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Report

MicroRNA Activity Is Suppressed in Mouse Oocytes

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Summary

MicroRNAs (miRNAs) are small endogenous RNAs that typically imperfectly base pair with 3′ untranslated regions (3′UTRs) and mediate translational repression and mRNA degradation. Dicer, which generates small RNAs in the miRNA and RNA interference (RNAi) pathways, is essential for meiotic maturation of mouse oocytes. We found that 3′UTRs of transcripts upregulated in *Dicer1*$^{-/-}$ oocytes are not enriched in miRNA binding sites, implicating a weak impact of miRNAs on the maternal transcriptome. Therefore, we tested the ability of endogenous miRNAs to mediate RNA-like cleavage or translational repression of reporter mRNAs. In contrast to somatic cells, endogenous miRNAs in oocytes poorly repressed translation of mRNA reporters, whereas their RNAi-like activity was much less affected. Reporter mRNA carrying let-7-binding sites failed to localize to P body-like structures in oocytes. Our data suggest that miRNA function is downregulated during oocyte development, an idea supported by normal meiotic maturation of oocytes lacking *Dgcr8*, which is required for the miRNA but not the RNAi pathway (Suh et al. [1], this issue of *Current Biology*). Suppressing miRNA function during oocyte growth is likely an early event in reprogramming gene expression during the transition of a differentiated oocyte into pluripotent blastomeres of the embryo.

Results and Discussion

Minimal Impact of MicroRNAs on Mouse Oocyte Transcriptome

The eight 5′-terminal nucleotides of a microRNA (miRNA) form a "seed," which hybridizes nearly perfectly with the target mRNA and nucleates the miRNA-mRNA interaction [2]. Whereas enrichment of motifs complementary to seeds of highly active miRNAs has been observed in 3′ untranslated regions (3′UTRs) of mRNAs whose relative abundance is increased (hereafter referred to as upregulated) upon depletion of *Dicer1* [3–5], transcriptome analysis of *Dicer1*$^{-/-}$ metaphase II (MII) eggs did not identify any miRNA-related motifs [6]. Because transcriptome remodeling during meiosis [7] could mask upregulation of primary miRNA targets, we performed an analysis of fully grown germinal vesicle-intact (GV) *Dicer1*$^{-/-}$ oocytes. Microarray profiling revealed a comparable number of upregulated (489, $p < 0.001$) and downregulated (628, $p < 0.001$) transcripts (Figure 1A). The magnitude of these changes was ~5 times smaller when compared to other studies of *Dicer1*-depleted mammalian cells [4, 5]. In fact, the loss of *Dicer1* in the oocyte caused a transcriptome change comparable to the effect of a single miRNA in embryonic stem (ES) cells (Figure 1A) [5].

We searched for heptamer motifs enriched in 3′UTRs of transcripts that were upregulated in the *Dicer1*$^{-/-}$ oocytes and that could explain the mRNA expression changes. One of the four motifs most significantly enriched (see Table S1 available online) was complementary to the seed of miR-1195 (GAACUCA, Figure 1B). This motif, however, is likely not associated with miRNA function, because miR-1195 was absent in deep sequencing of small RNAs from mouse oocytes [8]. Likewise, none of the predicted miR-1195 targets in the miRBase [9] was upregulated in the *Dicer1*$^{-/-}$ oocytes. Sylamer [10], an alternative approach to analyze miRNA signals in 3′UTRs, showed that none of the high-scoring motifs and none of the top five miRNA-related heptamers (Figure S1) match seed regions of miRNAs with a cloning frequency in oocytes > 0.1%.

We also examined motifs related to abundant miRNAs in transcriptomes of *Dicer1*$^{-/-}$ oocytes and ES cells. These motifs, which were selected based on deep sequencing data [8, 11], represent binding sites for more than half of all miRNAs cloned from these cells (Table S2). Interestingly, none of the motifs (including those for the let-7 family, which represents ~30% of maternal miRNAs [8, 12]) showed any enrichment or any statistical bias in 3′UTRs of transcripts upregulated in *Dicer1*$^{-/-}$ oocytes. This contrasts with *Dicer1*$^{-/-}$ ES cells, where the most significant motifs match a family of highly abundant miRNAs (~25% of cloned miRNAs [11]), and several motifs corresponding to other abundant miRNAs also showed enrichment and deviation from the statistical background (Figure 1C; Table S3).

Our data suggest limited miRNA-associated mRNA degradation in the oocyte and do not support the notion that miRNAs extensively modulate gene expression in oocytes [12, 13]. Our analysis of 3′UTRs of transcripts upregulated in *Dicer1*$^{-/-}$ oocytes does not provide evidence that the upregulation is associated with miRNA function via seed-mediated interaction with 3′UTRs. Likewise, we observed no significant enrichment of miRNA-associated motifs in 3′UTRs of intrinsically unstable mRNAs [14] and mRNAs degraded during meiosis [7]. Although miRNA binding sites were associated with specific transcript isoforms during meiotic mRNA degradation [15], it is unclear whether this observation reflects miRNA effects. It is possible that none of the maternal miRNAs is functionally dominant, and therefore none generates a strong signal, but this does not explain the low number of upregulated transcripts in *Dicer1*$^{-/-}$ oocytes. Alternatively, miRNA-mediated mRNA degradation is not robust, and the transcriptome change reflects the loss of endogenous small interfering RNAs (endo-siRNAs). We found that 42 of 489 upregulated but only 6 of the 628 downregulated transcripts in

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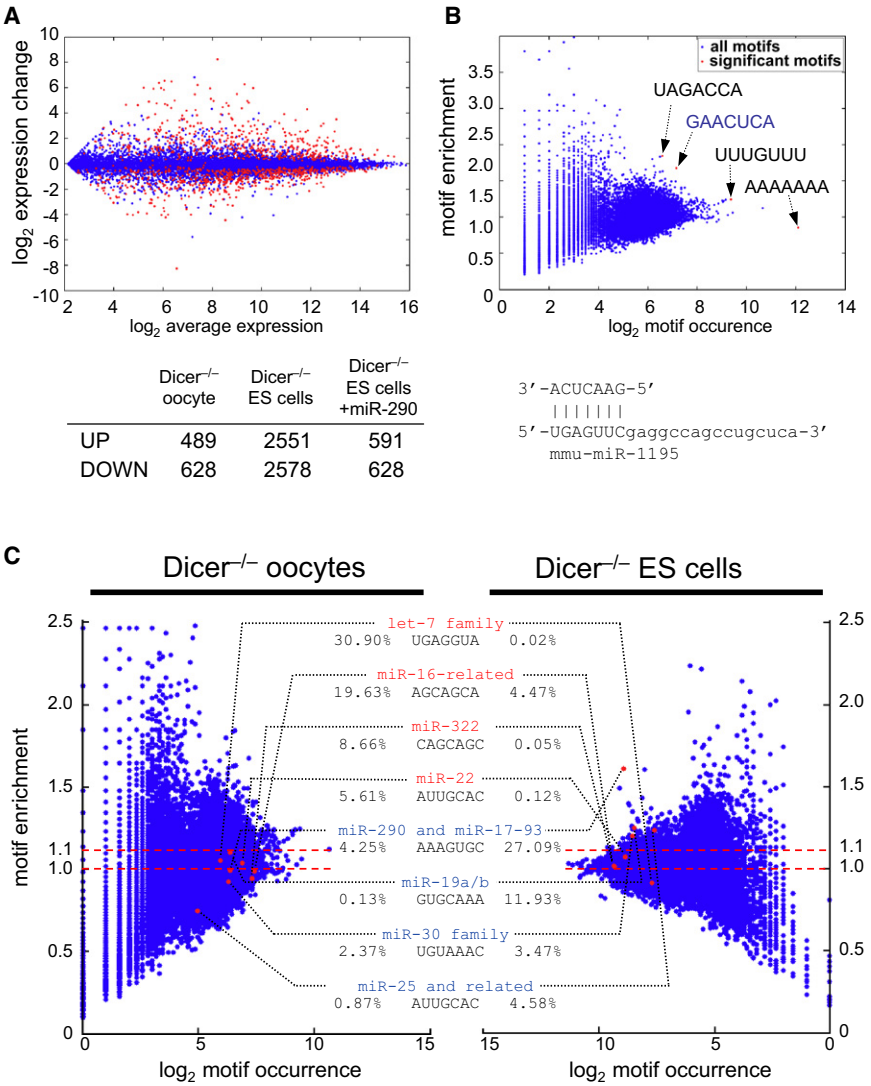

	Dicer$^{-/-}$ oocyte	Dicer$^{-/-}$ ES cells	Dicer$^{-/-}$ ES cells +miR-290
UP	489	2551	591
DOWN	628	2578	628

Figure 1. Transcriptome Analysis of *Dicer1*$^{-/-}$ Oocytes

(A) M [$\log_2$(fold change)] versus A [average $\log_2$(expression level)] plot for the *Dicer1*$^{-/-}$ versus *Dicer1*$^{+/+}$ fully grown germinal vesicle oocytes. Each dot represents a transcript. Significant expression changes ($p < 0.001$ computed from four replicate experiments) are shown in red.

(B) Heptamer motif analysis of upregulated transcripts. The motifs whose frequency in the 3′ untranslated regions (3′UTRs) of upregulated transcripts is significantly different from the frequency in the entire set of 3′UTRs are shown in red (see also Experimental Procedures). One of the significantly enriched motifs is complementary to positions 1–7 of the miR-1195.

(C) Comparison of heptamer motif analyses of *Dicer1*$^{-/-}$ oocytes (left) and embryonic stem (ES) cells (right, horizontally inverted); most-relevant motifs complementary to seeds of most-abundant microRNAs (miRNAs) in both cell types are highlighted. The most-abundant miRNAs in the oocyte and ES cells are shown in red and blue text, respectively. Note that none of the motifs corresponding to abundant maternal miRNAs is enriched more than 1.1 times in 3′UTRs of transcripts upregulated in *Dicer1*$^{-/-}$ oocytes, whereas all four motifs corresponding to miRNAs abundant in ES cells are enriched in *Dicer1*$^{-/-}$ ES cells. Posterior probability analysis shows a high significance (1.000) only for the GCACUUU motif. However, posterior probability for the other three motifs corresponding to ES cell miRNAs was one to three orders of magnitude higher than all other motifs, which scored within the statistical background ($\sim 10^{-5}$, Table S3). Abundance (%) of miRNAs related to individual motifs in both cell types is indicated next to each motif. Dashed lines mark 1.0- and 1.1-fold motif enrichment.

Dicer1$^{-/-}$ oocytes perfectly base pair (Table S4) with endo-siRNAs [16]. Because siRNA-guided cleavage by small RNAs requires less than complete base pairing and can occur without a perfect seed complementarity [17], it is plausible that inhibition of the RNAi pathway is the major cause of transcriptome changes in *Dicer1*$^{-/-}$ oocytes.

The idea that low activity of miRNA-mediated mRNA degradation is responsible for the absence of a miRNA signature in *Dicer1*$^{-/-}$ oocytes is supported by Suh et al. [1], who analyzed the maternal loss of *Dgcr8*, a component of the microprocessor complex involved in miRNA biogenesis. *Dgcr8*$^{-/-}$ oocytes show the same depletion of miRNAs as *Dicer1*$^{-/-}$ oocytes, yet the transcriptome of *Dgcr8*$^{-/-}$ oocytes is more similar to the wild-type, and mice with *Dgcr8*$^{-/-}$ oocytes are fertile, showing no meiotic spindle defects reported for *Dicer1*$^{-/-}$ and *Ago2*$^{-/-}$ oocytes. Therefore, the sterile phenotype of *Dicer1*$^{-/-}$ oocytes [6, 12] is likely due to misregulation of genes controlled by endo-siRNAs [8].

Endogenous miRNAs Poorly Repress Cognate mRNAs

To understand the function of maternal miRNAs, we used three sets of reporter mRNAs carrying binding sites for the endogenous miRNAs let-7a and miR-30c. let-7 is the most abundant miRNA family in the oocyte (~30% of maternal miRNAs [8, 12, 16]). The miR-30 family is less abundant; it represents ~8% of maternal miRNAs, as suggested by reverse transcriptase-polymerase chain reaction (RT-PCR) [12]. The deep-sequencing data suggest a lower abundance (~2.4% [8]), but such estimates are prone to errors [18].

To assess let-7 activity during oocyte growth and meiotic maturation, we used firefly luciferase reporters (Figure 2A) carrying a lin-41 fragment with two natural bulged let-7 binding sites (FL-2xlet-7), which were mutated in the control (FL-control) [19]. Because fully grown GV oocytes and MII eggs are transcriptionally quiescent, we microinjected in vitro-synthesized mRNAs instead of plasmid reporters. First, we compared let-7-mediated repression of FL-2xlet-7 mRNA microinjected into meiotically incompetent oocytes with repression of the FL-2xlet-7 plasmid or synthetic FL-2xlet-7 mRNA transfected into NIH 3T3 cells. FL-2xlet-7 expression was reduced by ~40% relative to FL-control in oocytes (Figure 2B). Although this was less than repression of FL-2xlet-7 reporters in NIH 3T3 cells (~50%, Figure 2B), it showed that reporter mRNA is repressed by endogenous let-7 in small, growing oocytes.

When FL-2xlet-7 mRNA was microinjected into fully grown GV oocytes, we observed inefficient let-7 repression, which