominate miRNA activities in ESCs and have a broader relationship to the control of cell growth and differentiation.

The role of microRNAs and endo-siRNAs in OZT

Mouse ovarian oocytes are highly specialized cells that are arrested in the prophase of the first meiotic division. The fully grown mouse oocyte can resume meiosis during ovulation but arrests again at metaphase II and dies unless fertilized. Even if diploidized and parthenogenetically activated, a zygotic genome of entirely maternal origin cannot support normal embryonic development (Surani *et al*, 1984). The fertilized egg, however, transforms into a cleaving zygote, the cells of which exhibit pluripotency. The factors required for genome reprogramming during OZT are present in the oocyte cytoplasm. OZT has two essential components: (i) control of stability and translation of maternal mRNAs and (ii) control of the transcriptional activation of zygotic genes. As no new mRNAs are produced before the two-cell stage (Zeng & Schultz, 2005), gene expression during OZT is mostly governed by post-transcriptional mechanisms such as reversible deadenylation coupled with transcriptional repression—which is the basis for controlling storage and recruitment of specific mRNAs for translation during meiosis and later (reviewed in Richter, 2007). RNA silencing pathways—that have essential roles elsewhere—are also involved in the regulation of mammalian OZT, but in a rather unexpected way.

MicroRNAs during OZT

The cloning of small RNAs from mouse oocytes has revealed three classes of small RNA: miRNAs, endo-siRNAs and piRNAs (Tam *et al*, 2008; Watanabe *et al*, 2008). Mammalian piRNAs are small RNAs produced by a *Dcr1*-independent mechanism that are essential for the male but not the female germline (reviewed in Klattenhoff & Theurkauf, 2008). The presence of all three known mammalian RNA silencing pathways in one cell type is unique. Interestingly, mouse oocytes do not contain any abundant oocyte-specific miRNAs and, due to a high abundance of members of the let-7 family and low levels of the miR-290 cluster miRNAs, the miRNA profile appears somatic-like. This is counterintuitive, considering the reprogramming ability of oocyte cytoplasm.

RT-PCR analysis of mouse miRNA expression during OZT (Tang *et al*, 2007) has revealed typical maternal, maternal-zygotic and zygotic expression patterns (Fig 3B). MicroRNA expression patterns are relatively unvarying when compared with mRNA expression patterns during OZT, and essentially depend on the 'entry' level of maternal miRNAs and the intensity of zygotic transcription. Maternal miRNAs undergo a common degradation on fertilization, and zygotic miRNAs increase their abundance from the two-cell stage onwards (Tang *et al*, 2007). This common degradation probably reflects the fact that miRNAs are too short to be selectively degraded on the basis of sequence motifs, but the common transcriptional activation during ZGA is peculiar because miRNA expression is controlled by polymerase II, which allows for variable expression patterns. This is in contrast to maternal mRNAs, which have diverse individual patterns of expression (Zeng *et al*, 2004; Zeng & Schultz, 2005).

The most abundant maternal miRNAs are members of the let-7 family, which account for about one-third of miRNAs in the oocyte (Tam *et al*, 2008; Tang *et al*, 2007; Watanabe *et al*, 2008). Such a high quantity of mature let-7 miRNAs is surprising considering that let-7 is typically found in adult and differentiated tissues, and its cloning frequency in *Xenopus* eggs is relatively low (Armisen *et al*, 2009). let-7 levels rapidly decline during OZT and let-7 expression is not restored in the early embryo, which is consistent with very low let-7 levels in ESCs. The most pronounced zygotic miRNAs are EEmiRC members

Fig 3 | miRNAs during mouse oocyte-to-zygote transition. (A) Total mRNA (poly(A) RNA) amount changes during OZT (based on Piko & Clegg, 1982). From left to right the following developmental stages are shown: fully grown oocyte, mature MII egg, one-cell, two-cell, four-cell and eight-cell. (B) Schematic miRNA expression profiles during OZT. From left to right the following developmental stages are shown: mature MII egg, one-cell, two-cell, four-cell and eight-cell. Graphs are based on previously published data (Tang *et al*, 2007). miRNA, microRNA; OZT, oocyte-to-zygote transition.

(the miR-290 cluster), which are transcribed during the two-cell stage (Zeng & Schultz, 2005) and accumulate throughout preimplantation development (Tang *et al*, 2007). There are also maternal-zygotic miRNAs (for example, the miR-17-92 cluster), which are present at considerably high levels in oocytes and embryos, with a transiently lower expression at the two-cell stage (Fig 3B). These data imply that there is a switch between maternal and zygotic miRNAs that involves the rapid degradation of maternal miRNAs between the fertilized egg and the two-cell embryo stages. The active degradation must cease at times during the two-cell stage to allow for expression of zygotic miRNAs. Presumably, the remaining maternal miRNAs that survive the degradation disappear as they are replaced with zygotic miRNAs.

Idling microRNAs

The loss of *Dcr1*-dependent small RNAs during oocyte growth causes defects in meiotic spindle formation and infertility (Murchison *et al*, 2007; Tang *et al*, 2007). This phenotype was originally attributed to the loss of maternal miRNAs, because mature miRNAs were abundant in the wild-type oocyte and it seemed unlikely that the loss of endo-siRNAs would cause a spindle defect (Murchison *et al*, 2007; Tang *et al*, 2007). More recent data, however, questions the activity

Fig 4 | Loss of P-bodies in fully grown oocytes. Confocal images of GW182 (green) and AGO2 (red) in meiotically incompetent mouse oocytes (from 12-day-old mice), fully grown preovulatory oocytes and blastocysts. The inset shows a detail of cytoplasmic staining, the part of the specimen covered by the insert is not any different from the rest of the image. Immunofluorescent staining of GW182 and AGO2 was carried out as previously described (Flemer *et al*, 2010). DNA is shown in blue (DAPI). Colocalization yields a yellow colour. Arrowheads depict P-bodies in blastocysts and somatic cells. Note the absence of GW182 and AGO2 colocalization and enhanced subcortical staining of GW182 in the subcortical region in fully grown oocytes. Scale bars, 20 µm.

and role of maternal miRNAs, and changes the view of RNA silencing in mouse oocytes and early embryos. A study of the maternal loss of *Dgcr8*—which is involved in the biogenesis of canonical miRNA—found that *Dgcr8*^{−/−} oocytes develop and ovulate normally, can be fertilized and give rise to viable mice (Suh *et al*, 2010). Although the loss of maternal miRNAs slightly reduced developmental competence, it became clear that oocytes have an impressive tolerance to the loss of miRNAs. This tolerance appears to extend into early development because *Dgcr8*^{−/−} oocytes fertilized with *Dgcr8*^{−/−} sperm develop to the blastocyst stage (Suh *et al*, 2010).

In parallel, a loss of P-bodies in fully grown mouse oocytes was discovered by indirect immunofluorescence and by visualizing mRNAs targeted to P-bodies (Flemer *et al*, 2010; Ma *et al*, 2010). During OZT, P-bodies disappear as meiotically incompetent small postnatal oocytes start to grow in size, and appear again at the morula and blastocyst stages (Fig 4; Flemer *et al*, 2010). Although P-bodies are absent in growing oocytes, their components—TNRC6, DDX6,

CPEB—and other proteins are found in subcortical ribonucleoprotein aggregates, which are thought to be a storage place for maternal mRNAs (Flemer *et al*, 2010).

The absence of P-bodies provoked a more detailed examination of maternal miRNA activities using luciferase-based reporters, which can distinguish between the abilities of endogenous miRNAs to induce RNAi-like cleavage and translational repression (Schmitter *et al*, 2006). Reporters targeted by endogenous let-7 and miR-30 showed that RNAi-like cleavage (that is, loading of miRNA on AGO2 and accessibility of cognate 3′ UTRs) is relatively normal, but the ability of miRNAs to repress translation is strongly reduced during oocyte growth and maturation (Ma *et al*, 2010). Interestingly, the less abundant miR-30 apparently retained more silencing activity, suggesting that an additional mechanism or mechanisms suppresses let-7 function in the oocyte (Ma *et al*, 2010). Suppression of miRNA activity is further supported by microarray data from *Dgcr8*^{−/−} and *Dcr1*^{−/−} oocytes (Suh *et al*, 2010). Taken together, this suggests that miRNAs are not

only non-essential for OZT, but also that their function is suppressed by the oocyte. Therefore, uncoupling miRNAs from the repression of translation is the earliest known event in OZT in the mouse.

The mechanism and benefits of idling miRNAs are unknown. Suppression of miRNA function seems to take place after AGO2 loading and possibly involves uncoupling of AGO2 and TNRC6 (GW182), the latter being necessary for miRNA-mediated translational repression (Eulalio *et al*, 2008). This model is supported by the loss of colocalization of AGO2 and GW182 in oocytes (Flemr *et al*, 2010). Interestingly, overexpression of AGO2 in early embryos induces formation of P-bodies (Lykke-Andersen *et al*, 2008). At the moment, it is unclear why the miRNA pathway is turned off during the largest genome reprogramming event in the mammalian life cycle. We can only speculate that it might contribute to OZT by facilitating the switch between maternal and zygotic miRNAs. Idling miRNA might be seen as a waste of resources, but such a gearshift mechanism is a simple solution to disengaging maternal miRNAs during oocyte growth and engaging zygotic miRNAs later in preimplantation development.

As mentioned above, members of the let-7 family constitute about one-third of maternal miRNome, and are negative regulators of pluripotency. Although *Lin-28*, an inhibitor of let-7 (Hagan *et al*, 2009), has high transcript levels in the oocyte (Zeng *et al*, 2004), it does not appear to prevent high levels of mature let-7 from accumulating. A global suppression of activity of maternal miRNAs might, therefore, be able to relieve let-7-mediated repression, and allow the expression of maternal factors, which will promote pluripotency in the zygote. Furthermore, as a considerable amount of let-7 survives maternal miRNA degradation between fertilization and the two-cell stage, continual suppression of the miRNA pathway into preimplantation development would further facilitate replacement of let-7 with zygotic miRNAs, particularly with the miR-290 cluster, which has a let-7 opposing role (Melton *et al*, 2010). In this situation, LIN28 would prevent zygotic expression of let-7, which could cause problems when the miRNA pathway becomes functional again.

Mammalian RNA interference in the spotlight

One important output of recent studies of maternal miRNAs is new data concerning endogenous RNAi in mammals. The mammalian RNAi pathway has so far been a mystery, as endo-siRNAs were found only in oocytes and ESCs (Babiarz *et al*, 2008; Tam *et al*, 2008; Watanabe *et al*, 2008). Oocyte endo-siRNAs derived from processed pseudogenes suggest that mammalian RNAi, in addition to roles in the suppression of mobile and repetitive sequences known from invertebrates, might also regulate endogenous genes (Tam *et al*, 2008; Watanabe *et al*, 2008). This hypothesis is now supported by the defective spindle phenotype of *Dcr1*^{-/-} and *Ago2*^{-/-} oocytes, which is absent in *Dgcr8*^{-/-} oocytes. Furthermore, the bioinformatic analysis of the *Dcr1*^{-/-} transcriptome shows that many upregulated transcripts have complementary sequences to endo-siRNAs found in the oocyte (Ma *et al*, 2010). Thus, data support a model in which the miRNA pathway becomes disengaged early during oocyte growth and RNAi becomes the dominant RNA silencing pathway essential for OZT. Here, it presumably targets genes that would interfere with proper meiotic spindle assembly. The requirement for endogenous RNAi might extend into early development, because Ago2 knock-down in early embryos causes arrests at the two-cell stage (Lykke-Andersen *et al*, 2008). The role of endogenous RNAi still needs to be validated by evidence that the 'slicer' activity of AGO2 is required for normal oocyte development. At present, it implies that the

Dcr1^{-/-} and *Ago2*^{-/-} phenotypes might be unique to the mouse and that the role of mammalian RNAi in the oocyte might not be well conserved because it relies on endo-siRNAs produced from transcribed, processed pseudogenes, which evolve rapidly.

MicroRNAs in the zygote

Although we have argued above that mammalian miRNAs are not needed for OZT, it is necessary to put the function of zygotic miRNAs into the context of other vertebrate systems. When such a comparison is made (Fig 5), it becomes apparent that vertebrate zygotes express AAGUGC miRNAs, but use them in a different spatiotemporal context.

In the mouse, the major reprogramming of the zygote by newly expressed genes initiates at the two-cell stage. Although the one-cell embryo is transcriptionally active, it does not seem to produce functional mRNAs (Zeng & Schultz, 2005). The final stage of degradation of maternal mRNAs is superimposed on the activation of the zygotic genome. It is initiated by the resumption of meiosis and is an integral part of the switch from the maternal to the zygotic programme, although a direct link between maternal mRNA degradation and establishment of pluripotency has not been documented. Maternal mRNA degradation is substantial and, by the two-cell stage, eliminates 75% of the poly(A) RNA that is originally found in the pre-ovulatory oocyte (Piko & Clegg, 1982). The zygotic miR-290 family transcription starts during the two-cell stage, but mature miRNA accumulation is first observed at the four-cell stage (Tang *et al*, 2007; Zeng & Schultz, 2005). Zygotic miRNAs are, therefore, unlikely to contribute significantly to maternal mRNA degradation in the mouse: first, because their activity is suppressed at the early stages of preimplantation development; and second, even if the zygotic miRNAs were active, they would appear when the majority of maternal mRNAs are already degraded.

This situation is different from that in the fish and the frog, in which development is very fast and zygotic miRNAs appear when maternal mRNAs are still abundant (Fig 5). Due to this fast development, there is likely to be greater pressure to eliminate maternal mRNAs so that they do not interfere with differentiation, and zygotic miRNAs offer a way to deal with this problem. A contribution of miRNAs to degradation of maternal mRNA was reported in *Drosophila* (Bushati *et al*, 2008). In the zebrafish, the miR-430 family becomes strongly upregulated a few hours after fertilization and targets up to an estimated 40% of maternal mRNAs (Giraldez *et al*, 2006). This suggests that the miR-430 family significantly contributes to maternal mRNA degradation. A similar role has also been proposed for miR-427 during *Xenopus* embryo development (Lund *et al*, 2009).

Despite the differences detailed above, there are also notable similarities. One example is a partly conserved role in Nodal signalling, which is important for germ layer specification (Choi *et al*, 2007; Rosa *et al*, 2009). Early vertebrate embryos express a set of related AAGUGC miRNAs during ZGA. This suggests that AAGUGC miRNAs have common and evolving roles in the regulation of development. In addition to *Lefty1* and *Lefty2* in Nodal signalling, only a small number of genes—including *Dazap2* and *Elavl2* (*HuB*)—are common among miR-290 and miR-430 targets (Giraldez *et al*, 2006; Sinkkonen *et al*, 2008). However, the general role of EEmiRCs and the miR-430 cluster is the same: they support development by targeting genes the expression of which should be kept low in the embryo. From this perspective, the difference is in the context: the miR-290 cluster targets mostly zygotic transcripts, whereas the miR-430 cluster deals also with a large amount of maternal mRNAs.

Fig 5 | Oocyte-to-zygote transition in the mouse, zebrafish and frog. Specific developmental stages, the course of maternal mRNA degradation, ZGA and expression of zygotic AAGUGC miRNAs in mouse, zebrafish and frog embryos is shown on the same timescale.

Variability among targets and specific roles of AAGUGC miRNAs across vertebrates should not be surprising, because early post-zygotic regulations might evolve fast during speciation.

Conclusions

It is remarkable that the molecular mechanisms underlying developmental potential are not strongly conserved. This can be contrasted with the example of *let-7* miRNA, which is found in differentiated cells from *Caenorhabditis elegans* to mammals, and zygotic AAGUGC miRNAs, which are found in vertebrates with considerable variation in conservation at the genomic level. RNA silencing mechanisms underlying OZT and pluripotency in mammals should be viewed as unique solutions superimposed on broader general principles. In the mouse, miRNA function is suppressed in oocytes: miRNAs do not contribute to zygotic genome activation, maternal mRNA degradation or establishment of the core pluripotency transcriptional network. However, the importance of endogenous RNAi in the oocyte has recently become apparent. MicroRNAs are essential for pluripotency, but whether this reflects the activity of ESC miRNAs or the requirement for other miRNAs during differentiation remains to be revealed.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

Sidebar A | In need of answers

- (i) Which miRNAs are essential for pluripotency in embryonic stem cells?
- (ii) How do miRNAs support pluripotency in embryonic stem cells?
- (iii) What is the role of RNAi in embryonic stem cells?
- (iv) How are miRNAs suppressed in the oocyte?
- (v) Would normal miRNA activity interfere with OZT?
- (vi) Is mammalian RNAi-mediated control of gene expression essential for meiosis?
- (vii) How conserved is the function of RNAi in the germline in mammals?

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