Published in final edited form as:

Mol Reprod Dev. 2009 September ; 76(9): 805–818. doi:10.1002/mrd.21038.

Gene expression during the oocyte-to-embryo transition in mammals

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Abstract

The seminal question in modern developmental biology is the origins of new life arising from the unification of sperm and egg. The roots of this question begin from 19th-20th century embryologists studying fertilization and embryogenesis. Although the revolution of molecular biology has yielded significant insight into the complexity of this process, the overall orchestration of genes, molecules, and cells, is still not fully formed. Early mammalian development, specifically the oocyte-to-embryo transition, is essentially under “maternal command” from factors deposited in the cytoplasm during oocyte growth, independent of *de novo* transcription from the nascent embryo. Many of the advances in understanding this developmental period occurred in tandem with application of new methods and techniques from molecular biology, from protein electrophoresis to sequencing and assemblies of whole genomes. From this bed of knowledge, it appears that precise control of mRNA translation is a key regulator coordinating the molecular and cellular events occurring during oocyte-to-embryo transition. Notably, oocyte transcriptomes share, yet retain some uniqueness, common genetic motifs among all chordates. The common genetic motifs typically define fundamental processes critical for cellular maintenance, whereas the unique genetic features may be a source of variation and a substrate for sexual selection, genetic drift, or gene flow. One purpose for this complex interplay among genes, proteins, and cells may allow for evolution to transform and act upon the underlying processes, at molecular, structural and organismal levels, to increase diversity, which is the ultimate goal of sexual reproduction.

Keywords

embryonic genome activation; gene duplication; maternal effect gene; translational control; reproductive isolation

I am strongly inclined to suspect that the most frequent cause of variability may be attributed to the male and female reproductive elements having been affected prior to the act of conception.

Charles Darwin, *The Origin of Species*

I. Introduction

The origin of individual life, fertilization, along with its causal and consequential molecular and cellular events, arguably remains one of the focal fields in modern developmental biology. Indeed, the fundamental issue of *cytoplasmic control of nuclear function in early development* (DiBerardino 1979; Gurdon and Woodland 1968) remains as essential as it was in the times of Boveri, Spemann, Briggs and other founding fathers of modern embryology

originating from the early 20th century. Setting aside the phenomenal discoveries of the recent decades and dissection of critical components and pathways in the system, the whole ‘unified’ picture remains elusive.

Some of the major reasons for this setback in major conceptual advance are (i) the (unexpected) complexity of a seemingly simple biological system, coupled with (ii) over-optimistic expectations that seminal discoveries (e.g., mammalian cloning) will translate into new paradigms, as well as (iii) the ungrounded shifts in the “community perception” of key biological concepts (e.g., “*ontogeny recapitulates phylogeny*”) from the ever-changing domains of *theories* to the firm grounds of *axioms*. In this review, we will focus mainly on one aspect of the complex mechanisms governing early mammalian development, the regulation of gene expression at the onset of oocyte maturation and into the early stages of development known as the oocyte-to-embryo transition (OET). We will also discuss the (ii) and (iii) points, as we believe that OET provides excellent, as well as enlightening examples on how dogmatism impedes scientific advance.

II. Gene expression during OET: a historical perspective

In metazoans, maternal factors accumulated in the ooplasm during the growth period of the egg are solely responsible for the control of maturation, fertilization and initial development of the newly formed embryo. In most non-mammalian species studied, the first nuclear divisions proceed rapidly and the ooplasm is programmed to progress through the first morphogenetic events without the need for *de novo* transcription from the embryonic genome (Davidson 1986). In contrast, the first cell cycles of a newly formed mammalian embryo are quite prolonged, with no differentiation events occurring prior to activation of the embryonic genome. For example, the developmental transition from oocyte maturation to embryonic genome activation occupies 40–44 hours, or about 8% of *Mus musculus* prenatal development (Braude et al. 1979; Howlett and Bolton 1985). The past decade of somatic cell nuclear transfer experiments to generate genetic clones of different mammalian species underscores that the oocyte cytoplasm contains factors pivotal for the remodeling of introduced nuclei. In mice, nuclear reprogramming must be completed by the end of the second cell cycle, the time when the *M. musculus* embryonic genome is completely activated, to produce viable embryos.

The studies of mammalian oocytes and embryos relied heavily, and evolved hand-in-hand with development and implementation of milestone molecular techniques. Indeed, the invention of SDS-PAGE electrophoresis by Laemmli (Laemmli 1970) spread quickly to study protein expression patterns in the oocytes and embryos (Braude et al. 1979; Eppig and Eckhardt 1976; Golbus and Stein 1976; Howlett and Bolton 1985; Petzoldt and Hoppe 1980; Petzoldt et al. 1981; Van Blerkom 1981; Van Blerkom and Brockway 1975; Van Blerkom and Manes 1974); further improvements in protein separation techniques such as 2D electrophoresis (O’Farrell 1975) and principally, the introduction of qualitative and quantitative tools for 2D electrophoregram analysis (Garrels 1983; Garrels 1989) had a similarly enthusiastic response from the embryological community (Bensaude et al. 1983; Christians et al. 1995; Evsikov and Solomko 1999; Howe and Solter 1979; Latham et al. 1991a; Latham et al. 1994; Latham et al. 1991b; Levinson et al. 1978; Richoux et al. 1991; Sanchez and Erickson 1985; Schultz and Wassarman 1977b; Shi et al. 1994). However exciting, at the time these techniques carried a major caveat: with an exception of a few known markers, the establishment of *protein identities* was essentially impossible. For these and other reasons, the cDNA library technology (Dworkin and Dawid 1980; Okayama and Berg 1982; Okayama and Berg 1983; Rougeon and Mach 1976), once optimized for microgram amounts of harvested mRNA (Okayama and Berg 1982) quickly became the dominant technique to capture and study gene expression patterns in oocytes and early embryos.

While the first attempts to construct cDNA libraries of mammalian oocytes and embryos from miniscule amounts of starting material were not very successful (Taylor and Piko 1987), optimization of the techniques lead to the production of representative *M. musculus* cDNA libraries (Rothstein et al. 1992) which are now the “gold standard” among high-quality mammalian oocyte and early embryonic libraries (Evsikov et al. 2006). Application of subtractive hybridization methods to these libraries (Sive and St John 1988) permitted production of specialized cDNA libraries enriched in stage-specific transcripts (Rothstein et al. 1993), and ultimately, isolation and characterization of genes differentially expressed during early mammalian development (Hwang et al. 1996; Hwang et al. 1997; Hwang et al. 1999; Oh et al. 1997). A modification of subtraction technique, known as suppression subtractive hybridization, SSH (Diatchenko et al. 1996) has been widely used to construct ‘stage-specific’ cDNA libraries for pre-implantation embryos of several mammalian species, including *Homo sapiens* (Bui et al. 2005; Leandri et al. 2009; Mohan et al. 2002; Morozov et al. 1999).

The next advance towards deciphering and enumeration of gene expression in oocytes and embryos came with advances in automated DNA sequencing, which enabled implementation of the large-scale Mouse Expressed Sequence Tag (EST) project and obtaining sequence information for tens of thousands of randomly picked cDNA clones from oocyte and pre-implantation embryo libraries (Marra et al. 1999). To date, this and similar projects (i.e., (Carninci et al. 2005; Kawai et al. 2001; Ko et al. 2000; Okazaki et al. 2002) provided sequence information for approximately 210,000 ESTs from oocytes and pre-implantation embryos of *M. musculus* alone. To facilitate the studies of changes in gene expression and move away from time-consuming procedures of SSH and subtracted cDNA libraries, the microarray technology developed in the 1990s – early 2000s (Fodor et al. 1993; Kargul et al. 2001; Lipshutz et al. 1999; Lockhart et al. 1996; VanBuren et al. 2002) proved very useful. Indeed, by now it is the most popular technique to study the dynamic changes of gene expression during early development (for recent reviews, see (Adjaye 2005; Aiba et al. 2006; Evans et al. 2008). At the same time, expansion of the genome sequencing projects for other chordate model organisms, together with production and representative sequencing of cDNA libraries from eggs or ovaries for these species permitted to add a second – phylogenetic – dimension to the studies of the oocyte cytoplasm’s role in early development (Evsikov et al. 2006; Evsikov and Marín de Evsikova 2009). Finally, recent developments in sensitivity of the mass spectrometry technology permitted establishment of peptide identities for individual spots on the 2D electrophoregrams of minute amounts of cell lysates, making feasible and reinvigorating research in the oocyte and pre-implantation embryo proteomics (Calvert et al. 2003; Coonrod et al. 2004; Coonrod et al. 2002; Hao et al. 2002; Ma et al. 2008; Vitale et al. 2007).

III. Study of the transcriptome during OET

As noted above, vast amounts of EST sequence data for the oocytes and pre-implantation embryos provided opportunity to establish identities of genes expressed during this critical period of development. The first published studies of oocyte and embryo transcriptome dynamics using EST analysis (Ko et al. 2000; Sasaki et al. 1998) had several caveats, such as relatively small amounts of annotated sequences in GenBank, lack of *Mus musculus* genome assembly, and relatively “primitive” data mining tools for unambiguous establishment of gene identities. Expansion of GenBank sequence data via projects like Mammalian Gene Collection (Gerhard et al. 2004; Strausberg et al. 2002; Strausberg et al. 1999) and RIKEN Mouse Gene Encyclopaedia Project (Kawai et al. 2001; Okazaki et al. 2002), massive expansion of the Mouse Genome Database effort to annotate all *M. musculus* genes (Blake et al. 2009; Nadeau et al. 1995), as well as publication of publicly available *M. musculus* genome assembly (Waterston et al. 2002) and development of genome navigation and mining tools such as ENSEMBL (Hubbard et al. 2002) allowed to increase the rate of precise gene identification in later studies. Indeed, in the “early post-genome era” gene identities could have been established

for 71% EST sequences from the 2-cell stage *M. musculus* embryo cDNA library (Evsikov et al. 2004), while just two years later, a methodologically identical analysis of the fully-grown *M. musculus* oocyte cDNA library established identities of 91% ESTs (Evsikov et al. 2006).

One of the most critical issues affecting the quality of EST-based gene expression analysis is the method used for cDNA library preparation. Due to low quantity of mRNA in mammalian oocytes and pre-implantation embryos, linear amplification of cDNA by PCR is often used to overcome this problem, especially in the studies of *H. sapiens* embryos (Adjaye et al. 1999; Adjaye et al. 1997; Adjaye et al. 1998; Goto et al. 2002; Serafica et al. 2005). While this technique is appropriate in the cases when the biological material is scarce, comparison of *M. musculus* oocyte and embryonic cDNA libraries generated using the PCR amplification step with those prepared by direct cloning reveals the superiority of the latter method in the preservation of original diversity and relative abundance of transcripts (Evsikov et al. 2006).

A key feature of the *M. musculus* oocyte and early embryo transcriptomes is the under-representation of “housekeeping” gene transcripts during OET. In fact, “normal” levels of housekeeping gene expression in *M. musculus* are established only by the blastocyst stage, and despite widespread unsubstantiated beliefs of the opposite, under-representation of housekeeping transcripts are also observed in the oocytes of African clawed frog *Xenopus laevis* as well (Evsikov et al. 2006). Arguably, this aspect of transcriptome reflects the uniqueness of the oocyte as a “reprogramming machine” designed to create a totipotent embryo. Among other intriguing characteristics of the *M. musculus* transcriptome dynamics during OET is profuse expression of the oocyte-specific genes (Table 1), high representation of novel “variants”, such as retrogenes and duplicated genes, and abundant usage of alternative promoters such as Long Terminal Repeats (LTRs) of retrotransposons to drive gene expression (Evsikov et al. 2004; Peaston et al. 2004). These enigmatic aspects of the oocyte biology are discussed in the last sections of this review.

As noted above, microarray analysis of gene expression has recently become a method of choice to study transcriptome dynamics during mammalian OET and pre-implantation embryogenesis. Particularly, development of commercial high-density, easy-to-use and relatively low-cost arrays like Affymetrix GeneChips opened the avenues not only to study changes in gene expression patterns in normal *M. musculus* development (Wang et al. 2004), but more importantly, in experimental systems such as oocytes and embryos harboring mutations in candidate fertility or maternal effect genes (Hao et al. 2009; Tang et al. 2007; Wan et al. 2008). Moreover, both commercial and “custom” arrays are now used in other mammalian species as well. Indeed, because embryonic genome activation in *Bos taurus* (bovine) and *Oryctolagus cuniculus* (rabbit) embryos occurs later than in the *M. musculus*, these agriculturally important species may more closely model *H. sapiens* OET and preimplantation development (Duranthon et al. 2008). For these reasons, studies of *O. cuniculus* and *B. taurus* OET and preimplantation development (Kues et al. 2008; Marjani et al. 2009; Vigneault et al. 2009), as well as the effects of *in vitro* maturation, fertilization and culture on the transcriptomes of *B. taurus* oocytes and embryos (Katz-Jaffe et al. 2009; Smith et al. 2009) are rapidly expanding. Moreover, microarrays have been used in studies aimed to uncover the effects of somatic nuclei reprogramming in cloned *B. taurus* embryos (Beyhan et al. 2007; Everts et al. 2008).

As any complex technological platform, microarray analysis has a number of caveats, and we will talk about only those pertinent to the studies of oocytes and embryos. Firstly, contrary to the common belief, microarrays *by definition* are *not* the tool for gene discovery, because individual probes on the arrays represent *known* (or sometimes putative) transcripts. Only the study of the transcriptome through direct sequence analysis, such as analysis of ESTs, is a valid tool for mining for novel expressed loci or transcripts during OET (Peaston et al. 2004).

Secondly, while microarrays are a perfect tool for validation and quantification of changes in *individual* gene expression across biological samples, they are unsuitable to quantify the transcript ratios *among* individual genes. Thirdly, due to the possibility of cross-hybridization, microarray technology is not the best tool to study gene families that have greater than 95% sequence identity among individual members and which are abundantly represented during OET (Evsikov et al. 2004; Evsikov et al. 2006). However, the most important caveats arise with experimental design and interpretation of microarray data. For example, mRNA content of the 2-cell stage *M. musculus* embryo, 0.26 pg, is almost four times lower than 0.95 pg of mRNA in the fully-grown oocyte (Piko and Clegg 1982) (Figure 1A). This reflects massive degradation of maternal mRNAs during OET discussed in the next section. The designs of microarray experiments usually employ *equal amounts of mRNA* from different biological samples. However, this methodology is unacceptable for oocytes and early embryos. In a putative experiment comparing the transcriptomes of fully-grown oocytes and 2-cell stage embryos normalization to the amount of mRNA would result in false “overexpression” of stable mRNAs (Figure 1B), and false “stability” of degrading mRNAs (Figure 1D). For this reason, only normalization to the number of oocytes and embryos (Figures 1C, 1E) results in the correct physiological representation of transcriptome dynamics (Su et al. 2007). Another important consideration often overlooked in the microarray studies of OET is differential polyadenylation of maternal transcripts. Indeed, common use of T7-oligo(dT) primer in the first step of sample preparation results in complex and un-interpretable gene expression patterns, due to selective bias for representation of mRNAs with longer poly(A) tails; however, usage of internally-priming oligonucleotides such as Full Spectrum™ MultiStart Primers overcomes this obstacle as well (Su et al. 2007).

IV. Regulatory mechanisms of OET

Commencing at oocyte maturation, and during the subsequent transcriptionally silent stages of development, three major mechanisms contribute to the dynamic changes that occur in the ooplasm: (i) the timely translation of stored maternal transcripts provides the cytoplasm with new proteins, (ii) post-translational modification of existing and/or newly synthesized proteins determines the exact timing of events during this period, and (iii) the machinery involved in degradation of proteins and mRNAs removes the no longer needed molecules. Indeed, early studies of specific changes in the patterns of synthesized proteins during oocyte maturation (Schultz and Wassarman 1977a; Schultz and Wassarman 1977b), demonstrated the necessity of protein synthesis for meiotic progression beyond premetaphase I (Schultz and Wassarman 1977a). Similarly, the adverse effects of protein phosphorylation inhibitors (Rime et al. 1989) and inhibitors of the major cellular protein-degrading organelle proteasome (Josefsberg et al. 2000; Josefsberg et al. 2001; Solter et al. 2004) imply the global involvement of these mechanisms from the onset of OET. Later studies, focused on the interplay among synthesis and degradation of cyclin B and phosphorylation status of p34^{cdc2} to orchestrate the oscillations in M-phase promoting factor (MPF) activity during oocyte maturation (Hampl and Eppig 1995) provided examples and understanding of particular molecular pathways affected by these three global mechanisms. Here, we will specifically focus on the mechanisms of mRNA translational recruitment and turnover during OET. Indeed, in the absence of *de novo* transcription, timely recruitment of mRNA for translation, as well as differential message stability, are the key governing mechanisms creating the dynamic changes in the molecular environment of the cytoplasm (Oh et al. 2000).

During oocyte growth, many transcribed mRNAs are de-adenylated and stored in the ooplasm for subsequent translation. During translational activation, their limited 3' poly(A) tails lengthen (Bachvarova 1992), a sign that active translation is occurring (Richter 1999). Half of the poly(A) mRNA found in the fully-grown oocyte is de-adenylated, or degraded, during maturation, and by the 2-cell stage, the embryo contains less than 30% original amount of

adenylated mRNAs found in the egg (Piko and Clegg 1982). A large number of maternal mRNAs degraded during the early phase of OET are ones with pivotal functions during oocyte growth and most likely have been retained in the ooplasm due to some unknown mechanism (s) for global inhibition of mRNA degradation (Evsikov et al. 2006). For example, mRNAs for structural genes of *zona pellucida* (*Zp1*, *Zp2* and *Zp3*) are highly abundant in fully-grown oocytes (Evsikov et al. 2006) but are not translated (Bleil and Wassarman 1980) and after maturation become virtually undetectable in ovulated, metaphase II (MII)-arrested oocytes. However, stable maternal mRNAs that are translated during OET are regulated by interacting with a number of RNA-binding regulatory proteins or other factors. Several seminal *cis*- and *trans*-acting factors involved in these interactions are discussed below.

Polyadenylation of dormant mRNAs

In the oocytes of *X. laevis*, as well as *M. musculus*, mRNAs that are translated in a time-dependent fashion contain certain “sequence signatures” in their 3'-untranslated regions (UTRs) (Fox et al. 1989; Fox and Wickens 1990; McGrew et al. 1989; Vassalli et al. 1989). These U-rich motifs, known as cytoplasmic polyadenylation elements, CPEs (consensus sequence is UUUUUAU), are located from several to about 120 nucleotides upstream of the nuclear polyadenylation signal AAUAAA in the *M. musculus* (Oh et al. 2000). The regulatory CPE-binding protein (CPEB), and several proteins with which it interacts, have been characterized in *X. laevis* and *M. musculus* (Hake and Richter 1994; Hodgman et al. 2001; Mendez et al. 2000; Stebbins-Boaz et al. 1999). Essentially, CPEB in its non-phosphorylated form binds to CPE and prevents, via interactions with other proteins, polyadenylation of mRNA (Figure 2A). Phosphorylation of CPEB triggers bound mRNA polyadenylation and its consequent translation (for recent reviews of this and related mechanisms, see (Richter and Sonenberg 2005; Vardy and Orr-Weaver 2007). Similar regulation of mRNA recruitment for translation is found in the synapto-dendritic compartment of neurons (Wells et al. 2000), suggesting the existence of a common mechanism for rapid molecular changes in either transcription-void systems, such as oocytes, or in cellular compartments located too far from a nucleus to employ typical translational activation mechanisms to be effective, such as in neurons. CPEB plays an essential role throughout oogenesis, since CPEB-knockout female mice lack oocytes due to meiosis failure in germ cells at the pachytene stage (Tay and Richter 2001), while conditional depletion of *Cpeb* mRNA in growing follicles results in grossly abnormal oocyte development (Racki and Richter 2006). The general nature of CPEB-mediated mRNA silencing/re-activation is supported by the existence of homologous proteins with similar functions across metazoans (Chang et al. 1999; Minshall et al. 1999). However, other alternative models for CPEB-dependent mRNA repression in oocytes (e.g., Figure 2B), as well as existence of multiple *Cpeb* paralogs in mammalian genomes (*Cpeb1* – *Cpeb4*), three of which are expressed in the oocytes, add a layer of complexity on CPEB-dependent translational regulation.

Two additional motifs have been identified in the 3'UTRs of the messages stored in *X. laevis* eggs, the “embryonic CPEs” (eCPEs; [U]_{10–18} or [C]_{10–18} tracts). mRNAs containing these motifs are recruited for translation only after fertilization (Paillard et al. 2000; Simon et al. 1992; Wu et al. 1997). eCPEs may be located up to several hundred nucleotides upstream of the poly(A) signal. Their known respective binding factors are *X. laevis* elrA, a member of the highly conserved ELAV family of RNA binding proteins, whose likely counterparts in mammalian oocytes are ELAVL1 and ELAVL2 proteins (Evsikov et al. 2004), and a 42 kDa protein, a homolog of mammalian poly(rC)-binding protein 2 (PCBP2), also highly expressed throughout OET in mammals (Evsikov et al. 2004; Evsikova et al. 2006). The high level of sequence conservation of these proteins among vertebrates suggests that mechanisms of mRNA recruitment for translation during OET are quite ancient and well conserved, despite significant differences in the mode of reproduction (i.e. oviparity vs. viviparity).

Factors affecting mRNA de-adenylation, stability and degradation

De-adenylation is a common mechanism for halting translation, and is often the first step in the degradation of mRNA. At least three distinct pathways are known to be involved in mRNA de-adenylation in oocytes and early embryos. Biochemical dissections of these pathways were mostly studied using *X. laevis* oocytes. The first pathway, “default” de-adenylation in maturing *X. laevis* oocytes, is relatively slow, and, as shown by microinjection of specific antibodies, is achieved by de-adenylating nuclease PARN (Korner et al. 1998). The second mechanism for “targeted” de-adenylation relies on the presence of AU-rich elements (AREs) in the 3'UTR of certain mRNAs. AREs were originally identified as motifs responsible for destabilization and rapid degradation of mRNAs in mammalian somatic cells (Chen and Shyu 1995). However, in oocytes and early embryos these elements seem to be responsible for fast de-adenylation, but not necessarily degradation, of mRNAs (Voeltz and Steitz 1998). AREs have been classified into three types on the basis of their sequence. Type I AREs have 1–3 copies of an AUUUA motif within a U-rich region; type II is characterized by at least two overlapping copies of an UUAUUUA(U/A)(U/A) motif, also within a U-rich region; type III AREs contain varying repeats of [U(A/G)]_n stretches, again in the context of the U-rich region of the UTR. Interestingly, in somatic cells ELAVL1, a potential eCPE binding protein (see above), binds to mRNAs that contain type I/II AREs and protects them from degradation but not de-adenylation (Fan and Steitz 1998; Peng et al. 1998). The list of proteins that have an effect on the stability and dynamics of ARE-containing mRNAs in oocytes and early embryos is constantly growing and include CUGBP1 (Paillard et al. 2002), ePAB (Voeltz et al. 2001), CCR4b/CNOT6L (Morita et al. 2007) and C3H-4/ZFP36L2 (Belloc and Mendez 2008). Genes encoding orthologs of these proteins are expressed in mammalian oocytes and early embryos as well (Evsikov et al. 2004; Evsikov et al. 2006; Ramos et al. 2004; Seli et al. 2005). Moreover, *Cugbp1*-null females are infertile (Kress et al. 2007), while *M. musculus* embryos from females homozygous for truncated *Zfp36l2* allele do not develop beyond the 2-cell stage (Ramos et al. 2004), pinpointing an important role for these proteins in the mammalian OET. A third mechanism for mRNA de-adenylation, occurring after mid-blastula transition in *X. laevis*, is α -amanitin sensitive and thus, depends on embryonic transcription (Audic et al. 2001; Audic et al. 2002). Specifically, maternal mRNAs for cyclin A1 and cyclin B2 are de-adenylated and degraded only after the initiation of zygotic transcription; interestingly, AREs present in the 3'UTRs regions of these mRNAs are dispensable for de-adenylation and thus other motifs in the 3'UTRs regulate this mRNA destabilization. Overall, these data are consistent with the observations of certain co-occurring sequence motifs found in the 3'UTRs of multiple stable maternal mRNAs and suggest the “combinatorial code” allowing precise spatio-temporal regulation of mRNAs during OET (Evsikov et al. 2006; Padmanabhan and Richter 2006; Pique et al. 2008).

Antisense and small RNA regulation of maternal transcripts

Recently, it has been shown that maternal microRNAs (miRNAs) are essential for OET in zebrafish, *Danio rerio* (Giraldez et al. 2006), and in *M. musculus* (Murchison et al. 2007; Tang et al. 2007). In particular, *D. rerio* miR-430 miRNA is responsible for de-adenylation and targeted degradation for a multitude of maternal messages (Giraldez et al. 2006). Another class of regulatory RNAs involved in the regulation of gene expression and mRNA turnover in oocytes are “small interfering RNAs” (siRNAs) (Tam et al. 2008; Watanabe et al. 2008). While miRNAs are encoded by their own loci and usually target a number of different mRNAs, the siRNAs are produced from long double-stranded RNAs (dsRNAs) and have more specific targets. In oocytes, the sources for sense strand of dsRNAs are mRNAs of cellular genes, while the antisense RNA strands are provided by retrogene inserts transcribed in “reverse” orientation, or antisense transcripts of cellular genes, such as *Suv39h1* antisense transcript; ESTs for both types were described in the oocyte cDNA library (Evsikov et al. 2006). Key proteins involved in miRNA and siRNA pathways during OET are DICER1 and AGO2/

EIF2C2 (Tam et al. 2008; Watanabe et al. 2008); indeed, loss of *Dicer1* in growing oocytes results in female sterility linked to abnormal oogenesis (Murchison et al. 2007; Tang et al. 2007). For recent review of this burgeoning field, see (Ghildiyal and Zamore 2009).

V. Phylogenetic aspects: conservation vs. uniqueness of oocyte transcriptomes

It has been well recognized for some time that eutherian mammals are quite unique with respect to early development, which does not rely, unlike most other metazoans, on antero-posterior and dorso-ventral axis specification during oogenesis or after fertilization, and that blastomeres of early mammalian embryos are highly regulative (Davidson 1990; Davidson 1991; Evsikov et al. 1994; Hiiragi and Solter 2004; Motosugi et al. 2005; Motosugi et al. 2006). This and other features of mammalian oocytes and early embryos, such as minute amounts of yolk, are likely attributed to viviparity, as embryos receive nutrition directly from mothers upon implantation. Indeed, eggs of placental viviparous reptiles are similar in morphology to mammalian oocytes (Blackburn et al. 1984; Gomez and Ramirez-Pinilla 2004; Hernandez-Franyutti et al. 2005). Despite this uniqueness, 80% of the genes expressed in the oocytes of *M. musculus* are also transcribed in the eggs from phylogenetically distant chordates such as *X. laevis* and sea squirt *Ciona intestinalis* (Evsikov et al. 2006). Similar results were reported in comparative genomics studies of *B. taurus*, *M. musculus* and *X. laevis* oocyte transcriptomes using custom multi-species or NIA 22K mouse cDNA microarrays (Vallee et al. 2008; Vallee et al. 2006). Remarkably, the “most conserved” subset of the *M. musculus* oocyte transcriptome overwhelmingly represents mRNAs stable throughout and thus required for the OET (Evsikov et al. 2006). Additional support for our interpretation arises from recent microarray studies of OET and preimplantation development in *B. taurus* (Kues et al. 2008). On the other hand, expression of genes whose transcripts rapidly disappear at the onset of *M. musculus* OET is the most divergent. Moreover, a substantial proportion of these genes underwent rapid molecular diversifications such as gene duplications (Figure 3), exaptation of retrogenes, or functional inactivation (Evsikov et al. 2004; Evsikov et al. 2006). Additionally, these “rapidly evolving” genes within the *M. musculus* oocyte transcriptome tend to be oocyte-specific, i.e. expressed *only* in oocytes, and often species-specific, i.e. present *only* in *M. musculus* genome. A disproportionate number of maternal effect genes identified to date in *M. musculus* also belong to this group (e.g., *Mater/Nlrp5* (Tong et al. 2000), *Oas1d* (Yan et al. 2005), and others), implying neofunctionalization as a strong evolutionary force behind duplications of oocyte-specific genes. These data imply phylogenetic plasticity of the mechanisms mediating oogenesis and may reflect, for example, the requirement for fast reproductive adaptation to a new ecological niche during speciation. At the same time, evolution of OET *per se* rests upon a stable molecular foundation of conserved gene interactions.

VI. Coda: oocytes as a playground of evolution

Analysis of oocyte transcriptomes gives hints about the processes underlying the cornerstones of biological evolution, specifically gametic selection and reproductive isolation. For instance, it has been established that genes associated with reproduction are subjected to strong selective pressure (Swanson and Vacquier 2002; Swanson et al. 2001). A general explanation for this phenomenon is that it serves as a primary mechanism attaining reproductive isolation during speciation. For example, the *zona pellucida* genes, which encode sperm receptors required for fertilization, are among the most rapidly evolving genes in mammals; in some instances, such as *M. musculus* *Zp4*, the “fast-paced” molecular evolution ultimately lead to functional elimination of the gene (Evsikov et al. 2006; Goudet et al. 2008). The rise of the oocyte-specific gene families, such as *Rfp14* and *Nlrp* (Figure 3), may reflect the other side of the same process: new genes arise by duplication, acquire a unique function, which facilitates reproductive isolation, thereby providing a substrate for natural selection to act upon and result in speciation.

Overall, this idea, which is obtaining more experimental and comparative genomics support (Bikard et al. 2009; Clark et al. 2007; Evsikov et al. 2006; Evsikov and Marín de Evsikova 2009; Semon and Wolfe 2007), implies that when it comes to the role of oocyte in early development, even subtle phylogenetic differences may play as big of a role as implied “evolutionary conserved mechanisms” that have been governing the field of experimental molecular embryology.

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Figure 1. Degradation of maternal mRNA during the oocyte-to-embryo transition: implications for interpretation of microarray data

Illustration of experimental bias commonly introduced in microarray and real-time PCR experiments by normalization to equal amount of starting RNA material. (A) Relative to oocytes, RNA levels are dramatically reduced in embryos, due to degradation of maternal transcripts during OET. Two cases of a “very stable” mRNA (i.e., which are not degraded during OET at all) and of an “average unstable” mRNA (i.e., which degrades at the same rate as “bulk” mRNA content) are illustrated. (B & D) When normalized to equal amounts of RNA, change in transcript ratio leads to the opposite interpretation of data – the levels of stable mRNA are “upregulated”, while those for unstable mRNA are unchanged. (C & E) Normalization to equal numbers of oocytes and embryos maintains the difference in absolute starting RNA material and preserves the appropriate change ratio for both stable and unstable transcripts.

Figure 2. Two models for CPEB-dependent translational repression

(A) In the “classic” model of translational control during OET, translation is repressed by tethering the mRNA cap-bound eukaryotic translation initiation factor 4E (eIF4E) to a “repressive” complex which includes Maskin and CPEB; this prevents poly(A) tail elongation required for translational initiation (Richter and Sonenberg 2005). (B) An alternative model for translational repression, occurring in *Tetrapoda* and at least some *Actinopterygii* (e.g., *Danio rerio*), involves direct sequestration of CPEB by a novel eIF4E1b via eIF4E transporter, 4E-T/iIF4ENIF. In this model, eIF4E1b, which has no detectable mRNA cap-binding activity, acts as a “direct” suppressor of CPEB-dependent poly(A) tail elongation in growing oocytes (Evsikov and Marín de Evsikova 2009; Minshall et al. 2007; Robalino et al. 2004; Standart and Minshall 2008).

Figure 3. Genomic duplications of oocyte-expressed genes: a mechanism for rapid tune-up of the oocyte-to-embryo transition program in phylogenesis

The evidence for rapid diversification of oocyte-specific genes comes with comparative analysis of the regions of “unbalanced synteny” among various mammals. In the example provided, comparison of syntenic *M. musculus* and *H. sapiens* regions on chromosomes 7 and 19, respectively, demonstrate expansion of the oocyte-specific *NLRP* and *RFPL4* gene families in *H. sapiens*. Typically, while the orthology and orientation of flanking loci in such cases is well preserved (exemplified here by *Epn1/EPN1* genes on the left and *Zfp787/ZNF787* and *Zfp444/ZNF444* genes on the right), unequal expansion of gene families among different mammalian lineages leads to unequal numbers of individually essential oocyte-specific genes (Evsikov et al. 2006).

Table 1

The highest expressed genes in *M. musculus* fully-grown oocytes (from (Evsikov et al. 2006)). TPM, transcripts per million. Asterisks denote genes whose expression is restricted to oocytes.

Gene symbol	Abundance, TPM	Description	Function in oocytes	Mutant phenotype	References
<i>Zhed3</i>	9,600	zinc finger, BED domain containing 3	Unknown	N/A	N/A
<i>Gdf9*</i>	4,400	growth differentiation factor 9	Oocyte-to-cumulus cell signaling	Female infertility	(Dong et al. 1996; Su et al. 2008; Su et al. 2004)
<i>Eif4e1b*</i>	4,000	eukaryotic translation initiation factor 4E family member 1B	Translational repressor	N/A	(Evsikov and Marín de Evsikova 2009; Minshall et al. 2007)
<i>AU022751*</i>	3,900	expressed sequence AU022751	Unknown	N/A	N/A
<i>H1foo*</i>	3,600	H1 histone family, member O, oocyte-specific	Chromatin organization	N/A	(Furuya et al. 2007; Tanaka et al. 2001)
<i>Ubb</i>	3,600	ubiquitin B	Targeted protein degradation	Female and male infertility; adult-onset obesity	(Ryu et al. 2008a; Ryu et al. 2008b)
<i>Spin1</i>	3,500	spindlin 1	Cell cycle	N/A	(Oh et al. 1998; Oh et al. 1997; Yuan et al. 2008)
<i>Tle6*</i>	3,400	transducin-like enhancer of split 6	Unknown; forms a protein complex with NLRP5/MATER and OOE/FLOPED	N/A	(Li et al. 2008)
<i>Bmp15*</i>	3,400	bone morphogenetic protein 15	Oocyte-to-cumulus cell signaling	Female infertility	(Galloway et al. 2000; Su et al. 2008; Su et al. 2004; Sugitara et al. 2007; Yan et al. 2001)
<i>Padi6*</i>	3,300	peptidyl arginine deiminase, type VI	Ribosomal storage	Female infertility	(Esposito et al. 2007; Yurtas et al. 2008)