Biology of Reproduction, 2018, 99(1), 160–174
doi:10.1093/biolre/ioy013
Review
Advance Access Publication Date: 15 February 2018

OXFORD

Review

The oocyte-to-embryo transition in mouse: past, present, and future†

Richard M. Schultz[1,2,*], Paula Stein[3] and Petr Svoboda[4]

[1]Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania, USA; [2]Department of Anatomy, Physiology, Cell Biology, School of Veterinary Medicine, University of California, Davis, Davis, California, USA; [3]Reproductive and Developmental Biology Laboratory, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina, USA and [4]Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic

*__Correspondence:__ Department of Anatomy, Physiology, and Cell Biology, Veterinary Medicine 3B, Room 4017, School of Veterinary Medicine, University of California, Davis, 1089 Garrod Drive, Davis, CA 95616. E-mail: rschultz@sas.upenn.edu

†**Grant support:** This work was supported by a grant from NIH (HD022681 to RMS), in part by the Intramural Research Program of the National Institutes of Health, NIEHS (1ZIAES102985 to Paula Stein), and the Czech Science Foundation grant (GACR P305/12/G034 to Petr Svoboda).

Received 20 November 2017; Revised 8 January 2018; Accepted 3 February 2018

Abstract

The oocyte-to-embryo transition (OET) arguably initiates with formation of a primordial follicle and culminates with reprogramming of gene expression during the course of zygotic genome activation. This transition results in converting a highly differentiated cell, i.e. oocyte, to undifferentiated cells, i.e. initial blastomeres of a preimplantation embryo. A plethora of changes occur during the OET and include, but are not limited to, changes in transcription, chromatin structure, and protein synthesis; accumulation of macromolecules and organelles that will comprise the oocyte's maternal contribution to the early embryo; sequential acquisition of meiotic and developmental competence to name but a few. This review will focus on transcriptional and post-transcriptional changes that occur during OET in mouse because such changes are likely the major driving force for OET. We often take a historical and personal perspective, and highlight how advances in experimental methods often catalyzed conceptual advances in understanding the molecular bases for OET. We also point out questions that remain open and therefore represent topics of interest for future investigation.

Summary Sentence

We review, often with a historical perspective, transcriptional and post-transcriptional changes that underlie the oocyte-to-embryo transition in mouse.

Key words: oocyte development, preimplantation embryo development, gene expression, RNA, degradation, genome activation, reprogramming gene expression, small RNA, siRNA, miRNA, lncRNA, dormant maternal mRNA, RNAi.

Oocytes, which are terminally differentiated cells, become even more specialized during the course of their growth phase. Nevertheless, following maturation and fertilization, oocyte identity is lost such that the early blastomeres are totipotent. This remarkable transition, the oocyte-to-embryo transition (OET), has fascinated reproductive biologists, developmental biologists, cell biologists, molecular biologists, to name a few, and has been the object of their research efforts for decades. Although a detailed understanding of this transition at the molecular and cellular levels still remains elusive substantial progress has been made in the recent past. Our understanding of the OET has often been driven not only by major advances in our conceptual understanding of transcriptional and post-transcriptional regulation of gene expression but also by methods to study these biological processes. This review focuses on the evolution of our

© The Author(s) 2018. Published by Oxford University Press on behalf of Society for the Study of Reproduction. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

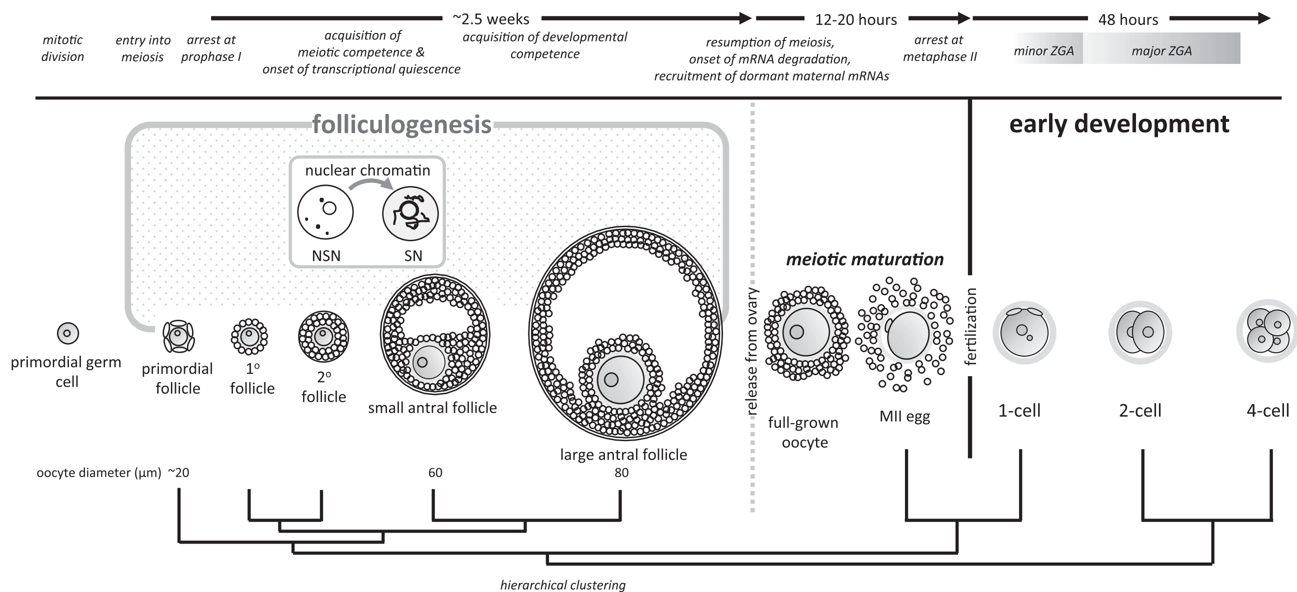

Figure 1. Schematic diagram of events occurring during oocyte growth and early development. See the text for further discussion of specific events and developmental transitions. Initiation of follicle growth is accompanied by a transition of the surrounding somatic cells from an epithelial to cuboidal shape and oocyte maturation is accompanied by cumulus cell expansion.

understanding of the OET from a regulation of gene expression perspective and we hope it will spark future research that will further our understanding of the OET.

Changes in gene expression and transcription during oocyte growth

Oocytes grow within follicles and initially oocyte growth coordinates with follicle growth, but as the oocyte approaches its final volume, growth of the follicle continues with antrum formation, i.e. increase in follicle size with little increase in oocyte size (Figure 1). Development of methods to isolate oocytes at different stages of growth that exploited the relatively synchronous first wave of follicle growth in pre-pubertal mice [1] coupled with in vitro culture systems that supported both acquisition of meiotic (ability to resume meiosis and progress to and arrest at metaphase II) and developmental competence (ability to be fertilized and develop to term) [2] was central to defining changes in gene expression during oocyte development described below. Initial studies, which capitalized on the commercial availability of high specific activity [^{35}S]methionine and high-resolution two-dimensional gel electrophoresis, captured for the first time extensive changes in the pattern of protein synthesis—both increases and decreases—that presumably reflected, to a large degree, changes in the oocyte's transcriptome [3]. The identity of the genes encoding these proteins, however, was essentially unknown and extant methods were highly biased to detect expression of abundant transcripts, e.g. structural proteins such as tubulin, rather than regulators of transcription.

Analysis of gene expression during oogenesis and preimplantation development exploded with the advent of the polymerase chain reaction coupled with reverse transcription by permitting analysis of changes in relative transcript abundance of individual genes [4], including low-abundance transcripts, and multiplexing methods permitting simultaneous analysis of several transcripts. With the introduction of microarrays, whose development required genome sequencing and bioinformatics to generate transcript-specific probes, it finally became possible to define changes in the transcriptome during oocyte growth [5]. Dramatic changes accompanied the primordial to primary follicle transition and are far more pronounced than subsequent changes that progressively occur during oocyte growth. The transcriptomes of oocytes within primary and secondary oocytes cluster separately from oocytes within small and large antral follicles. This change in clustering coincides with a number of developmental changes that occur during oocyte growth. Acquisition of meiotic competence occurs during the secondary to small antral follicle transition [1, 6, 7] whereas acquisition of developmental competence occurs during the small to large antral transition [2], with the ability of matured oocytes to develop to the blastocyst stage acquired during the last days of oocyte growth. Analysis of the transcriptomes of these oocytes identified a plethora of genes that could drive these transitions [5] and highlighted how such analyses were critical for discovering/identifying candidate genes for functional studies.

Changes in chromatin structure clearly underlie reprogramming gene expression during oocyte development, with changes in histone modifications being a key player in either establishing or maintaining remodeled chromatin. Availability of highly specific antibodies that recognize specific histone modifications such as acetylation, methylation, and phosphorylation, which led to numerous studies describing such changes during oocyte growth, e.g. see [8], coupled with development of oocyte-specific promoters such as *Gdf9* (active in primordial oocytes) and *Zp3* (active shortly after the onset of oocyte growth) [9] made possible conditional mutagenesis of histone modifying genes to assess their function in reprogramming gene expression during oocyte development. For example, detailed analyses using mice lacking different combinations of *Hdac1* and *Hdac2* and microarrays revealed that these HDACs are critical for proper patterns of gene expression that support oocyte development, and in particular, that HDAC2 is more critical for oocyte development than HDAC1, a situation reversed in the preimplantation embryo [10]. The phenotype of female mice in which *Hdac1* and *Hdac2* were

conditionally deleted is small ovary size, arrest of follicle growth before antrum formation—the mice are infertile—a decrease in global transcription and histone H3K4 methylation, a transcription activating mark.

It was recognized early on that transcription declines toward the end of oocyte growth such that the full-grown oocyte is essentially transcriptionally inactive. In the late 1960s and 1970s, the availability of high specific [^{3}H]uridine permitted autoradiographic analysis of [^{3}H]uridine incorporation by oocytes within follicles at different stages of growth following intraperitoneal injection [11, 12]. During oocyte growth, incorporation initially increased for both the nucleoplasm (POLR2) and nucleolus (POLR1), indicating a coordinate increase in transcription for both mRNA and rRNA. As the oocyte approached its full size, incorporation rapidly decreased. This decline is associated with the onset of antrum formation and hence with acquisition of meiotic competence. The decrease in incorporation could not be attributed to changes in [^{3}H]uridine uptake or metabolism because similar results were obtained using ovarian sections and an assay that measured endogenous POLR2 and POLR1 activity in oocytes of different sizes; the assay captures polymerases engaged in transcription, i.e. is effectively a nuclear run-on assay [13]. Sensitive fluorometric methods to measure total RNA, coupled with the availability of [^{3}H]poly(U) for in situ hybridization to ovarian sections, demonstrated that the kinetics of accumulation of total RNA, mainly rRNA, and mRNA were consistent with the aforementioned changes in RNA polymerase activity [14]. A growth-related decrease in transcription was also detected for expression of an SP1-dependent plasmid-borne reporter gene [15].

The molecular basis for the decline in transcription during oocyte growth, which is an evolutionary conserved feature of oocytes of all species examined to date, is poorly understood. Transcriptional quiescence was faithfully recapitulated using an in vitro culture system and moreover demonstrated a role for cumulus cells [16]. Oocytes isolated from 12-day-old mice revealed a time-dependent decline in transcriptional activity during the following 8 days of culture, whereas no decline was observed for oocytes liberated from their attached cumulus cells and then cultured for an additional 8 days. The ability to quantify transcription in single oocytes by monitoring incorporation of modified ribonucleotides detectable by immunocytochemistry propelled such studies [17, 18]. Consistent with a role for cumulus cells in generating a transcriptionally quiescent state is that cumulus cells are essentially unattached from *Msy2*$^{-/-}$ oocytes [19], which do not undergo transcriptional quiescence. Whether the communication between the oocyte and its companion cumulus cells is mediated by a juxtacrine pathway or by gap junctions is not known.

During oocyte growth both in vivo and in vitro, oocytes undergo a change in DNA configuration from the nonsurrounded nucleolus (NSN) to surrounded nucleolus (SN) that is temporally linked with transcriptional quiescence [20]. Developmental competence of full-grown oocytes that do not undergo the NSN-to-SN transition is compromised [21, 22], and likely reflects changes in their transcriptome as determined by microarrays [23]. Full-grown NSN oocytes remain transcriptionally active and although the abundance of many transcripts differs between NSN and SN oocytes, no clear candidates emerged as critical for the difference in transcriptional activity. It should also be noted that transcription, as assessed by BrUTP incorporation, of cumulus cell-enclosed NSN oocytes is about 3-fold less than denuded NSN oocytes, which is consistent with a role for cumulus cells to suppress transcription in oocytes [24].

This change in DNA configuration, coupled with the acquisition of M-phase traits during oocyte growth, e.g. chromatin condensation [7], was initially thought to underlie transcriptional quiescence. Several lines of experimentation demonstrate that such is not the case because NSN-to-SN transition and transcriptional quiescence can be uncoupled. For example, *Npm2*$^{-/-}$ oocytes do not undergo the NSN-to-SN transition but become transcriptionally quiescent [25], whereas *Mll2*$^{-/-}$ oocytes—MLL2 methylates H3K4me1—undergo the transition but remain transcriptionally active [26]. Furthermore, full-grown transcriptionally quiescent SN oocytes remain transcriptionally inert following treatment with trichostatin A [25], a histone deacetylase inhibitor that induces histone hyperacetylation that is associated with transcriptionally permissive chromatin and chromatin decondensation. Although the NSN-to-SN transition is not responsible for transcriptional silencing, inhibiting transcription stimulates conversion of NSN oocytes to SN oocytes [27, 28]. Thus, transcription is required for the transition but whether this is the result of inhibiting expression of specific genes required for the transition or reflects a requirement for transcription per se is not known.

Like real estate, transcription is all about location, in this case RNA polymerase gaining access to a promoter, which is a function of the interplay between transcription factors and chromatin structure. The TATA-binding protein TBP is a general transcription factor central for transcription mediated by RNA polymerase II and is also involved in transcription mediated by RNA polymerases I and III [29]. Oocytes express TBP [15] but also an oocyte-specific form, TBPL2 [30, 31]; *Tbpl2*$^{-/-}$ mice are infertile indicating a critical role for TBPL2 in oocyte development, whereas mice in which *Tbp* is conditionally deleted in oocytes are fertile [32]. Interestingly, immunohistochemistry indicates that both TBP and TBPL2 proteins decline during oocyte development—TBP declining during the primordial to primary follicle transition and TBPL2 during the secondary antral follicle to preovulatory follicle transition—such that neither is detected in preovulatory full-grown oocytes [30]. Thus, the decline in TBPL2 could contribute to transcriptional quiescence. It should be noted, however, that based on TBPL2 histochemistry staining intensity the decline in transcriptional activity precedes the decline in TBPL2 protein. Although overexpression of TBPL2, but not TBP, in zygotes compromises development to the blastocyst stage, whether expressing TBPL2 in full-grown oocytes restores transcription is not known.

The above discussion focused on POLR2-mediated transcription. Nevertheless, rDNA transcription also ceases with oocyte growth. Although transcription factors involved in rDNA transcription decrease with oocyte growth, e.g. upstream-binding factor decreases between the secondary follicle and small antral follicle stage [5], the role, if any, of such decreases in cessation of rDNA transcription is not known.

A key insight for the basis for the onset of transcriptional quiescence during oocyte growth is that POLR2 remains in the nucleus following nuclear membrane permeabilization of NSN growing oocytes as well as full-grown oocytes that have not undergone the transition [27]; whether such changes occur for POLR1/POLR3 is not known. In contrast, POLR2 is lost from the nucleus following nuclear permeabilization of full-grown SN oocytes. Of note, loss of nuclear localization following nuclear membrane permeabilization correlates with loss of phosphorylation on both S2 and S5 of the carboxyl-terminal domain (CTD) of POLR2A, the largest POLR2 subunit; S2 phosphorylation is linked with transcription elongation and S5 phosphorylation with transcription initiation [33]. Not known is whether observed decreases in transcript abundance of key players that regulate CTD phosphorylation (e.g. *Ccnt1*, which encodes cyclin T2)

lead to declines in the encoded proteins; such decreases could be contributing factors should they occur.

Development of microarrays to assess changes in the transcriptome during oocyte development identified genes whose expression is extinguished during growth [5]. With RNA-seq now being readily performed with single oocytes and providing a complete description of the transcriptome [34] coupled with development of highly sensitive methods to perform chromatin immunoprecipitation (ChIP-seq) [35–37] and Transposase-Accessible Chromatin (ATAC-seq) to assess the state of chromatin accessibility at the genome-wide level [38]—regulatory regions, like promoters and enhancers, are generally more accessible—on readily obtainable numbers of oocytes it should now be possible to link changes in gene expression, e.g. repression, with changes in specific modified histones that regulate expression of those genes with chromatin structure. And likely sooner, rather than later, ChIP-seq will be feasible on growing oocytes to locate developmental changes in the localization of specific transcription factors, e.g. oocyte-specific transcription factors such as FIGLA, NOBOX, LHX8, TAF4B, SOHLH1/2, YY1, FOXO3A, and TBPL2. Such informative analyses will result in a far better understanding how transcriptional quiescence (as well as developmental changes in gene expression) arises during oocyte growth and identify potential genes for functional analyses.

Genome activation and reprogramming gene expression

High specific [^{35}S]methionine also made possible metabolic radiolabeling to assess changes in protein synthesis during oocyte maturation and preimplantation development as an indirect means to assess changes in transcript abundance. What became apparent early on is that inhibiting transcription with α-amanitin, a POLR2 inhibitor, not only inhibited development of mouse embryos beyond the two-cell stage but also inhibited the appearance of a set of proteins readily detected following one-dimensional PAGE [39]. No effect on the pattern of protein synthesis was observed when one-cell embryos were treated. These results were the first to describe that genome activation and reprogramming of gene expression definitely occurred during the two-cell stage. In addition, similar experiments using rabbit embryos defined waves of transcriptional changes associated with developmental transitions, e.g. genome activation, compaction, blastocyst formation with its associated cell differentiation [40].

Microarrays and RNA-seq made readily feasible identifying genes expressed during these developmental transitions. With respect to genome activation and reprogramming gene expression, microarrays were first used to identify ~3000 α-amanitin-sensitive genes, i.e. genes activated during zygotic gene activation (ZGA) [41], which is likely an underestimate because the arrays did not detect noncoding RNAs. Analyses of these genes revealed several processes that occur during genome activation that would not be apparent from analysis of single genes and provided a deeper understanding of processes that occur during this critical transition. For example, genes involved in transcription and mRNA splicing were over-represented. Expression of such genes makes intuitive sense because their expression could potentially create a positive feedback to ensure that genome activation is irreversible and robust and generates functional transcripts. Genes involved in nucleotide metabolism are also over-represented. Expression of these genes would make certain the new demand for nucleotides to support not only transcription but also DNA replication is met. Although it is uncertain "why" rRNA and transcripts encoding ribosomal proteins are degraded during oocyte maturation, genes involved in ribosome biogenesis are also over-represented; their expression would provide a safety net to meet the demand for increased protein synthesis for the developing embryo [3].

Superimposed on genome activation is development of a transcriptionally repressive state that is mediated, at least in part, by chromatin. Critical to coming to this understanding was demonstrating that a reporter gene encoding the *Xenopus* 5S genes was faithfully transcribed following microinjection into the germinal vesicle (GV) of growing oocytes [42]. Results of experiments assaying expression of microinjected plasmid borne reporter genes demonstrated that efficient expression in two-cell embryos required an enhancer; one-cell embryos do not exhibit this requirement [43–45]. Enhancers function by overcoming chromatin-mediated repression of transcription, suggesting development of a chromatin-mediated transcriptionally repressive state. Consistent with this proposal is that an enhancer is no longer required to drive efficient expression of a plasmid-borne reporter when histone acetylation is induced by butyrate treatment. Furthermore, the transcriptionally repressive state becomes greater as development proceeds, i.e. the stimulatory effect of the enhancer decreases with development [45]. Results of fluorescence recovery after photobleaching strikingly demonstrate that the high mobility of nucleosomes in two-cell embryos is markedly reduced with development, a finding consistent with changes in chromatin structure underlying formation of the transcriptionally repressive state [46].

Analysis of single genes, e.g. eIF1A, as well as microarray studies, identified many genes that are transiently expressed during the two-cell stage [47, 48], providing indirect evidence that a transcriptionally repressive state develops during the course of genome activation. Small-interfering RNA (siRNA)-mediated reduction of HDAC1 protein in two-cell embryos results not only in histone hyperacetylation but also in an increased relative abundance of transcripts that are normally transiently expressed during genome activation [10], a finding consistent with the basis for the repressive state being at the level of chromatin structure. Furthermore, that DNA replication is required for formation of the transcriptionally repressive state for both reporter genes and endogenous genes presumably reflects replication-dependent changes in chromatin structure [44, 47]. Going forward, experiments employing ATAC-seq coupled with the emerging advances in chromosome capture techniques will provide the opportunity to identify and define changes in chromatin structure, and how such changes underlie genome activation and reprogramming of gene expression.

Evidence is rapidly emerging that changes in histone modifications and the location of nucleosomes harboring these modified histones are also critical for OET. As described above, ChIP-seq can now locate the position of histones on chromatin of oocytes and preimplantation embryos. What emerged from a series of recent studies examining changes in location of H3K4me3 was that the distribution of this activating mark changes during oocyte growth and genome activation in a very unanticipated manner [35–37]. Typically H3K4me3 maps to a highly defined narrow region over a promoter of most active genes, a so-called canonical distribution. Although this distribution is observed early on during oocyte growth, around the time of antrum formation, and onset of transcriptional quiescence, the distribution becomes broader in promoter regions and distal regions, a so-called noncanonical distribution. Genome activation during the two-cell stage is associated with re-establishment of the canonical distribution and is highly correlated with genes activated at this time. This transition occurs when DNA

replication is inhibited, i.e. not due to replication-dependent dilution, but rather is an active process, mainly catalyzed by KDM5A and B; the decrease in H3K4me3 that occurs during the two-cell stage is not observed following siRNA/morpholino targeting of these histone lysine demethylases and moreover, results in retention of the noncanonical distribution. The transition from a noncanonical to canonical distribution also requires transcription in two-cell embryos and likely reflects that both *Kdm5a* and *Kdm5b* are zygotically expressed [48]. These results suggest that the noncanonical to canonical transition is a consequence of genome activation that then plays an important role in governing which genes are expressed during genome activation. Integrating ATAC-seq with data describing changes in H3K4me3 distribution should be highly informative in understanding how genes are selected for expression during OET, and in particular, conducting such studies during the one-cell stage when the genome is starting to be promiscuously transcribed and there are distinct differences in chromatin structure between the physically separated maternal and paternal genomes. Last, the transition from a canonical to noncanonical distribution may contribute to the global cessation of transcription during oocyte growth because overexpressing KDM5B, but not KDM5A or other demethylases, in full-grown oocytes restores transcription in a substantial fraction of injected oocytes [37]. A proposed possible mechanism is that H3K4me3 located in distal regions could sequester transcription factors. The connection, if any, by which POLR2 no longer remains associated with oocyte chromatin and these changes in the distribution of H3K4me3 is not known.

Genome activation is also associated with a change in promoter utilization. Experiments using both plasmid-borne reporter genes that either contain or do not contain a TATA-box [49] or the endogenous *Eif1a* gene [50], which contains both a proximal TATA-containing promoter and a distal TATA-less promoter, demonstrated that prior to genome activation TATA-containing promoters are more efficiently used than TATA-less ones, whereas following genome activation the reverse is observed. Should this change reflect a global change in promoter utilization it could also contribute significantly to reprograming gene expression during genome activation. Another consequence could be increased expression of housekeeping genes to meet the increasing demands on energy production of the developing embryo—many housekeeping genes employ a TATA-less promoter [51]—as well as *Pou5f1*, whose expression is driven by a TATA-less promoter and essential for blastocyst formation [52].

How does a maternally inherited transcription machinery generate a pattern of gene expression in a two-cell embryo that dramatically differs from that in the oocyte? As noted above, changes in chromatin structure are likely critical for genome activation and reprogramming gene expression by making promoters accessible to POLR2. Recruitment of dormant maternal mRNAs encoding chromatin remodelers and transcription factors is likely a major strategy employed by the oocyte to direct the OET—dormant maternal mRNAs are discussed more fully below—and in fact proteomic analysis of mouse oocytes, eggs, and one-cell embryos indicated that some transcription factors and chromatin remodelers are more abundant in MII eggs than in GV oocytes [53]. For example, inhibiting the maturation-associated increase in SIN3A [54] or OBOX1/2 (Stein and Schultz, unpublished observations) using a combined siRNA/morpholino approach inhibits expression of a subset of genes normally activated during OET and compromises development beyond the two-cell stage. SIN3A is a scaffolding protein for HDAC1/2 complexes, i.e. chromatin remodeler, and OBOX1/2 is a homeodomain-containing transcription factor preferentially expressed in oocytes. Messenger RNAs encoding proteins that catalyze histone modifications are also likely involved. KDM1A demethylates H3K4 and H3K9, and oocyte-specific deletion of KDM1A results in arrest at the two-cell stage with unfaithful reprogramming of gene expression as assayed by RNA-seq [55, 56]. *Kdm1a* mRNA has all the hallmarks of a dormant maternal mRNA, e.g. cytoplasmic polyadenylation element (CPE) closely located to the polyadenylation site and microarray data reveal an increase in the relative abundance of *Kdm1a* mRNA between the GV oocyte and one-cell stages that likely reflects elongation of the poly (A) tail during maturation that in turn leads to more efficient oligo (dT) priming when generating the cDNA libraries (see below for further discussion).

Oocyte-derived transcription factors are also involved, e.g. BRG1/SMARCA4, which is the catalytic subunit of SWI-SNF chromatin remodeling complexes. Conditional deletion of *Brg1/Smarca4* in oocytes does not prevent oocyte development but does result in developmental arrest at the two-cell stage with failure to reprogram correctly about 1/3 of the genes normally activated as assessed by microarrays [57]. The developmental arrest is not due to lack of BRG1/SMARCA4 during oocyte growth that becomes manifest later because developmental arrest at the two-cell stage is observed when BRG1/SMARCA4 protein in full-grown oocytes is knocked down by RNA interference (RNAi).

As noted above, the first products of transcription in two-cell embryos are enriched in genes involved in transcription and critical for reprogramming, e.g. *Kdm5a* and *Kdm5b* (see above). Because these factors are the products of transcription, they intrinsically cannot initiate reprogramming—a responsibility that falls on the oocyte's dowry to the marriage of sperm and egg—but rather participate in a feedback loop that sculpts the pattern of gene expression. In addition, loss of oocyte-specific transcription factors by the time oocytes become full grown, e.g. NOBOX [58], would ensure that their presence does not compromise proper reprogramming of gene expression.

Transcriptional control of gene expression during OET is evolving; genes become engaged in the maternal and/or zygotic programs or lost from them during evolution. This view can be illustrated by a simple comparison of maternal and zygotic expression of core pluripotency transcription factors during mouse and bovine OET [59]. Repetitive sequences, particularly long terminal repeats (LTRs) of retrotransposons, constitute a key mechanism contributing to remodeling of maternal and zygotic gene expression during evolution. It was recognized more than a decade ago by transcriptome analyses in oocytes and early mouse embryos that LTRs can contribute to 5′ exons of dozens of maternal and zygotic genes [60]. Subsequent studies provided strong evidence that LTRs were repeatedly co-opted for stage-specific enhancers, promoters, and first exons [61–63], and a recent systematic annotation revealed that the mouse genome carries over a thousand LTR promoters from a single LTR family, many of which represent mouse-specific co-options of LTRs for control of expression of noncoding RNAs and protein-coding genes [63]. Furthermore, LTR sequences were implicated as binding sites for transcriptional factors and repressors setting up transcriptional control of pluripotency [62, 64, 65]. Integration of LTR insertions into wiring of OET in mice, which occurred during the last 60 million years of evolution, implies that wiring of OET in other mammals would be evolving to a significant extent by convergent evolution. Thus, one should be careful when interpreting data from mouse OET as different regulations of gene expression control may be underlying the same principles of development.

Some 15 years after it was clearly established that mouse genome activation definitively occurs during the two-cell stage [39], the question whether one-cell embryos are transcriptionally active was revisited using a number of different approaches [18, 66, 67] and the data clearly demonstrated that one-cell embryos are transcriptionally active. Activation of transcription in mouse zygotes is unique among animal embryos, which typically undergo several cleavages before ZGA (reviewed in [68]). Delayed onset of minor and major ZGA is also common in mammals, which typically undergo genome activation in 4-cell to 16-cell zygotes, although next generation sequencing (NGS) data show that transcription initiates earlier than suggested by earlier studies [69, 70]. However, in terms of time since fertilization, mice undergo genome activation later than zebrafish (∼4 h), *Xenopus* (∼6 h), or *Drosophila* (∼2.5 h) (reviewed in [71]).

Mouse ZGA is divided into minor ZGA, occurring during the one-cell stage, and major ZGA during the two-cell stage. This traditional division reflects that although the two-cell stage was identified as the first burst of zygotic gene expression, new RNAs of unclear origin could be detected in mouse zygotes as early as ∼7 h upon pronucleus formation [17, 18, 66]. With some exceptions, such as MuERV-L retrotransposon transcripts at the late one-cell stage [72], RNAs produced during minor ZGA remained unknown even upon transcriptome analysis using expression microarrays [41, 73]. These results supported the notion, which emerged from previous studies of minor ZGA, that the first transcription in mouse zygotes yields low levels of transcripts and that the transcription may be promiscuous. The nature of transcripts produced during minor ZGA was finally revealed by NGS [74, 75] where it was shown that the first wave of transcription is relatively promiscuous, low level, genome wide, and produces transcripts from thousands of protein-coding genes that are inefficiently spliced and polyadenylated [74, 75]. At the same time, although the one-cell transcription requires only minimal promoter features, it is partially selective as it is biased toward genes transcribed upon major ZGA and against exclusively maternally expressed genes [74]. Although the significance of minor ZGA in mouse zygotes remains unclear, it does not seem to be just opportunistic transcription producing nonfunctional protein-coding transcripts. One of the gene candidates with high functional significance during minor ZGA is *Dux4*, an intronless, multicopy gene encoding a double-homeobox transcription factor implicated in major ZGA [64, 76, 77]. *Dux4* is transcribed during the minor ZGA [74] and its features make it well adapted for protein production at the one-cell stage because being intronless it is resistant to inefficient splicing and its multiple copies would allow for mRNA accumulation even at low levels of transcription.

Chromatin organization and histone/DNA modifications also provide mechanisms to regulate gene expression and make a unique contribution to OET in mice. A long-known characteristic unique feature of transcription in mouse zygotes is parent-of-origin asymmetry of transcription where male pronuclei are more transcriptionally active than female pronuclei [17] and exhibit high levels of histone acetylation [78]. This difference in transcriptional activity has been attributed to the unique chromatin history of the male pronucleus, which undergoes protamine/histone exchange yielding in a "naïve" accessible chromatin structure with a minimum of transcriptionally repressive marks. While histone modifications emerge during the one-cell stage in the male pronucleus, formation of heterochromatin continues into the late two-cell stage (reviewed for in [79]). Female pronuclei also undergo chromatin remodeling, which reduces their heterochromatin [80–82]. Recent analysis of spatial genome organization in male and female pronuclei using a single-nucleus high-resolution chromosome conformation capture (Hi-C) revealed absence of compartmentalization of female pronuclei into active and inactive compartments (possibly due to transcriptionally inactive extended G1) and a weak but significant compartmentalization of the paternal genome [83]. In male pronuclei, compartmentalization into active compartments aligns with H4 hyperacetylation [83] described above [78]. De novo nuclear compartmentalization in zygotes thus concerns both genomes and continues into the two-cell stage.

Transition from mRNA stability to instability

High specific activity [^{3}H]uridine and in vitro culture systems also enabled in vivo and in vitro studies to assess RNA stability using pulse-chase experiments. Results of these experiments indicated that all classes of RNA (e.g. mRNA, rRNA, tRNA) were remarkably stable during oocyte growth [84–86]. For example, there was virtually no loss of non-poly(A)-containing RNA over the course of 8 days of culture and poly(A)-containing RNA had a half-life of about 10 days. Such stability makes sense in light of oocytes growing without dividing and the onset of transcriptional quiescence that starts around the time of antrum formation in the growing follicle. Nevertheless, although the overall population of maternal mRNAs is quite stable, mRNA turnover does occur during oocyte growth, contributes to changes in the transcriptome [5], and is essential to produce oocytes capable of successful maturation [87]. Uridylation of the poly(A) tail catalyzed by ZCCHC6 and ZCCHC11 is critical for turnover of oocyte mRNAs because conditionally deleting *Zcchc6/Zcchc11* in oocytes stabilizes mRNAs normally degraded during oocyte growth.

These early studies also indicated that onset of oocyte maturation results in a global decrease in RNA. In particular, oocyte maturation was associated with a loss of about 50% of poly(A)-containing RNAs, with most mRNAs being lost by the two-cell stage [88–90]. Thus, oocyte maturation initiates a transition from mRNA stability to instability. MSY2, a germ-cell specific RNA-binding protein, and recruitment of dormant maternal mRNAs encoding components of the RNA degradation machinery play critical roles in this transition. MSY2 constitutes ∼2% of oocyte protein and by the two-cell stage cannot be detected by immunoblotting [91]. Calculations using estimated intracellular concentrations of MSY2, mRNA, and average mRNA length suggest that most oocyte mRNAs are complexed with MSY2. *Msy2*$^{-/-}$ female mice are infertile [92, 93] and their oocytes have ∼75% of the total amount of mRNA present in wild-type oocytes [94]. The latter result suggested that MSY2 could be involved in mRNA stability and consistent with such a role is that a cRNA injected into *Msy2*$^{-/-}$ oocytes is less stable than when injected into wild-type oocytes and co-injection into *Msy2*$^{-/-}$ oocytes of a cRNA-encoding MSY2, but not a mutant form unable to bind to RNA, restore stability of the reporter cRNA [19].

MSY2 is phosphorylated by CDK1 with the onset of germinal vesicle breakdown (GVBD)—the increase in CDK1 activity initiates GVBD—and this phosphorylation likely contributes to the transition from mRNA stability to instability that initiates after GVBD [94]. For example, inhibiting CDK1 shortly after GVBD not only results in a conversion of phosphorylated MSY2 to its nonphosphorylated form but also prevents the normal decrease in relative abundance of mRNAs assayed by qRT-PCR after GVBD. The connection between MSY2 phosphorylation and mRNA instability is presumably causal because over-expressing a nonphosphorylatable form of MSY2 inhibits the decrease in relative abundance of the assayed mRNAs

during maturation, whereas overexpressing a putative constitutively active form of MSY2 in oocytes prevented from resuming maturation by inclusion of milrinone in the culture medium induces degradation of these mRNAs.

Phosphorylation of MSY2 may make mRNAs more accessible to the RNA degradation machinery. Treating permeabilized GV oocytes with concentrations of RNase that do not result in degradation of specific mRNAs as determined by qRT-PCR does result in their degradation in permeabilized MII eggs [94]. The connection between mRNA instability and MSY2 phosphorylation is strengthened by the observation that expressing the putative constitutively active form in GV-arrested oocytes also results in RNase susceptibility of the assayed mRNAs. MSY2 phosphorylation could in principle weaken its interaction with RNA due to charge repulsion or cause it to dissociate, in either case making the target RNA more susceptible to degradation. Whether such is the case remains to be demonstrated.

The pathway for mRNA degradation in somatic cells is typically initiated by deadenylation mediated by the sequential action of the PAN2/PAN3 and CCR4-NOT deadenylases [95–98]. Deadenylation, which is followed by further degradation in the 3′-5′ direction by the exosome complex, also triggers decapping of the transcript by the DCP1/DCP2 complex. The unprotected 5′ end is now accessible to the 5′-3′ exonuclease XRN that degrades further the transcript. In somatic cells, deadenylation is usually the rate-limiting step and once deadenylation commences the mRNA is rapidly degraded within minutes.

Recruitment of dormant maternal mRNAs encoding components of the RNA degradation machinery is likely another factor contributing to the transition from mRNA stability to instability initiated by resumption of meiosis [99, 100]. DCP1A (regulatory subunit) and DCP2 (catalytic subunit) are encoded by dormant maternal mRNAs, and resumption of meiosis triggers a dramatic increase in the amount of these components of the decapping complex. Inhibiting the maturation-associated increase in DCP1A/2 using siRNAs/morpholinos not only retards degradation of a microinjected reporter cRNA but also retards degradation of endogenous mRNAs, as assessed by microarrays, that are normally degraded during maturation. Components of the CCR4-NOT complex are also encoded by dormant maternal mRNAs, e.g. CNOT7, a catalytic component of the complex, and inhibiting the maturation-associated increase in CNOT7 using siRNAs inhibits deadenylation of mRNAs as assessed by RACE-PAT (rapid amplification of cDNA ends) assays. Reciprocally, overexpressing CNOT7 by microinjecting a *Cnot7* cRNA into GV oocytes prevented from resuming meiosis by milrinone results in deadenylation of mRNAs.

Messenger RNA stability in growing oocytes is likely attributed to key components of the RNA degradation machinery being encoded by dormant maternal mRNAs. The low level of RNA degradation activity would facilitate accumulation of mRNAs during the course of oocyte growth and enable mRNAs to maintain their previous steady-state level for several days in the face of transcriptional quiescence commencing with initiation of antrum formation. The increase in activity of the RNA degradation machinery during oocyte maturation would initiate loss of oocyte identity at precisely the correct time and thereby facilitate the transition of a highly differentiated oocyte into its totipotent "offspring."

Advent of sensitive techniques to measure the amount of RNA in small amounts of biological material, e.g. ethidium bromide fluourescence, led to the observation that a decrease in total RNA occurs during oocyte maturation, with a loss of 50% of poly(A) RNA (see above). These studies also revealed that mRNA comprises about 20% of total RNA present in full-grown oocytes, a relative abundance much greater than the typical 1–2% in somatic cells. With northern blot analysis appearing on the scene in the early 1980s and the ability to generate extremely high specific activity probes, it became possible to quantify changes in relative abundance of individual mRNA in oocytes and preimplantation embryos, but such studies required large numbers of oocytes (e.g. 1000–2000) [88–90]. What emerged from these early studies was that mRNAs were often deadenylated but then not further degraded, in stark contrast to somatic cells in which deadenylation typically triggers rapid and complete mRNA degradation. These deadenylated mRNAs were finally eliminated by the late two-cell stage. Quantitative RT-PCR using random priming also confirmed that many mRNAs were only partially degraded, and a global picture of partial mRNA degradation emerged from microarray studies that examined the relative abundance of mRNAs in oocytes and MII eggs [101, 102].

RNA-seq experiments have further refined the properties of the stable deadenylated mRNAs (Ma, Vandivier, Gregory, and Schultz, unpublished observations). The 3′-end of the mRNA is "nibbled" into the 3′ UTR at which point degradation apparently stops. In contrast, there is little degradation from the 5′-end into the 5′ UTR. Assuming that the 5′ cap is removed as a consequence of the increase in DCP1A and DCP2, microarray data suggest the lack of further degradation at the 5′-end may reflect the absence (or very low amounts) of XRN1 in oocytes [5, 48]; XRN1 is the major 5–3′ exonuclease involved in mRNA decay [103]. Of interest going forward is identifying why degradation in the 3′ direction stops. Are there RNA-binding proteins (and consensus binding sequences for these proteins) that inhibit processivity of exosome-mediated degradation? Are critical components of the exosome missing but are expressed during OET and thereby contribute to the loss of maternal mRNAs by the end of the two-cell stage? What is the role, if any, of MSY2? Of note, MSY2 protein persists until the two-cell stage but is lost by the late two-cell stage, raising the question that loss of MSY2 triggers the observed final degradation by the end of the two-cell stage [91]. It will be interesting to see if maintaining MSY2 in two-cell embryos prevents the final degradation of maternal mRNAs and what is the effect on development beyond the two-cell stage.

Partial degradation of mRNAs may serve two purposes. First, decapping and deadenylation will generate a nonfunctional mRNA that can no longer support translation and protein synthesis. This loss of function would initiate irreversibly loss of oocyte identity. In addition, these partially degraded mRNAs could be a storage form of nucleotides with their degradation during the two-cell stage providing nucleotides to support transcription and thus affording a mechanism to circumvent the poor ability of one-cell and two-cell embryos to transport ribonucleosides [104].

Going forward, how certain mRNAs escape degradation until much later and what are the consequences on development if these mRNAs are prematurely degraded remains an open and interesting question. In addition, with emergence of m^{6}A (N^6-methyladenosine) modification of mRNA regulating mRNA stability [105, 106]—m6A modification destabilizes mRNAs in embryonic stem cells [107]—the role of this post-transcriptional RNA modification in mRNA stability and degradation during oocyte growth and maturation is an area ripe for exploration. Analysis of the distribution of m^{6}A in mRNAs indicated that this modification is enriched in the 3′UTR and that A's within GAC (and to a lesser extent AAC) motifs are methylated [105]. Whether any relationship exists between m^{6}A methylation in the 3′UTR and partial degradation of the transcript remains to be established.

The m^6A-binding protein YTHDF2 is essential for degradation of ∼1/3 maternal mRNAs in zebrafish [108]. In mouse, conditionally deleting *Ythdf2* results in failure to degrade a subset of mRNAs normally degraded during maturation [109]. In addition, because *Ythdf2* expression initiates during the two-cell stage [48], YTHDF2 could in principle be involved in degradation of maternal mRNAs from the two-cell stage and beyond. Last, microarray data suggest that a dormant maternal mRNA encodes WTAP, a member of the METTL3-METTL14-WTAP complex that catalyzes m^6A methylation. Although the bulk of m^6A methylation occurs in the nucleus, cytoplasmic methylation does occur [110] and following GVBD mRNAs would be accessible to the methylating complex. Thus, recruitment of *Wtap* mRNA during oocyte maturation could destabilize maternal mRNAs and determining the effect of inhibiting the presumed increase in WTAP during maturation on maternal mRNA degradation and development could address this question.

Dormant maternal mRNAs

Known for more than 50 years is that full-grown oocytes contain a pool of stored transcripts that are not translated until after resumption of meiosis or fertilization [111, 112]. These mRNAs, initially termed “masked” mRNAs, are designated dormant maternal mRNAs. The first such transcript identified in mammalian oocytes was *Plat*, which encodes tissue-type plasminogen activator [113]. Dormancy of this class of transcripts is due to their short poly-A tail, which makes them not translatable or poorly translated. During oocyte maturation, or in some cases after fertilization, these transcripts undergo cytoplasmic polyadenylation, leading to their recruitment to polysomes [114]. Microarray data from mouse oocytes and preimplantation embryos allowed the systematic identification of this class of transcripts [48, 73, 115]. As described above, because oligo-dT priming was used for the reverse transcription step, longer poly-A tails result in a more efficient hybridization that leads to a higher relative transcript abundance but not its absolute abundance. Thus, a higher relative abundance of a transcript in one-cell embryos compared to GV oocytes makes it a likely candidate to be a dormant maternal mRNA. Similar data can be obtained from studies comparing relative abundance of transcripts associated with polysomes in GV oocytes and metaphase II eggs [116].

One hallmark of this class of transcripts is their high relative abundance in oocytes, which presumably enables synthesis of significant amounts of protein during the course of oocyte maturation. Another hallmark is the presence in their 3′UTR of a CPE, an AU-rich sequence upstream of the AAUAAA polyadenylation hexanucleotide. CPE binding proteins (CPEBs) specifically recognize CPEs and repress translation of dormant messages. Upon resumption of meiosis, CPEB phosphorylation induces cytoplasmic polyadenylation and translation [114]. Deleted in azoospermia-like elements cooperate with CPEB1 to regulate maternal mRNA translation [117]. Yet another layer of regulation of translation during oocyte maturation is utilization of transcripts with different 3′UTRs containing different regulatory elements, which provides temporal regulation of translational activation [118].

Examination of these transcripts reveals that they encode functions not needed for oocyte development (or for a function whose activity is required at a low level of activity; see below) but are required for an MII egg or early embryo. For example, DNA replication should not occur in full-grown GV oocytes, but must occur following fertilization. Two components of the DNA replication machinery, CDC6 and ORC6L, are encoded by dormant maternal messages [119, 120], and inhibiting the maturation-associated increase in ORC6L using RNAi inhibits DNA replication in the one-cell embryo [120]. Egg activation, a series of events that converts an MII egg into a developing embryo, is triggered in all species studied to date by an increase in intracellular Ca^{2+}. In mammals, this calcium increase is mediated by inositol 1,4,5-trisphosphate (IP_3), through binding to its receptor in the endoplasmic reticulum [121]. The transcript for IP_3 receptor, *Itpr1*, is recruited during oocyte maturation such that the amount of ITPR1 increases 1.5-fold to 2-fold, enhancing the sensitivity to IP_3-mediated calcium release. RNAi knockdown of *Itpr1*, which renders IP_3 receptor levels in MII eggs comparable to those in GV oocytes, results in impaired cortical granule exocytosis, and defects in calcium oscillations after fertilization [122]. The downstream effector of calcium during egg activation is calcium/calmodulin-dependent protein kinase II (CaMKIIγ). CaMKIIγ is also encoded by a recruited mRNA, and the amount and activity of the protein is ∼2-fold higher in eggs than in GV oocytes [123, 124]. This mode of regulation of two key players of egg activation ensures that the events comprising egg activation do not occur prematurely, which would result in a nonviable egg.

As described above, oocyte maturation initiates a process in which oocyte identity is lost with clearance of maternal mRNAs playing a critical role. A role for proteasome-mediated degradation of maternal proteins is presumably the other side of the coin to ensure erasure of the oogenic program [125–127]. In fact, ubiquitin-dependent protein degradation by the proteasome is an over-represented pathway in the GV oocyte [128]. Several E3 ubiquitin ligases are among the most abundant proteins in mouse oocytes, both at the transcript level [48] and at the protein level [53, 129], and some of them (*Fbxw24*, *Rnf114*, *Rnf141*, *Smurf2*, *Cul1*) are recruited during oocyte maturation. E3 ubiquitin ligases catalyze ubiquitination of their target proteins, marking them for degradation by the proteasome. A recent study found that the E3 ubiquitin ligase RNF114 (ring finger 114), a protein encoded by a recruited message, is essential for the OET in mouse and knockdown of *Rnf114* by siRNAs results in developmental arrest at the two-cell stage. In addition, RNF114-mediated ubiquitination and degradation of TAB1 (TGF-beta-activated kinase 1-binding protein 1) activate the NF-κB pathway during OET [118]. Going forward identifying the E3 ligases, in particular those encoded by dormant maternal mRNAs, and their targets will provide further insight in how oocyte identity is lost during the course of oocyte maturation and following fertilization.

Small RNAs

The discovery of RNAi revolutionized biology similar to the seismic effects of the discovery of restriction enzymes and polymerase chain reaction. This pioneer work described sequence-specific gene silencing triggered by long (500–1000 base pairs) double-stranded RNA (dsRNA) in *Caenorhabditis elegans* [130]. The mechanism of interference involves reduction or disappearance of the endogenous transcript and is restricted to exonic sequences. Soon after the initial discovery similar effects were reported in *Drosophila* [131], *Trypanosoma* [132], planarians [133], *Hydra* [134], and zebrafish [135]. In mammals, dsRNA activates the interferon (IFN) response, an antiviral mechanism that results in a block of protein synthesis and ultimately apoptosis [136]. Long dsRNA molecules induce expression of IFN α and β, which via the JAK-STAT pathway transcriptionally activate hundreds of genes. Among these genes are those encoding dsRNA-dependent protein kinase (PKR) and 2′,5′

oligoadenylate synthetase (OAS). PKR in turn phosphorylates several substrates, one being the α subunit of the eukaryotic translation initiation factor 2 (eIF2A); this post-translational modification inhibits protein synthesis. OAS, on the other hand, by generating 2′5 ′-linked oligoadenylates (2–5A), activates RNase L, a latent ribonuclease that catalyzes nonspecific degradation of cellular or viral RNAs [136]. This nonspecific response to dsRNA raised doubt that RNAi operated in mammals but such was not the case in mouse oocytes and preimplantation embryos [137, 138]. The likely reason for a lack of an interferon response is that oocytes do not express *Pkr*, *Rnasel*, or any of the catalytically active *Oas* isoforms, but express high levels of catalytically inactive *Oas* isoforms, which likely act as dominant negatives [139].

Functional RNAi in oocytes quickly led to development of a transgenic approach to study gene function by expressing a hairpin driven by either the ubiquitous cytomegalovirus (*Cmv*) promoter or the oocyte-specific *zona pellucida* 3 (*Zp3*) promoter [140]. As proof of principle a transgenic RNAi approach targeted *Mos*, an upstream activator of mitogen-activated protein kinase (MAPK), by expressing a *Mos* hairpin driven by the *Zp3* promoter [141]. This transgenic knockdown approach recapitulated the known *Mos* null phenotype, namely parthenogenetic activation. In addition, the approach generated founder mice with different degrees of knockdown, similar to a hypomorphic allelic series of mutants, which enabled detecting a threshold level of MAPK activity necessary to maintain MII arrest. This oocyte-specific transgenic knockdown model, which was simpler and less time consuming than the existing conditional knockout approach, was subsequently used to elucidate the role of several proteins in mouse oocytes and early embryos (reviewed in [142]).

Work from several laboratories shed light on the mechanism of RNAi. Long dsRNA molecules are cleaved into 21–23 bp long duplexes, known as siRNAs, by the RNase III enzyme DICER [143]. These siRNAs get incorporated into a multiprotein effector complex, termed RISC (RNA-induced silencing complex), where the two strands of the duplexes are unwound by a helicase activity. One of the strands, the “guide” strand, remains in the complex, while the other one, the “passenger” strand, is degraded or recycled [144]. Target recognition by the guide strand leads to target mRNA cleavage by an endonuclease activity associated with the ARGONAUTE (AGO) family (AGO2 in mammals) in the center of the region of complementarity between the transcript and the siRNA [145].

Two other classes of small RNAs have been identified in metazoans: microRNAs (miRNAs) and PIWI-interacting RNAs (piRNAs). MicroRNAs are similar in size to siRNAs, encoded in the genome, and generated from stem-loop-containing primary transcripts (pri-miRNAs) [146]. Another RNase III enzyme, DROSHA, acting in a complex with the double-stranded RNA binding protein DiGeorge syndrome critical region 8 (DGCR8), cleaves the pri-miRNA at the base of the stem loop to generate a 70-bp miRNA precursor (pre-miRNA). This pre-miRNA is then exported to the cytoplasm by EXPORTIN 5, and further processed by DICER into 21–24 nucleotide long duplexes, the mature miRNAs. MicroRNAs also exert their silencing role via the RISC complex (termed miRISC in this case) [146]. The outcome of this interaction depends on the degree of similarity between the miRNA and its target transcript. If they are a perfect match, endonucleolytic cleavage of the message ensues, just like siRNAs. If, on the other hand, the miRNA is not totally complementary to its target, the mode of silencing is translational repression, followed by 5′-to-3′ mRNA decay [145]. The vast majority of miRNAs in animals binds to partially complementary sites in the 3′UTR of endogenous transcripts and triggers their translational repression and degradation. Once AGO-bound miRNAs recognize their target transcript, AGO recruits two deadenylase complexes (PAN2-PAN3 and CCR4-NOT) to the mRNA, via the adaptor protein TNRC6. Recruitment of the decapping complex (DCP1/DCP2) results in removal of the 5′-cap and further digestion by the exonuclease XRN1. The mechanism of translational repression is not completely understood, but likely it is at the level of translation initiation and involves the eukaryotic translation initiation factor eIF4A [147].

PIWI-interacting RNAs were originally isolated from murine testes by five groups [148–152]. PIWI-interacting RNAs are longer than siRNAs and miRNAs (23–31 bp), are not derived from double-stranded RNA precursors (as miRNAs and siRNAs do), largely match transposable elements and repeats, and are expressed almost exclusively in the germline [153]. PIWI-interacting RNAs are generated by transcription from large genomic clusters, followed by 5′ trimming, loading onto PIWI protein-containing complexes, 3′ trimming, export to the cytoplasm, and amplification by a Ping-Pong cycle [153]. PIWI-interacting RNAs interact with both nuclear and cytoplasmic members of the PIWI clade of ARGONAUTE proteins. These interactions result in piRNA processing, as well as in silencing target mRNAs. The main role of piRNAs is to repress transposons by both transcriptional and post-transcriptional mechanisms, but piRNAs have also been postulated to regulate protein coding genes [154]. For recent reviews of piRNA biosynthesis and function, see [154, 155].

Because the RNAi pathway was functional in mouse oocytes and early embryos when exogenous dsRNA was introduced, it was likely that this machinery had a biological role during oogenesis/preimplantation development. In fact, such is the case. An oocyte-specific knockout of DICER generated by combining a conditional allele of *Dicer* with *Cre* recombinase driven by the *Zp3* promoter was used to assess the role of miRNAs and siRNAs in mouse oocytes [156]. *Dicer*$^{f/f}$; *Zp3-cre* females are infertile due to defects during meiotic maturation, including disorganized spindles, and chromosome misalignment. Transcriptomic analysis of in vitro-matured oocytes by microarrays revealed misregulation of thousands of genes (18.4% of transcripts), and overexpression of a subset of transposable elements, indicating a role for small RNAs in genome defense. Similar results were obtained by [157]. Thus, DICER-dependent small RNAs are essential for mouse oocyte development.

To characterize better the function of small RNAs in female meiosis, the small RNA profile of full-grown mouse oocytes was determined by RNA-seq [158, 159] and established that mouse oocytes express the three main classes of small RNAs: miRNAs, endogenous (endo-) siRNAs, and piRNAs, which in mammals had only been described in the male germline. Two populations of siRNAs were uncovered: one that maps to transposons and one that corresponds to protein-coding genes. Furthermore, some siRNAs are derived from dsRNAs formed by annealing of transcripts from protein-coding genes to antisense transcripts from homologous pseudogenes and these endo-siRNAs then regulate expression of the endogenous gene.

The phenotype of *Dicer*-deficient oocytes could be due to a depletion of miRNAs or siRNAs, or both. The transcriptome of *Dicer* null oocytes does not contain a miRNA signature, i.e. a relative increase of transcripts that contain binding sites for those miRNAs present in oocytes. Such an miRNA signature is observed in different cells, tissues, and organs after disruption of the miRNA pathway [160–162]. This observation suggested that the miRNA pathway might not function in mouse oocytes. Using miRNA reporters, miRNA activity

decreases during oocyte growth, such that full-grown oocytes and MII eggs exhibit a very modest translational repression and no detectable mRNA degradation in response to miRNAs [34]. In agreement with this finding, mice with an oocyte-specific deletion of *Dgcr8* are fertile, produce oocytes with no defects in meiotic maturation or preimplantation embryo development, and with a normal transcriptome [163]. A recent study using highly sensitive small RNA sequencing indicates that repression by miRNAs is reactivated at the two-cell stage and increases progressively at later stages [164]. In fact, the full knockout of *Dgcr8* is embryonic lethal at embryonic day 6.5, indicating an essential function for miRNAs post-implantation [165].

The reason why miRNAs are not functional in mouse oocytes is not clear. A handful of possible explanations have been postulated: insufficient amounts of AGO proteins and/or TNRC6 proteins; restricted accessibility of AGO proteins to bind to their target mRNAs; impaired interaction between AGO and TNRC6, either by post-translational modifications or cellular compartmentalization; RNA-binding proteins may interact with miRNAs and inhibit binding to their targets [163]; low activity of the RNA degradation machinery may limit miRNA-driven gene silencing. The concentration of endo-siRNAs in mouse oocytes, as inferred by the number of reads in RNAseq experiments, is substantially greater than that of miRNAs [164]. A plausible scenario is that the silencing machinery is being "sequestered" by the endo-siRNAs, rendering the miRNA pathway virtually not functional.

The aforementioned studies indicated that depletion of endo-siRNAs in *Dicer* null oocytes is likely responsible for the observed phenotype. This prediction was confirmed using mice carrying a catalytically inactive knock-in allele of *Ago2* ($Ago2^{ADH}$) in their oocytes to ablate endo-siRNA function without altering the miRNA pathway [166]. $Ago2^{ADH}$ females had defects in meiosis I, with severe abnormalities in spindle formation and chromosome alignment that caused meiotic catastrophe and infertility. The transcriptome of $Ago2^{ADH}$ oocytes was extensively perturbed and was very similar to the transcriptome of *Dicer* null oocytes. Expression of the mouse transcript (*Mt*), the most abundant transposable element in mouse oocytes, was also increased. Thus, endo-siRNAs are essential during meiosis I in mouse females.

Mouse and rat oocytes express a shorter transcript of *Dicer* driven by an MT-C retrotransposon promoter contained within intron 6 of the *Dicer* gene and lack the N-terminal DExD helicase domain of the protein [167]. This oocyte-specific form of DICER ($DICER^{O}$) is more abundant than the full-length somatic form ($DICER^{S}$), and processes long dsRNAs much more efficiently than $DICER^{S}$. $Dicer^{O}$ null mice (which lack the MT-C element, but express normal levels of $Dicer^{S}$) are viable, males are fertile, but females are sterile. The phenotype of $Dicer^{\circ}$ females is virtually identical to the $Dicer^{f/f}$; *Zp3-cre* females, which lack both $Dicer^{S}$ and $Dicer^{O}$. These results demonstrate that this alternative form of *Dicer* driven by a promoter provided by a retrotransposon insertion is essential for the female germline. In addition, such studies provide a real incentive to expression profile oocytes and preimplantation embryos using long RNA-seq to identify unequivocally oocyte/embryo-specific isoforms and splice variants, because such forms are likely to execute more efficiently functions critical for central processes of oocyte/preimplantation development than their somatic cell counterparts.

Mutant mice lacking any one of the PIWI proteins exhibit male sterility, but females are fertile. In flies and fish, on the other hand, both male and female *Piwi* mutants are sterile [168]. The likely reason for the piRNA pathway being dispensable in female mice is the presence of endo-siRNAs that fulfil similar functions, mainly genomic defense against transposons. In fact, in mammals, an essential role for endo-siRNAs has only been described in mouse oocytes, which possess a very unique environment due to the presence of a *Dicer* isoform highly specialized in converting long dsRNA precursors into endo-siRNAs, and the absence of an interferon response.

Long noncoding RNAs

Long non-coding RNAs (LncRNAs) are a heterogeneous largely unexplored group of RNAs > 200 nt, which do not encode proteins (reviewed in more detail in [169–171]). Intergenic and intragenic mammalian lncRNA emerged in large numbers from high-throughput transcriptome and chromatin analyses. In mammalian oocytes and early embryos, many thousands of lncRNA loci were annotated [172–175]. LncRNAs expressed during OET show lower median expression than mRNA, usually exhibit expression restricted to either the maternal or the zygotic phase of OET, evolve rapidly, and often co-opt functional elements from repetitive elements [63, 172, 173]. Interestingly, a subset of maternal lncRNAs apparently undergo cytoplasmic polyadenylation during meiotic maturation but the biological significance of this phenomenon remains unknown [172].

Although a systematic classification of lncRNAs has not been developed, one can distinguish five categories of lncRNA modes of action (i) scaffolding, (ii) guiding/tethering, (iii) signaling/allosteric effects, (iv) decoying/sequestering, and (v) precursors of smaller RNAs, such as small nucleolar RNAs, or small RNAs in RNA silencing. The last category has particular significance for mouse oocytes because as described above abundant siRNAs derived from lncRNAs carrying antisense pseudogene sequence [63, 158, 159] may underlie an essential role of RNAi for mouse OET [156, 157, 166, 176]. However, the role of most lncRNAs expressed during OET remains unknown and there has been rather slow progress in this area. In fact, given the rapid lncRNA evolution, it is possible that only a minority of lncRNAs acquired a significant biological role. For example, a loss-of-function analysis of two selected lncRNAs—one was an endo-siRNA substrate—yielded no apparent phenotype [172]. Among the functional studies revealing lncRNA functions during OET are identification of promoter associated noncoding RNAs, which appeared essential for early development [177], and implication of LincGET lncRNA in regulating alternative splicing during the two-cell stage [178].

Coda

The future of understanding molecular mechanisms underlying OET is very bright, built on a solid foundation of research spanning almost 50 years. Research tools that one could only imagine a few years ago are now in place and will permit the upcoming generation of investigators the opportunity to answer unresolved long-standing questions mentioned above. With our ever-increasing understanding of transcriptional and post-transcriptional regulation, there is every reason to believe that within the next decade we will understand how a highly differentiated oocyte is transformed into totipotent blastomeres. And such an understanding will likely lead to more efficient ways to reprogram somatic cells and result in advances in regenerative medicine.

Acknowledgments

RMS thanks all lab members who contributed to the work on OET as well as numerous colleagues for fruitful conversations over the course of many years. The author order is alphabetical based on last name.

References

1. Sorensen RA, Wassarman PM. Relationship between growth and meiotic maturation of the mouse oocyte. *Dev Biol* 1976; **50**:531–536.
2. Eppig JJ, Schroeder AC. Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development to live young after growth, maturation, and fertilization in vitro. *Biol Reprod* 1989; **41**:268–276.
3. Schultz RM, Letourneau GE, Wassarman PM. Program of early development in the mammal: changes in the patterns and absolute rates of tubulin and total protein synthesis during oocyte growth in the mouse. *Dev Biol* 1979; **73**:120–133.
4. Rappolee DA, Brenner CA, Schultz R, Mark D, Werb Z. Developmental expression of PDGF, TGF-alpha, and TGF-beta genes in preimplantation mouse embryos. *Science* 1988; **241**:1823–1825.
5. Pan H, O'Brien M J, Wigglesworth K, Eppig JJ, Schultz RM. Transcript profiling during mouse oocyte development and the effect of gonadotropin priming and development in vitro. *Dev Biol* 2005; **286**:493–506.
6. Wickramasinghe D, Albertini DF. Centrosome phosphorylation and the developmental expression of meiotic competence in mouse oocytes. *Dev Biol* 1992; **152**:62–74.
7. Wickramasinghe D, Ebert KM, Albertini DF. Meiotic competence acquisition is associated with the appearance of M-phase characteristics in growing mouse oocytes. *Dev Biol* 1991; **143**:162–172.
8. Kageyama S, Liu H, Kaneko N, Ooga M, Nagata M, Aoki F. Alterations in epigenetic modifications during oocyte growth in mice. *Reproduction* 2007; **133**:85–94.
9. Lan ZJ, Xu X, Cooney AJ. Differential oocyte-specific expression of Cre recombinase activity in GDF-9-iCre, Zp3cre, and Msx2Cre transgenic mice. *Biol Reprod* 2004; **71**:1469–1474.
10. Ma P, Schultz RM. HDAC1 and HDAC2 in mouse oocytes and preimplantation embryos: specificity versus compensation. *Cell Death Differ* 2016; **23**:1119–1127.
11. Moore GP, Lintern-Moore S, Peters H, Faber M. RNA synthesis in the mouse oocyte. *J Cell Biol* 1974; **60**:416–422.
12. Oakberg EF. Relationship between stage of follicular development and RNA synthesis in the mouse oocyte. *Mutat Res* 1968; **6**:155–165.
13. Moore GP, Lintern-Moore S. Transcription of the mouse oocyte genome. *Biol Reprod* 1978; **18**:865–870.
14. Sternlicht AL, Schultz RM. Biochemical studies of mammalian oogenesis: kinetics of accumulation of total and poly(A)-containing RNA during growth of the mouse oocyte. *J Exp Zool* 1981; **215**:191–200.
15. Worrad DM, Ram PT, Schultz RM. Regulation of gene expression in the mouse oocyte and early preimplantation embryo: developmental changes in Sp1 and TATA box-binding protein, TBP. *Development* 1994; **120**:2347–2357.
16. De La Fuente R, Eppig JJ. Transcriptional activity of the mouse oocyte genome: companion granulosa cells modulate transcription and chromatin remodeling. *Dev Biol* 2001; **229**:224–236.
17. Aoki F, Worrad DM, Schultz RM. Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Dev Biol* 1997; **181**:296–307.
18. Bouniol C, Nguyen E, Debey P. Endogenous transcription occurs at the 1-cell stage in the mouse embryo. *Exp Cell Res* 1995; **218**:57–62.
19. Medvedev S, Pan H, Schultz RM. Absence of MSY2 in mouse oocytes perturbs oocyte growth and maturation, RNA stability, and the transcriptome. *Biol Reprod* 2011; **85**:575–583.
20. Bouniol-Baly C, Hamraoui L, Guibert J, Beaujean N, Szollosi MS, Debey P. Differential transcriptional activity associated with chromatin configuration in fully grown mouse germinal vesicle oocytes. *Biol Reprod* 1999; **60**:580–587.
21. Zuccotti M, Piccinelli A, Giorgi Rossi P, Garagna S, Redi CA. Chromatin organization during mouse oocyte growth. *Mol Reprod Dev* 1995; **41**:479–485.
22. Monti M, Zanoni M, Calligaro A, Ko MS, Mauri P, Redi CA. Developmental arrest and mouse antral not-surrounded nucleolus oocytes. *Biol Reprod* 2013; **88**:2.
23. Ma JY, Li M, Luo YB, Song S, Tian D, Yang J, Zhang B, Hou Y, Schatten H, Liu Z, Sun QY. Maternal factors required for oocyte developmental competence in mice: transcriptome analysis of non-surrounded nucleolus (NSN) and surrounded nucleolus (SN) oocytes. *Cell Cycle* 2013; **12**:1928–1938.
24. Liu H, Aoki F. Transcriptional activity associated with meiotic competence in fully grown mouse GV oocytes. *Zygote* 2002; **10**:327–332.
25. De La Fuente R, Viveiros MM, Burns KH, Adashi EY, Matzuk MM, Eppig JJ. Major chromatin remodeling in the germinal vesicle (GV) of mammalian oocytes is dispensable for global transcriptional silencing but required for centromeric heterochromatin function. *Dev Biol* 2004; **275**:447–458.
26. Andreu-Vieyra CV, Chen R, Agno JE, Glaser S, Anastassiadis K, Stewart AF, Matzuk MM. MLL2 is required in oocytes for bulk histone 3 lysine 4 trimethylation and transcriptional silencing. *PLoS Biol* 2010; **8**: e1000453.
27. Abe K, Inoue A, Suzuki MG, Aoki F. Global gene silencing is caused by the dissociation of RNA polymerase II from DNA in mouse oocytes. *J Reprod Dev* 2010; **56**:502–507.
28. Liu YJ, Nakamura T, Nakano T. Essential role of DPPA3 for chromatin condensation in mouse oocytogenesis. *Biol Reprod* 2012; **86**:40.
29. Hernandez N. TBP, a universal eukaryotic transcription factor? *Genes Dev* 1993; **7**:1291–1308.
30. Gazdag E, Rajkovic A, Torres-Padilla ME, Tora L. Analysis of TATA-binding protein 2 (TBP2) and TBP expression suggests different roles for the two proteins in regulation of gene expression during oogenesis and early mouse development. *Reproduction* 2007; **134**:51–62.
31. Xiao L, Kim M, DeJong J. Developmental and cell type-specific regulation of core promoter transcription factors in germ cells of frogs and mice. *Gene Expr Patterns* 2006; **6**:409–419.
32. Gazdag E, Santenard A, Ziegler-Birling C, Altobelli G, Poch O, Tora L, Torres-Padilla ME. TBP2 is essential for germ cell development by regulating transcription and chromatin condensation in the oocyte. *Genes Dev* 2009; **23**:2210–2223.
33. Phatnani HP, Greenleaf AL. Phosphorylation and functions of the RNA polymerase II CTD. *Genes Dev* 2006; **20**:2922–2936.
34. Tang F, Barbacioru C, Nordman E, Li B, Xu N, Bashkirov VI, Lao K, Surani MA. RNA-Seq analysis to capture the transcriptome landscape of a single cell. *Nat Protoc* 2010; **5**:516–535.
35. Dahl JA, Jung I, Aanes H, Greggains GD, Manaf A, Lerdrup M, Li G, Kuan S, Li B, Lee AY, Preissl S, Jermstad I et al. Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic transition. *Nature* 2016; **537**:548–552.
36. Liu X, Wang C, Liu W, Li J, Li C, Kou X, Chen J, Zhao Y, Gao H, Wang H, Zhang Y, Gao Y et al. Distinct features of H3K4me3 and H3K27me3 chromatin domains in pre-implantation embryos. *Nature* 2016; **537**:558–562.
37. Zhang B, Zheng H, Huang B, Li W, Xiang Y, Peng X, Ming J, Wu X, Zhang Y, Xu Q, Liu W, Kou X et al. Allelic reprogramming of the histone modification H3K4me3 in early mammalian development. *Nature* 2016; **537**:553–557.
38. Wu J, Huang B, Chen H, Yin Q, Liu Y, Xiang Y, Zhang B, Liu B, Wang Q, Xia W, Li W, Li Y et al. The landscape of accessible chromatin in mammalian preimplantation embryos. *Nature* 2016; **534**:652–657.
39. Flach G, Johnson MH, Braude PR, Taylor RA, Bolton VN. The transition from maternal to embryonic control in the 2-cell mouse embryo. *EMBO J* 1982; **1**:681–686.
40. Van Blerkom J. Molecular differentiation of the rabbit ovum. *Dev Biol* 1979; **72**:188–194.

41. Zeng F, Schultz RM. RNA transcript profiling during zygotic gene activation in the preimplantation mouse embryo. *Dev Biol* 2005; **283**:40–57.
42. Brinster RL, Chen HY, Trumbauer ME. Mouse oocytes transcribe injected Xenopus 5S RNA gene. *Science* 1981; **211**:396–398.
43. Majumder S, Miranda M, DePamphilis ML. Analysis of gene expression in mouse preimplantation embryos demonstrates that the primary role of enhancers is to relieve repression of promoters. *EMBO J* 1993; **12**:1131–1140.
44. Majumder S, DePamphilis ML. A unique role for enhancers is revealed during early mouse development. *Bioessays* 1995; **17**:879–889.
45. Henery CC, Miranda M, Wiekowski M, Wilmut I, DePamphilis ML. Repression of gene expression at the beginning of mouse development. *Dev Biol* 1995; **169**:448–460.
46. Boskovic A, Eid A, Pontabry J, Ishiuchi T, Spiegelhalter C, Raghu Ram EV, Meshorer E, Torres-Padilla ME. Higher chromatin mobility supports totipotency and precedes pluripotency in vivo. *Genes Dev* 2014; **28**:1042–1047.
47. Davis W, Jr, De Sousa PA, Schultz RM. Transient expression of translation initiation factor eIF-4C during the 2-cell stage of the preimplantation mouse embryo: identification by mRNA differential display and the role of DNA replication in zygotic gene activation. *Dev Biol* 1996; **174**:190–201.
48. Zeng F, Baldwin DA, Schultz RM. Transcript profiling during preimplantation mouse development. *Dev Biol* 2004; **272**:483–496.
49. Majumder S, DePamphilis ML. TATA-dependent enhancer stimulation of promoter activity in mice is developmentally acquired. *Mol Cell Biol* 1994; **14**:4258–4268.
50. Davis W, Jr, Schultz RM. Developmental change in TATA-box utilization during preimplantation mouse development. *Dev Biol* 2000; **218**:275–283.
51. Bird AP. CpG-rich islands and the function of DNA methylation. *Nature* 1986; **321**:209–213.
52. Pesce M, Gross MK, Scholer HR. In line with our ancestors: Oct-4 and the mammalian germ. *Bioessays* 1998; **20**:722–732.
53. Wang S, Kou Z, Jing Z, Zhang Y, Guo X, Dong M, Wilmut I, Gao S. Proteome of mouse oocytes at different developmental stages. *Proc Natl Acad Sci USA* 2010; **107**:17639–17644.
54. Jimenez R, Melo EO, Davydenko O, Ma J, Mainigi M, Franke V, Schultz RM. Maternal SIN3A regulates reprogramming of gene expression during mouse preimplantation development. *Biol Reprod* 2015; **93**:89.
55. Ancelin K, Syx L, Borensztein M, Ranisavljevic N, Vassilev I, Briseno-Roa L, Liu T, Metzger E, Servant N, Barillot E, Chen CJ, Schule R et al. Maternal LSD1/KDM1A is an essential regulator of chromatin and transcription landscapes during zygotic genome activation. *Elife* 2016; **5**: e08851.
56. Wasson JA, Simon AK, Myrick DA, Wolf G, Driscoll S, Pfaff SL, Macfarlan TS, Katz DJ. Maternally provided LSD1/KDM1A enables the maternal-to-zygotic transition and prevents defects that manifest postnatally. *Elife* 2016; **5**: e08848.
57. Bultman SJ, Gebuhr TC, Pan H, Svoboda P, Schultz RM, Magnuson T. Maternal BRG1 regulates zygotic genome activation in the mouse. *Genes Dev* 2006; **20**:1744–1754.
58. Belli M, Cimadomo D, Merico V, Redi CA, Garagna S, Zuccotti M. The NOBOX protein becomes undetectable in developmentally competent antral and ovulated oocytes. *Int J Dev Biol* 2013; **57**:35–39.
59. Svoboda P, Franke V, Schultz RM. Sculpting the transcriptome during the oocyte-to-embryo transition in mouse. *Curr Top Dev Biol* 2015; **113**:305–349.
60. Peaston AE, Evsikov AV, Graber JH, de Vries WN, Holbrook AE, Solter D, Knowles BB. Retrotransposons regulate host genes in mouse oocytes and preimplantation embryos. *Dev Cell* 2004; **7**:597–606.
61. Bourque G, Leong B, Vega VB, Chen X, Lee YL, Srinivasan KG, Chew JL, Ruan Y, Wei CL, Ng HH, Liu ET. Evolution of the mammalian transcription factor binding repertoire via transposable elements. *Genome Res* 2008; **18**:1752–1762.
62. Macfarlan TS, Gifford WD, Driscoll S, Lettieri K, Rowe HM, Bonanomi D, Firth A, Singer O, Trono D, Pfaff SL. Embryonic stem cell potency fluctuates with endogenous retrovirus activity. *Nature* 2012; **487**: 57–63.
63. Franke V, Ganesh S, Karlic R, Malik R, Pasulka J, Horvat F, Kuzman M, Fulka H, Cernohorska M, Urbanova J, Svobodova E, Ma J et al. Long terminal repeats power evolution of genes and gene expression programs in mammalian oocytes and zygotes. *Genome Res* 2017; **27**:1384–1394.
64. Hendrickson PG, Dorais JA, Grow EJ, Whiddon JL, Lim JW, Wike CL, Weaver BD, Pflueger C, Emery BR, Wilcox AL, Nix DA, Peterson CM et al. Conserved roles of mouse DUX and human DUX4 in activating cleavage-stage genes and MERVL/HERVL retrotransposons. *Nat Genet* 2017; **49**:925–934.
65. Huang Y, Kim JK, Do DV, Lee C, Penfold CA, Zylicz JJ, Marioni JC, Hackett JA, Surani MA. Stella modulates transcriptional and endogenous retrovirus programs during maternal-to-zygotic transition. *Elife* 2017; **6**:e22345.
66. Matsumoto K, Anzai M, Nakagata N, Takahashi A, Takahashi Y, Miyata K. Onset of paternal gene activation in early mouse embryos fertilized with transgenic mouse sperm. *Mol Reprod Dev* 1994; **39**:136–140.
67. Ram PT, Schultz RM. Reporter gene expression in G2 of the 1-cell mouse embryo. *Dev Biol* 1993; **156**:552–556.
68. Tadros W, Lipshitz HD. The maternal-to-zygotic transition: a play in two acts. *Development* 2009; **136**:3033–3042.
69. Graf A, Krebs S, Zakhartchenko V, Schwalb B, Blum H, Wolf E. Fine mapping of genome activation in bovine embryos by RNA sequencing. *Proc Natl Acad of Sci* 2014; **111**:4139–4144.
70. Xue Z, Huang K, Cai C, Cai L, Jiang CY, Feng Y, Liu Z, Zeng Q, Cheng L, Sun YE, Liu JY, Horvath S et al. Genetic programs in human and mouse early embryos revealed by single-cell RNA sequencing. *Nature* 2013; **500**:593–597.
71. Lee MT, Bonneau AR, Giraldez AJ. Zygotic genome activation during the maternal-to-zygotic transition. *Annu Rev Cell Dev Biol* 2014; **30**:581–613.
72. Kigami D, Minami N, Takayama H, Imai H. MuERV-L is one of the earliest transcribed genes in mouse one-cell embryos. *Biol Reprod* 2003; **68**:651–654.
73. Hamatani T, Carter MG, Sharov AA, Ko MSH. Dynamics of global gene expression changes during mouse preimplantation development. *Dev Cell* 2004; **6**:117–131.
74. Abe K, Yamamoto R, Franke V, Cao M, Suzuki Y, Vlahovicek K, Svoboda P, Schultz RM, Aoki F. The first murine zygotic transcription is promiscuous and uncoupled from splicing and 3' processing. *EMBO J* 2015; **34**:1523–1537.
75. Park SJ, Komata M, Inoue F, Yamada K, Nakai K, Ohsugi M, Shirahige K. Inferring the choreography of parental genomes during fertilization from ultralarge-scale whole-transcriptome analysis. *Genes Dev* 2013; **27**:2736–2748.
76. De Iaco A, Planet E, Coluccio A, Verp S, Duc J, Trono D. DUX-family transcription factors regulate zygotic genome activation in placental mammals. *Nat Genet* 2017; **49**:941–945.
77. Whiddon JL, Langford AT, Wong CJ, Zhong JW, Tapscott SJ. Conservation and innovation in the DUX4-family gene network. *Nat Genet* 2017; **49**:935–940.
78. Adenot PG, Mercier Y, Renard JP, Thompson EM. Differential H4 acetylation of paternal and maternal chromatin precedes DNA replication and differential transcriptional activity in pronuclei of 1-cell mouse embryos. *Development* 1997; **124**:4615–4625.
79. Burton A, Torres-Padilla ME. Chromatin dynamics in the regulation of cell fate allocation during early embryogenesis. *Nat Rev Mol Cell Biol* 2014; **15**:723–735.
80. Daujat S, Weiss T, Mohn F, Lange UC, Ziegler-Birling C, Zeissler U, Lappe M, Schubeler D, Torres-Padilla ME, Schneider R. H3K64 trimethylation marks heterochromatin and is dynamically remodeled during developmental reprogramming. *Nat Struct Mol Biol* 2009; **16**:777–781.
81. Kourmouli N, Jeppesen P, Mahadevhaiah S, Burgoyne P, Wu R, Gilbert DM, Bongiorni S, Prantera G, Fanti L, Pimpinelli S, Shi W, Fundele R

et al. Heterochromatin and tri-methylated lysine 20 of histone H4 in animals. *J Cell Sci* 2004; **117**:2491–2501.
82. Puschendorf M, Terranova R, Boutsma E, Mao X, Isono K, Brykczynska U, Kolb C, Otte AP, Koseki H, Orkin SH, van Lohuizen M, Peters AH. PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. *Nat Genet* 2008; **40**:411–420.
83. Flyamer IM, Gassler J, Imakaev M, Brandao HB, Ulianov SV, Abdennur N, Razin SV, Mirny LA, Tachibana-Konwalski K. Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition. *Nature* 2017; **544**:110–114.
84. Bachvarova R, De Leon V. Polyadenylated RNA of mouse ova and loss of maternal RNA in early development. *Dev Biol* 1980; **74**:1–8.
85. De Leon V, Johnson A, Bachvarova R. Half-lives and relative amounts of stored and polysomal ribosomes and poly(A)+ RNA in mouse oocytes. *Dev Biol* 1983; **98**:400–408.
86. Brower PT, Gizang E, Boreen SM, Schultz RM. Biochemical studies of mammalian oogenesis: synthesis and stability of various classes of RNA during growth of the mouse oocyte in vitro. *Dev Biol* 1981; **86**:373–383.
87. Morgan M, Much C, DiGiacomo M, Azzi C, Ivanova I, Vitsios DM, Pistolic J, Collier P, Moreira PN, Benes V, Enright AJ, O'Carroll D. mRNA 3' uridylation and poly(A) tail length sculpt the mammalian maternal transcriptome. *Nature* 2017; **548**:347–351.
88. Bachvarova R, Cohen EM, De Leon V, Tokunaga K, Sakiyama S, Paynton BV. Amounts and modulation of actin mRNAs in mouse oocytes and embryos. *Development* 1989; **106**:561–565.
89. Bachvarova R, De Leon V, Johnson A, Kaplan G, Paynton BV. Changes in total RNA, polyadenylated RNA, and actin mRNA during meiotic maturation of mouse oocytes. *Dev Biol* 1985; **108**:325–331.
90. Paynton BV, Rempel R, Bachvarova R. Changes in state of adenylation and time course of degradation of maternal mRNAs during oocyte maturation and early embryonic development in the mouse. *Dev Biol* 1988; **129**:304–314.
91. Yu J, Hecht NB, Schultz RM. Expression of MSY2 in mouse oocytes and preimplantation embryos. *Biol Reprod* 2001; **65**:1260–1270.
92. Yang J, Medvedev S, Yu J, Schultz RM, Hecht NB. Deletion of the DNA/RNA-binding protein MSY2 leads to post-meiotic arrest. *Mol Cell Endocrinol* 2006; **250**:20–24.
93. Yu J, Deng M, Medvedev S, Yang J, Hecht NB, Schultz RM. Transgenic RNAi-mediated reduction of MSY2 in mouse oocytes results in reduced fertility. *Dev Biol* 2004; **268**:195–206.
94. Medvedev S, Yang J, Hecht NB, Schultz RM. CDC2A (CDK1)-mediated phosphorylation of MSY2 triggers maternal mRNA degradation during mouse oocyte maturation. *Dev Biol* 2008; **321**:205–215.
95. Balagopal V, Fluch L, Nissan T. Ways and means of eukaryotic mRNA decay. *Biochim Biophys* 2012; **1819**:593–603.
96. Houseley J, Tollervey D. The many pathways of RNA degradation. *Cell* 2009; **136**:763–776.
97. Schoenberg DR, Maquat LE. Regulation of cytoplasmic mRNA decay. *Nat Rev Genet* 2012; **13**:246–259.
98. Yamashita A, Chang TC, Yamashita Y, Zhu W, Zhong Z, Chen CY, Shyu AB. Concerted action of poly(A) nucleases and decapping enzyme in mammalian mRNA turnover. *Nat Struct Mol Biol* 2005; **12**:1054–1063.
99. Ma J, Flemr M, Strnad H, Svoboda P, Schultz RM. Maternally recruited DCP1A and DCP2 contribute to messenger RNA degradation during oocyte maturation and genome activation in mouse. *Biol Reprod* 2013; **88**:11.
100. Ma J, Fukuda Y, Schultz RM. Mobilization of dormant Cnot7 mRNA promotes deadenylation of maternal transcripts during mouse oocyte maturation. *Biol Reprod* 2015; **93**:48.
101. Salisbury J, Hutchison KW, Wigglesworth K, Eppig JJ, Graber JH. Probe-level analysis of expression microarrays characterizes isoform-specific degradation during mouse oocyte maturation. *PLoS ONE* 2009; **4**:e7479.
102. Su YQ, Sugiura K, Woo Y, Wigglesworth K, Kamdar S, Affourtit J, Eppig JJ. Selective degradation of transcripts during meiotic maturation of mouse oocytes. *Dev Biol* 2007; **302**:104–117.
103. Nagarajan VK, Jones CI, Newbury SF, Green PJ. XRN 5'→3' exoribonucleases: structure, mechanisms and functions. *Biochim Biophys Acta* 2013; **1829**:590–603.
104. Daentl DL, Epstein CJ. Developmental interrelationships of uridine uptake, nucleotide formation and incorporation into RNA by early mammalian embryos. *Dev Biol* 1971; **24**:428–442.
105. Meyer KD, Jaffrey SR. The dynamic epitranscriptome: N6-methyladenosine and gene expression control. *Nat Rev Mol Cell Biol* 2014; **15**:313–326.
106. Yue Y, Liu J, He C. RNA N6-methyladenosine methylation in post-transcriptional gene expression regulation. *Genes Dev* 2015; **29**:1343–1355.
107. Wang Y, Li Y, Toth JI, Petroski MD, Zhang Z, Zhao JC. N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. *Nat Cell Biol* 2014; **16**:191–198.
108. Zhao BS, Wang X, Beadell AV, Lu Z, Shi H, Kuuspalu A, Ho RK, He C. m6A-dependent maternal mRNA clearance facilitates zebrafish maternal-to-zygotic transition. *Nature* 2017; **542**:475–478.
109. Ivanova I, Much C, Di Giacomo M, Azzi C, Morgan M, Moreira PN, Monahan J, Carrieri C, Enright AJ, O'Carroll D. The RNA m 6 A reader YTHDF2 is essential for the post-transcriptional regulation of the maternal transcriptome and oocyte competence. *Mol Cell* 2017; **67**:1059–1067.e4 e1054.
110. Harper JE, Miceli SM, Roberts RJ, Manley JL. Sequence specificity of the human mRNA N6-adenosine methylase in vitro. *Nucleic Acids Res* 1990; **18**:5735–5741.
111. Gross PR, Malkin LI, Moyer WA. Templates for the first proteins of embryonic development. *Proc Natl Acad Sci USA* 1964; **51**:407–414.
112. Spirin AS. “Masked” forms of mRNA. *Curr Top Dev Biol* 1966; **1**:1–38.
113. Huarte J, Belin D, Vassalli JD. Plasminogen activator in mouse and rat oocytes: Induction during meiotic maturation. *Cell* 1985; **43**:551–558.
114. Mendez R, Richter JD. Translational control by CPEB: a means to the end. *Nat Rev Mol Cell Biol* 2001; **2**:521–529.
115. Wang QT, Piotrowska K, Ciemerych MA, Milenkovic L, Scott MP, Davis RW, Zernicka-Goetz M. A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo. *Dev Cell* 2004; **6**:133–144.
116. Chen J, Melton C, Suh N, Oh JS, Horner K, Xie F, Sette C, Blelloch R, Conti M. Genome-wide analysis of translation reveals a critical role for deleted in azoospermia-like (Dazl) at the oocyte-to-zygote transition. *Genes Dev* 2011; **25**:755–766.
117. Sousa Martins JP, Liu X, Oke A, Arora R, Franciosi F, Viville S, Laird DJ, Fung JC, Conti M. DAZL and CPEB1 regulate mRNA translation synergistically during oocyte maturation. *J Cell Sci* 2016; **129**:1271–1282.
118. Yang Y, Yang C-r, Han SJ, Daldello EM, Cho A, Martins JPS, Xia G, Conti M. Maternal mRNAs with distinct 3′ UTRs define the temporal pattern of Ccnb1 synthesis during mouse oocyte meiotic maturation. *Genes Dev* 2017; **31**:1302–1307.
119. Anger M, Stein P, Schultz RM. CDC6 requirement for spindle formation during maturation of mouse oocytes. *Biol Reprod* 2005; **72**:188–194.
120. Murai S, Stein P, Buffone MG, Yamashita S, Schultz RM. Recruitment of Orc6l, a dormant maternal mRNA in mouse oocytes, is essential for DNA replication in 1-cell embryos. *Dev Biol* 2010; **341**:205–212.
121. Ducibella T, Schultz RM, Ozil JP. Role of calcium signals in early development. *Semin Cell Dev Biol* 2006; **17**:324–332.
122. Xu Z, Williams CJ, Kopf GS, Schultz RM. Maturation-associated increase in IP3 receptor type 1: role in conferring increased IP3 sensitivity and Ca2+ oscillatory behavior in mouse eggs. *Dev Biol* 2003; **254**:163–171.
123. Abbott AL, Fissore RA, Ducibella T. Identification of a translocation deficiency in cortical granule secretion in preovulatory mouse oocytes. *Biol Reprod* 2001; **65**:1640–1647.
124. Backs J, Stein P, Backs T, Duncan FE, Grueter CE, McAnally J, Qi X, Schultz RM, Olson EN. The gamma isoform of CaM kinase II controls mouse egg activation by regulating cell cycle resumption. *Proc Natl Acad Sci USA* 2010; **107**:81–86.

125. DeRenzo C, Seydoux G. A clean start: degradation of maternal proteins at the oocyte-to-embryo transition. *Trends Cell Biol* 2004; **14**: 420–426.
126. Josefsberg LB-Y. The proteasome is involved in the first metaphase-to-anaphase transition of meiosis in rat oocytes. *Biol Reprod* 2000; **62**:1270–1277.
127. Solter D, Hiiragi T, Evsikov AV, Moyer J, De Vries WN, Peaston AE, Knowles BB. Epigenetic mechanisms in early mammalian development. *Cold Spring Harb Symp Quant Biol* 2004; **69**:11–18.
128. Evsikov AV, Graber JH, Brockman JM, Hampl A, Holbrook AE, Singh P, Eppig JJ, Solter D, Knowles BB. Cracking the egg: molecular dynamics and evolutionary aspects of the transition from the fully grown oocyte to embryo. *Genes Dev* 2006; **20**:2713–2727.
129. Zhang P, Ni X, Guo Y, Guo X, Wang Y, Zhou Z, Huo R, Sha J. Proteomic-based identification of maternal proteins in mature mouse oocytes. *BMC Genomics* 2009; **10**:348.
130. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 1998; **391**:806–811.
131. Kennerdell JR, Carthew RW. Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. *Cell* 1998; **95**:1017–1026.
132. Ngo H, Tschudi K, Gull K, Ullu E. Double-stranded RNA induces mRNA degradation in *Trypanosoma brucei*. *Proc Natl Acad Sci USA* 1998; **95**:14687–14692.
133. Sanchez-Alvarado A, Newmark PA. Double-stranded RNA specifically disrupts gene expression during planarian regeneration. *Proc Natl Acad Sci USA* 1999; **96**:5049–5054.
134. Lohmann JU, Endl I, Bosch TC. Silencing of developmental genes in Hydra. *Dev Biol* 1999; **214**:211–214.
135. Wargelius A, Ellingsen S, Fjose A. Double-stranded RNA induces specific developmental defects in zebrafish embryos. *Biochem Biophys Res Commun* 1999; **263**:156–161.
136. Samuel CE. Antiviral actions of interferons. *Clin Microbiol Rev* 2001; **14**:778–809, table of contents.
137. Svoboda P, Stein P, Hayashi H, Schultz RM. Selective reduction of dormant maternal mRNAs in mouse oocytes by RNA interference. *Development* 2000; **127**:4147–4156.
138. Wianny F, Zernicka-Goetz M. Specific interference with gene function by double-stranded RNA in early mouse development. *Nat Cell Biol* 2000; **2**:70–75.
139. Stein P, Zeng F, Pan H, Schultz RM. Absence of non-specific effects of RNA interference triggered by long double-stranded RNA in mouse oocytes. *Dev Biol* 2005; **286**:464–471.
140. Svoboda P, Stein P, Schultz RM. RNAi in mouse oocytes and preimplantation embryos: effectiveness of hairpin dsRNA. *Biochem Biophys Res Commun* 2001; **287**:1099–1104.
141. Stein P, Svoboda P, Schultz R. Transgenic RNAi in mouse oocytes: a simple and fast approach to study gene function. *Dev Biol* 2003; **256**:188–194.
142. Svoboda P. RNA silencing in mammalian oocytes and early embryos. *Curr Top Microbiol Immunol* 2008; **320**:225–256.
143. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 2001; **409**:363–366.
144. Czech B, Hannon GJ. Small RNA sorting: matchmaking for Argonautes. *Nat Rev Genet* 2011; **12**:19–31.
145. Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. *Cell* 2009; **136**:642–655.
146. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol* 2009; **10**:126–139.
147. Izaurralde E. Breakers and blockers–miRNAs at work. *Science* 2015; **349**:380–382.
148. Grivna ST, Beyret E, Wang Z, Lin H. A novel class of small RNAs in mouse spermatogenic cells. *Genes Dev* 2006; **20**:1709–1714.
149. Aravin A, Gaidatzis D, Pfeffer S, Lagos-Quintana M, Landgraf P, Iovino N, Morris P, Brownstein MJ, Kuramochi-Miyagawa S, Nakano T, Chien M, Russo JJ et al. A novel class of small RNAs bind to MILI protein in mouse testes. *Nature* 2006; **442**:203–207.
150. Lau NC, Seto AG, Kim J, Kuramochi-Miyagawa S, Nakano T, Bartel DP, Kingston RE. Characterization of the piRNA complex from rat testes. *Science* 2006; **313**:363–367.
151. Girard A, Sachidanandam R, Hannon GJ, Carmell MA. A germline-specific class of small RNAs binds mammalian Piwi proteins. *Nature* 2006; **442**:199–202.
152. Watanabe T, Takeda A, Tsukiyama T, Mise K, Okuno T, Sasaki H, Minami N, Imai H. Identification and characterization of two novel classes of small RNAs in the mouse germline: retrotransposon-derived siRNAs in oocytes and germline small RNAs in testes. *Genes Dev* 2006; **20**:1732–1743.
153. Ishizu H, Siomi H, Siomi MC. Biology of PIWI-interacting RNAs: new insights into biogenesis and function inside and outside of germlines. *Genes Dev* 2012; **26**:2361–2373.
154. Iwasaki YW, Siomi MC, Siomi H. PIWI-interacting RNA: its biogenesis and functions. *Annu Rev Biochem* 2015; **84**:405–433.
155. Czech B, Hannon GJ. One loop to rule them all: the ping-pong cycle and piRNA-guided silencing. *Trends Biochem Sci* 2016; **41**:324–337.
156. Murchison EP, Stein P, Xuan Z, Pan H, Zhang MQ, Schultz RM, Hannon GJ. Critical roles for Dicer in the female germline. *Genes Dev* 2007; **21**:682–693.
157. Tang F, Kaneda M, O'Carroll D, Hajkova P, Barton SC, Sun YA, Lee C, Tarakhovsky A, Lao K, Surani MA. Maternal microRNAs are essential for mouse zygotic development. *Genes Dev* 2007; **21**:644–648.
158. Tam OH, Aravin AA, Stein P, Girard A, Murchison EP, Cheloufi S, Hodges E, Anger M, Sachidanandam R, Schultz RM, Hannon GJ. Pseudogene-derived small interfering RNAs regulate gene expression in mouse oocytes. *Nature* 2008; **453**:534–538.
159. Watanabe T, Totoki Y, Toyoda A, Kaneda M, Kuramochi-Miyagawa S, Obata Y, Chiba H, Kohara Y, Kono T, Nakano T, Surani MA, Sakaki Y et al. Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. *Nature* 2008; **453**:539–543.
160. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M. Silencing of microRNAs in vivo with 'antagomirs'. *Nature* 2005; **438**:685–689.
161. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel D, Linsley PS, Johnson JM. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature*; **433**:769–773.
162. Schmitter D, Filkowski J, Sewer A, Pillai RS, Oakeley EJ, Zavolan M, Svoboda P, Filipowicz W. Effects of Dicer and Argonaute down-regulation on mRNA levels in human HEK293 cells. *Nucleic Acids Res* 2006; **34**:4801–4815.
163. Suh N, Baehner L, Moltzahn F, Melton C, Shenoy A, Chen J, Blelloch R. MicroRNA function is globally suppressed in mouse oocytes and early embryos. *Curr Biol* 2010; **20**:271–277.
164. Yang Q, Lin J, Liu M, Li R, Tian B, Zhang X, Xu B, Liu M, Zhang X, Li Y, Shi H, Wu L. Highly sensitive sequencing reveals dynamic modifications and activities of small RNAs in mouse oocytes and early embryos. *Sci Adv* 2016; **2**:e1501482–e1501482.
165. Wang Y, Medvid R, Melton C, Jaenisch R, Blelloch R. DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nat Genet* 2007; **39**:380–385.
166. Stein P, Rozhkov NV, Li F, Cardenas FL, Davydenko O, Vandivier LE, Gregory BD, Hannon GJ, Schultz RM. Essential role for endogenous siRNAs during meiosis in mouse oocytes. *PLoS Genet* 2015; **11**:e1005013.
167. Flemr M, Malik R, Franke V, Nejepinska J, Sedlacek R, Vlahovicek K, Svoboda P. A retrotransposon-driven dicer isoform directs endogenous small interfering RNA production in mouse oocytes. *Cell* 2013; **155**:807–816.
168. Cook MS, Blelloch R. Small RNAs in germline development. *Curr Top Dev Biol* 2013; **102**:159–205.
169. Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. *Nature* 2012; **482**:339–346.

170. Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet* 2016; **17**:47–62.
171. Ulitsky I, Bartel DP. lincRNAs: genomics, evolution, and mechanisms. *Cell* 2013; **154**:26–46.
172. Karlic R, Ganesh S, Franke V, Svobodova E, Urbanova J, Suzuki Y, Aoki F, Vlahovicek K, Svoboda P. Long non-coding RNA exchange during the oocyte-to-embryo transition in mice. *DNA Res* 2017; **24**:219–220.
173. Veselovska L, Smallwood SA, Saadeh H, Stewart KR, Krueger F, Maupetit-Mehouas S, Arnaud P, Tomizawa S, Andrews S, Kelsey G. Deep sequencing and de novo assembly of the mouse oocyte transcriptome define the contribution of transcription to the DNA methylation landscape. *Genome Biol* 2015; **16**:209.
174. Yan L, Yang M, Guo H, Yang L, Wu J, Li R, Liu P, Lian Y, Zheng X, Yan J, Huang J, Li M et al. Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. *Nat Struct Mol Biol* 2013; **20**:1131–1139.
175. Zhang K, Huang K, Luo Y, Li S. Identification and functional analysis of long non-coding RNAs in mouse cleavage stage embryonic development based on single cell transcriptome data. *BMC Genomics* 2014; **15**:845.
176. Kaneda M, Tang F, O'Carroll D, Lao K, Surani MA. Essential role for Argonaute2 protein in mouse oogenesis. *Epigenet Chromatin* 2009; **2**:9.
177. Hamazaki N, Uesaka M, Nakashima K, Agata K, Imamura T. Gene activation-associated long noncoding RNAs function in mouse preimplantation development. *Development* 2015; **142**:910–920.
178. Wang J, Li X, Wang L, Li J, Zhao Y, Bou G, Li Y, Jiao G, Shen X, Wei R, Liu S, Xie B et al. A novel long intergenic noncoding RNA indispensable for the cleavage of mouse two-cell embryos. *EMBO Rep* 2016; **17**:1452–1470.