[Frontiers in Bioscience 12, 3713-3726, May 1, 2007]

Mechanisms of maternal mRNA regulation: implications for mammalian early embryonic development

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1. ABSTRACT

Mammalian oocytes accumulate a large pool of mRNA molecules that orchestrate subsequent embryonic development. The transcriptional machinery is silent during oocyte meiotic maturation and early embryogenesis, and thereby the early decisive events in embryo development prior to initiation of transcription from the embryonic genome are directed by the translation of pre-existing maternal mRNAs. Oocytes display remarkable post-transcriptional regulatory mechanisms that control mRNA stability and translation. The regulatory mechanisms are generally negative, and target mRNAs are either subjected to degradation or repressed from undergoing translation until specifically activated. Such negative regulatory mechanisms generally are mediated by transcript deadenylation, interaction of transcripts with RNA-binding proteins in a nonspecific or sequence-specific fashion, and/or potentially via actions of microRNA and repeat-associated small interfering RNA, which degrade maternal RNA transcripts. In contrast, translational activation is initiated via cytoplasmic polyadenylation of maternal transcripts facilitated via the binding of embryo-specific poly(A)-binding proteins (ePABs). In certain instances, translational regulation (positive or negative) is dictated by the balance of positive and negative trans-acting factors that compete for specific sequence motifs present in maternal transcripts. Coordinate post-transcriptional regulation of the oocyte mRNA pool is critical for normal progression of early embryonic development.

2. INTRODUCTION

The growing mammalian oocyte arrested at prophase I of meiosis is transcriptionally active and produces a store of mRNA and proteins required for early embryogenesis. During oocyte meiotic maturation and the initial stages of embryonic development, the transcriptional machinery is silent, and in mammals, depending on the species, embryonic genome activation (EGA) occurs coincident with the first one to four cell cycles following fertilization (1-3). Genome activation initiates transcriptional activity within the embryonic nucleus, and subsequent development is dependent upon newly synthesized mRNA and protein. Prior to EGA, key developmental events in the oocyte, pre- and post-fertilization (e.g., meiotic maturation, fertilization, initial cleavage divisions, and programming of EGA), are totally dependent on maternally derived mRNA and proteins (4). The maternal mRNAs are translated temporally during these critical stages into functional proteins to ensure normal embryonic development. The mechanisms whereby the maternal mRNAs are stored and translationally repressed in the prophase-I-arrested oocyte, and precisely how the ultimate fate of maternal transcripts is decided in the developing embryo, are the focuses of this review. These post-transcriptional regulatory mechanisms tightly control the translation of maternal mRNAs during early embryonic development. The maternal mRNAs are translationally repressed until specifically activated, and the repression is promoted by transcript deadenylation,

association of maternal transcripts with RNA-binding proteins in a nonspecific or sequence-specific manner, and mRNA degradation. The degradation of the untranslated maternal mRNA pool is presumed critical to early embryonic development, and recent evidence suggests it may be achieved via microRNA (miRNA) or repeat-associated small interfering RNA (rasiRNA) pathways (5, 6). During initiation of translation, the repressive effects of RNA-binding proteins are overcome via signals triggered during progression of meiosis and (or) fertilization. As a result, the maternal mRNAs are translationally activated by cytoplasmic polyadenylation. In certain scenarios, translational regulation is facilitated by the competitive binding of positively or negatively acting trans-factors at sequence-specific motifs present within the secondary structure of the maternal mRNA transcript. During early embryonic development, the inhibitory factors are displaced, and consequently the positive factors mediate translational activation of maternal transcripts. How the above events are coordinated, culminating in the progression of an oocyte into a developmentally competent embryo following fertilization, is an area of active investigation, and many questions remain unresolved. Biochemical and mechanistic information on the majority of the RNA-binding factors implicated in translational regulation is derived from studies of oocytes and embryos of lower vertebrates. Accumulating data indicates that most of these factors seem to have similar yet distinct roles in oocytes of evolutionarily distant mammals, like the mouse.

The following sections of this review will discuss the translational regulatory mechanisms critical to early development, with emphasis primarily on mechanistic information obtained from mammalian model systems. Gaps in knowledge and established mechanisms in other vertebrate models are also highlighted for comparison. Components of the general translational machinery pertinent to described mechanisms of translational regulation in oocytes and early embryos are outlined where essential, but the reader is referred to other reviews (7, 8) for a detailed discussion of the translational machinery and how translation is mediated.

3. POST-TRANSCRIPTIONAL CONTROL MECHANISMS MEDIATING MATERNAL mRNA REPRESSION

3.1. Deadenylation of maternal mRNA

The translational potential of a maternal mRNA transcript is determined by the length of the poly(A) tail. Accordingly, an increase in translation is associated with poly(A) tail elongation, whereas translational repression correlates with shortening of the poly(A) tail (9). Most cellular mRNAs receive a poly(A) tail in the nucleus and associate with ribosomes for translation after export to the cytoplasm (10). However, some maternal mRNAs are translationally silenced through a cytoplasmic deadenylation mechanism involving shortening of the poly(A) tail at the 3' end of the mRNA (11). Regulation of tissue plasminogen activator (tPA) mRNA in growing mouse oocytes is a classical example of translational repression by cytoplasmic deadenylation. The newly synthesized tPA transcript receives a long poly(A) tail [~300 nucleotides (nt)] and subsequently undergoes poly(A) shortening (40-60 nt) in the cytoplasm, preventing translation (12). Until resumption of meiosis, tPA mRNA with a short poly(A) tail is stored in the cytoplasm in a dormant form (12). The mechanisms targeting specific maternal mRNAs for deadenylation in mammalian oocytes and the mediators involved are not known. In Xenopus oocytes, truncation of the poly(A) tails of cyclin B1 and Mos mRNAs is catalyzed via a cytoplasmic poly(A)-specific ribonuclease (PARN), also termed deadenylating nuclease (DAN) (13). Although a truncated poly(A) tail generally interferes with translation initiation, it is insufficient to completely prevent translation of certain maternal transcripts. For example, maternally expressed histone B4 mRNA in immature Xenopus oocytes has a short poly(A) tail, but the protein accumulates to a substantial level during oogenesis (14, 15). The mechanisms whereby such transcripts escape translational inhibition in the presence of a short poly(A) tail are unknown.

3.2. Maternal mRNA masking: Nonspecific interaction of Y-box proteins

During oogenesis, the oocyte genome is transcriptionally active, and the newly synthesized maternal mRNAs are either translated or stored in a dormant form. For instance, the mRNAs for the zona pellucida genes (ZP1, ZP2 and ZP3) are actively transcribed and translated during mouse oogenesis and are barely detectable at ovulation, when eggs are transcriptionally inert (16). The dormant mRNAs are recruited for translation at defined periods of early development in response to physiological cues (4). In Xenopus, 80 percent of maternal mRNAs are dormant and are associated with maternal ribonucleoprotein (mRNP) particles, which repress translation by preventing the binding of mRNA to polyribosomes via a process referred to as "masking of maternal mRNAs" (11, 17-20). The major constituent of mRNP particles is the Y-box proteins, a group of nonspecific RNA-binding proteins (11, 21-23). The germ-cell-specific Y-box protein FRGY2 in Xenopus binds to nonpolysomal RNA with an average density of one protein molecule per 40 to 50 nucleotide residues (24). FRGY2 is phosphorylated by the mRNP-associated protein kinase, and this increases the stability of the FRGY2-RNA complex (25, 26). Microinjection of protein kinase inhibitors into growing Xenopus oocytes stimulates the rate of endogenous protein synthesis by two- to threefold (27). This may be due to destabilization of the RNA-protein interaction resulting in the release of oocyte mRNAs accessible for translation (25, 27). Therefore, it appears that phosphorylation of FRGY2 may inhibit translation and the dephosphorylation of FRGY2 may initiate translational activation of maternal mRNAs (27).

Masking of maternal mRNA may be a conserved mechanism of translational repression in mammals, because Y-box proteins (MSY1, MSY2 and MSY4) have been identified in mouse oocytes and early embryos (28-30). MSY2, the murine homologue of Xenopus FRGY2, is specifically expressed in male and female germ cells and is maternally inherited in early embryos; both the mRNA and

protein are degraded in two-cell embryos concomitant with activation of the embryonic genome (29, 31). The loss of MSY2 at embryonic genome activation coincides with bulk degradation of maternal mRNAs, suggesting that MSY2 may play a role in the storage, stability and regulation of maternal mRNAs. Targeted disruption of the MSY2 gene results in female infertility in mutant animals, with early loss of oocytes and defects in ovulation (32). MSY2 constitutes 2 percent of the total protein in fully grown mouse oocytes, and 75 percent of this protein is localized to the cytoplasm. Recombinant MSY2 protein inhibits translation of luciferase reporter mRNAs to a modest level in an *in vitro* (rabbit reticulocyte lysate) translation system (33), providing further support for a potential role in translational repression of maternal mRNAs in the mouse oocyte.

Biochemical evidence also supports a potential role for MSY2 in RNA masking. The N-terminal domain of MSY2 has a cold-shock domain and a basic/aromatic amino acid island in the C-terminus. Therefore, 75 percent of the MSY2 associates with the Triton-insoluble portion of mouse oocytes, suggesting that MSY2 sequesters maternal mRNA from the translational machinery (34). RNase-A treatment of the Triton-permeablized mouse oocytes or microinjection of RNase-A into oocytes releases MSY2 proteins bound to mRNAs (34), which indirectly supports a role for MSY2 in binding and stabilization of maternal transcripts. Murine MSY2 contains several potential phosphorylation sites for protein kinases, as observed in FRGY2 (29, 31), and the MSY2 protein is phosphorylated during meiotic maturation and dephosphorylated following fertilization (31). However, the mediators of the above post-translational modifications of MSY2 and the functional significance of such modifications are unclear.

It is not clear whether MSY2 binds to a selective class of maternal transcripts or has a global role in binding to multiple mRNAs in the oocyte. MSY2 is implicated in storage of paternal protamine mRNAs in mouse spermatids (30, 35). Protamines are small arginine-rich proteins involved in condensation of DNA in the nuclei of mature spermatids. MSY2 protein recognizes a consensus sequence (UCCAUCA) in the 3'-untranslated region (3' UTR) of protamine mRNA, raising the possibility that murine MSY2 may have a previously unknown sequence-specific RNA-binding activity (35). In contrast, another study demonstrated that mouse recombinant MSY2 protein binds at multiple sites *in vitro*, with limited or no sequence specificity, to full-length protamine mRNA (33). Further, MSY2 has approximately tenfold higher affinity for binding to full-length synthetic protamine-1 mRNA compared to the short protamine mRNA sequences derived from its 3' UTR (33), indirectly indicating that length of the mRNA maybe a prerequisite for its action. Specific transcripts bound by MSY2 in mouse oocytes have not been described.

3.3. Sequence-specific interaction of maternal mRNA-binding proteins

The translation of specific maternal mRNA is one of the hallmarks for activation of meiosis in mammalian oocytes. The maternal transcripts required for progression of meiosis are translationally repressed by sequence-specific interaction with RNA-binding proteins. In response to gonadotropins, several events are initiated in the prophase-I-arrested oocyte (36). For example, synthesis of cyclin B1 (a cofactor of maturation-promoting factor) and activation of cyclin-dependent kinase (CDK) and maturation-promoting factor (MPF) are the early events during meiotic maturation (36). MPF activity further stimulates translation of specific maternal transcripts such as Mos (a serine threonine kinase), which in turn is required for activation of mitogen-activated protein kinase (MAPK), which enables the oocyte to progress through meiosis and finally arrest at metaphase II, awaiting fertilization (36). During meiotic maturation, the maternal mRNAs are translated in a sequential, well-controlled manner with a complex network of translational regulatory mechanisms, but the chronological events are not characterized in mammals. In the Xenopus oocyte, a rapid inducer of G2/M progression in oocytes known as RINGO/Spy is implicated as one of the earliest maternal mRNAs to be translated. RINGO/Spy translation precedes Mos synthesis because overexpression of RINGO/Spy in Xenopus oocytes induces Mos synthesis, MAPK activation and maturation (37, 38). In contrast, these events are blocked if RINGO/Spy mRNA is knocked down in progesterone-stimulated Xenopus oocytes (37). Further, RINGO/Spy protein can bind and activate CDK1 and CDK2 and has a role in cell cycle regulation (38, 39). The mRNA encoding a protein related to RINGO/Spy has been detected in mouse oocytes, but the mechanism of its action during meiotic maturation is not clear (40).

Translational repression of RINGO/Spy and Mos mRNA in oocytes is conferred by sequence-specific interactions of repressive factors bound to the 3' UTRs, with the proteins of the eukaryotic initiation factor 4E (eIF4E) family bound to the 5' end of the mRNA. The molecular mechanism for translational repression of RINGO/Spy mRNA in mammalian oocytes is not known but is well described in Xenopus oocytes. Two pumilio-binding elements (PBEs), UGUAUAAA and UGUAAAUA, residing at 3' UTR of Xenopus mRNA are necessary for the repression (Figure 1). The PBEs are bound by pumilio-2 (Pum-2), a member of the pumilio and FBF (Fem-3 mRNA-binding factor) (PUF) family of RNA-binding proteins (41). PUF proteins belong to family of evolutionarily conserved translational regulators in eukaryotes (42). Pum-2 is expressed in oocytes both before and after maturation, and injection of Pum-2 antibody relieves the repression and induces synthesis of endogenous RINGO/Spy even in the absence of progesterone stimulation (41). Likewise, injection of the dominant negative form of Pum-2 stimulates RINGO/Spy protein synthesis (41). Pum-2 not only interacts with PBE in oocytes, but also interacts with two other proteins: DAZL (Deleted in Azoospermia-like), an RNA-binding protein, and embryonic poly(A)-binding protein (ePABP) (41). Human Pum-2 also interacts with human DAZ and mouse DAZL (mouse homologue to DAZ) (43), but the effects of such interactions are not clear. DAZL expression is exclusive to germ cells of gonads, and gene-targeting of

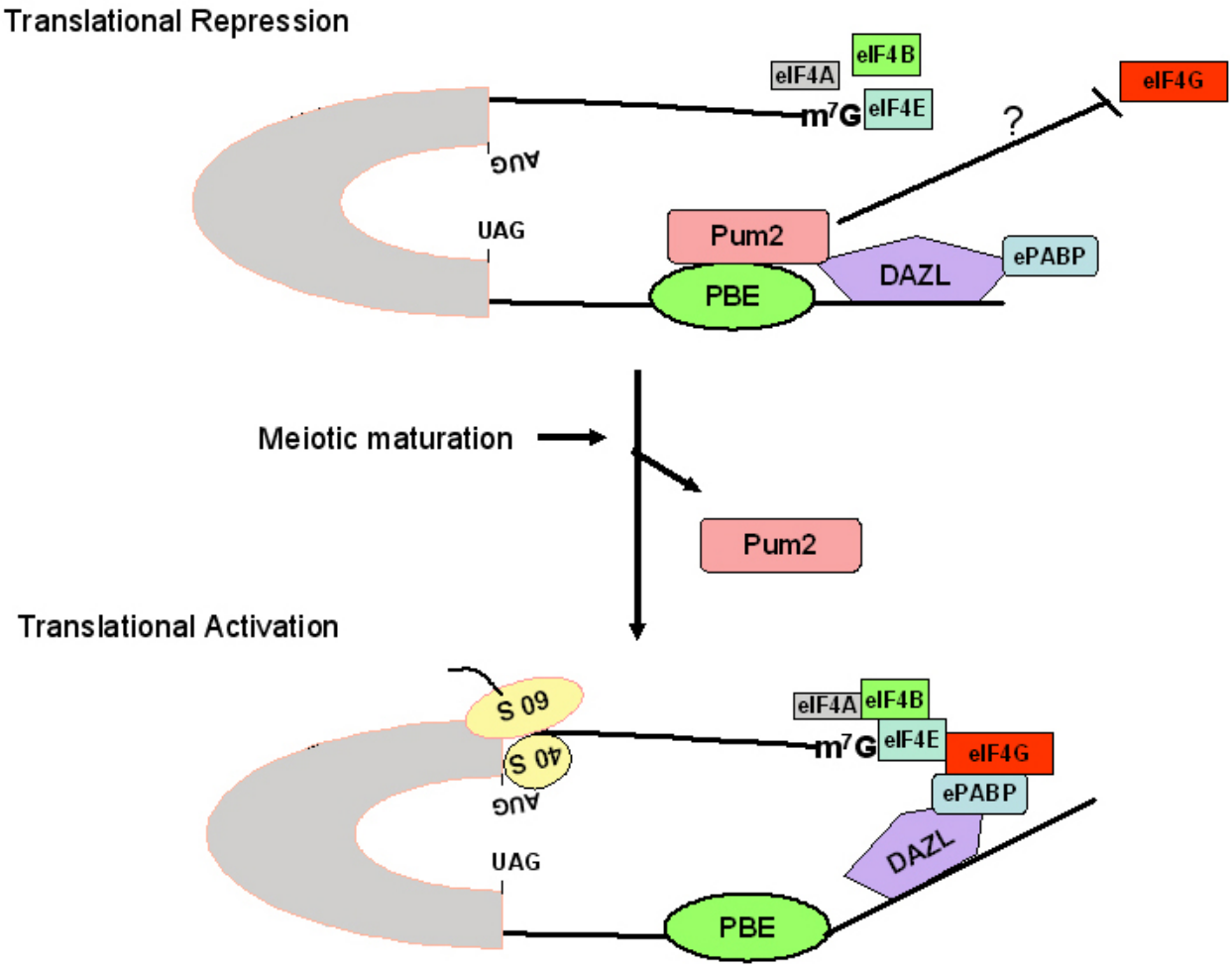

Figure 1. Schematic model for regulation of pumilio-2 (Pum-2) bound RINGO/Spy mRNA translation during oocyte maturation. The RINGO/Spy mRNA is repressed by the binding of Pum-2 to the pumilio-binding element (PBE) in the 3' untranslated region (UTR). Pum2 resides in a complex consisting of embryonic poly (A)-binding protein (ePABP) and the RNA binding protein deleted in Azoospermia-like (DAZL) and interferes with the interaction between translation initiation factors (e.g., eIF4G) and the 5' end of the mRNA. During meiotic maturation, Pum-2 dissociates from the protein complex bound to mRNA, and thereby ePABP interacts with eukaryotic initiation factor-4G (eIF4G) at the 5'end, resulting in translational activation.

DAZL disrupts fertility, with loss of germ cells in both male and female mice (44, 45). DAZ/DAZL has a highly conserved RNA recognition motif (RRM) and resides in a complex of Pum-2, DAZL and ePAB bound to RINGO/Spy mRNA in Xenopus oocytes (Figure 1) (41, 46). Although ePAB classically binds to the poly(A) tail and interacts with eukaryotic initiation factor 4G (eIF4G) to stimulate translation of polyadenylated transcripts, Pum-2 binding at PBE may have overriding effects to prevent translation of RINGO/Spy mRNA in prophase-I-arrested oocytes (41).

Another well-studied example of mRNA repression in mouse oocyte cytoplasm is cytoplasmic polyadenylation element (CPE) mediated regulation of Mos and tPA mRNAs (12, 47). CPEs (also termed adenylation control elements [ACEs]) are uridine-rich sequences (consensus sequence UUUUUAU) in the 3'-UTR of some maternal mRNAs that can either repress translation by recruiting a repressive complex or direct polyadenylation and resumption of translation (12, 47). One other mechanism by which CPE containing transcripts achieve translational repression is through deadenylation, reducing the poly(A) tail lengths to 20 to 40 nt (12, 48). The translational repression of CPE-containing transcripts is dependent on a CPE-binding protein (CPEB), an RNA-binding protein with high affinity to CPE elements (47, 49). Mouse CPEB mRNA is present in the ovary, testis, brain and kidney, and it encodes for a 62-kDa protein (49). Within the ovary, CPEB mRNA expression is exclusively restricted to oocytes (49). The additional factors required for CPEB-mediated repression are present in mouse oocytes (50), but the regulatory mechanism of CPEB action is better understood in Xenopus. In Xenopus oocytes, CPEB anchors Maskin, a repressor protein which blocks cap-dependent translation (Figure 2) (51). Maskin behaves as an eukaryotic initiation factor (eIF) 4E-binding protein (eIF4E-BP) and competitively binds to eIF4E at the same

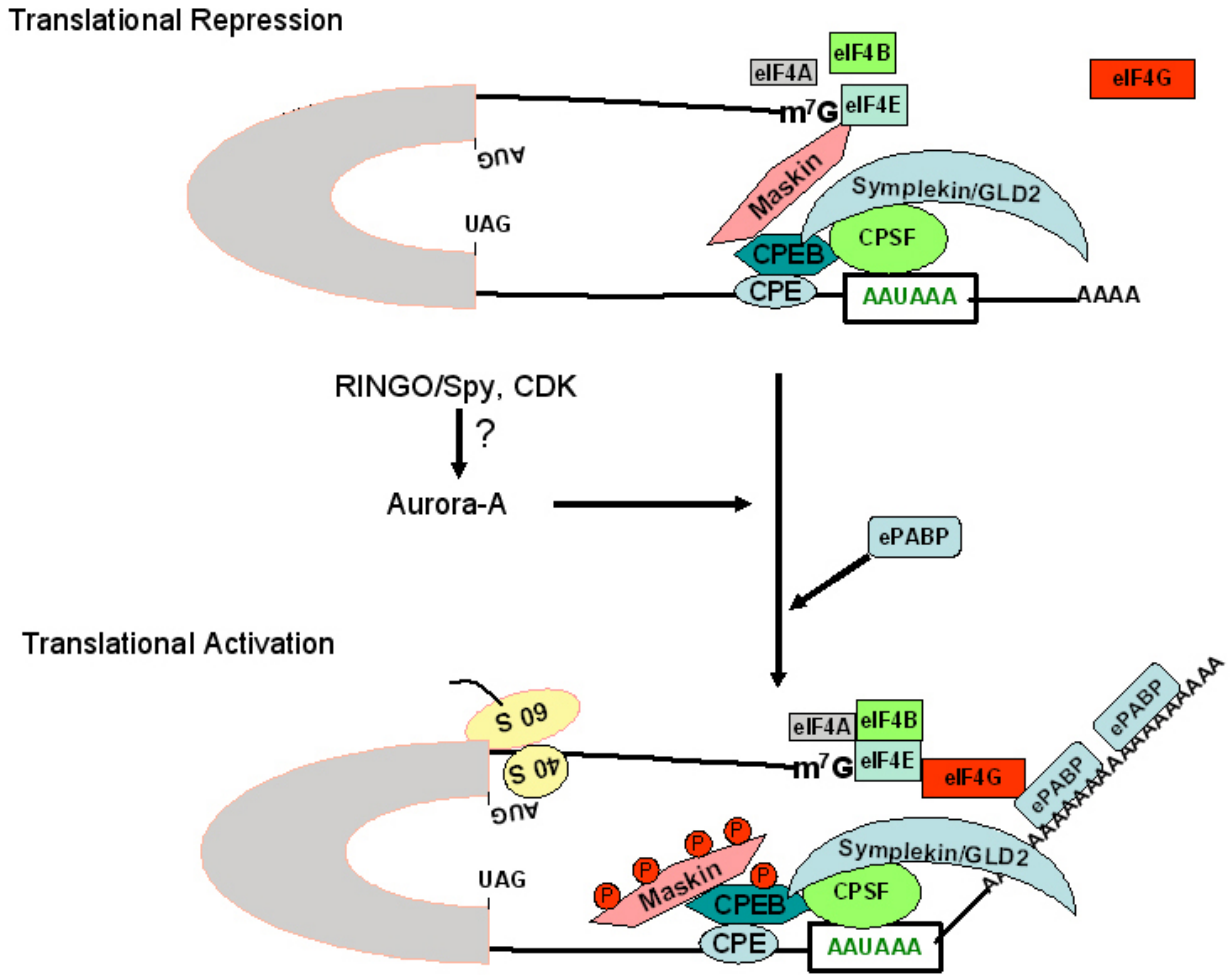

Figure 2. Schematic model for regulation of cytoplasmic polyadenylation element (CPE)-containing maternal mRNA during oocyte maturation. CPE-binding protein (CPEB), an RNA binding factor binds to CPE sequence and further interacts with Maskin, a repressor protein that prevents cap-dependent translation. Maskin acts as a eukaryotic initiation factor-4E (eIF4E) binding protein and competitively binds to eIF4E at the same region as eukaryotic initiation factor-4G (eIF4G) and thereby prevents the access of translation initiation factors to the 5' end of the mRNA. CPEB further interacts with three other proteins; (a) Cleavage and polyadenylation specificity factor (CPSF), a protein bound to poly(A) signal; (b) symplekin, a scaffold protein; and (c) germline development deficient-2 (GLD2), a cytoplasmic poly(A) polymerase. During meiotic maturation, RINGO/Spy protein acts as a cofactor for cyclin dependent kinase (CDK) activity and presumably activates Aurora-A kinase, which in turn activates CPEB by phosphorylation. CPEB phosphorylation leads to activation of CPSF/Symplekin/GLD2 protein complex, which in turn elongates the short poly (A) tail of CPE-containing transcripts. Maskin undergoes differential phosphorylation at many residues by CDK and thereby releases the repressive effects of maskin at the 5'end of the mRNA. The longer poly(A) tail binds to embryonic poly(A) binding protein (ePABP) and in turn recruits eIF4G to the cap-structure of the mRNA consequently activating translation.

region as eIF4G, thereby inhibiting interaction of eIF4E and eIF4G (47, 51). The blockade of such interaction further prevents the eIF4F group of initiation factors from initiating translation on CPE-containing mRNAs in the prophase-I-arrested oocyte. A recently described novel gene named eukaryotic translation initiation factor 4E-like (*Eif4eloo*) is specifically expressed in mouse oocytes and embryos (52). *Eif4eloo* encodes for seven mRNA variants that arise predominantly because of alternative splicing at the 5' end of the gene (52). The predominant *Eif4eloo* mRNA encodes for a 244-amino acid protein similar to eIF4E, and the mRNA is not detected beyond the early two-cell stage, indicating its maternal origin in the embryo, with a potential role in maternal mRNA translation (52). CPEB interacts with three other factors in Xenopus oocytes; (a) symplekin, a protein that appears to act as a scaffold for other proteins to associate with; (b) CPSF (cleavage and polyadenylation specificity factor), a group of four proteins that also bind to a poly(A) signal; and (c) GLD2 (germline development deficient), a cytoplasmic poly(A) polymerase which elongates the short poly(A) tail of CPE-containing transcripts (Figure 2) (53). Specific

roles of symplekin, CPSF and GLD2 in mediating mRNA repression are not established, but they are necessary components of CPEB during polyadenylation-induced translational activation.

CPEB has several roles in development. CPEB-null mice have defects in learning and memory and carry defective female germ cells that are arrested at the pachytene stage of prophase I by embryonic day (E) 16.5 (54). Defective germ cells are attributed to failure in polyadenylation and translation of CPE-containing synaptonemal complex protein (SCP1 and SCP3) mRNAs (54). CPEB is phosphorylated at E16.5 (pachytene) at residue T171 and is dephosphorylated by protein phosphatase 1 at E18.5 (55), implying that post-translational modification of CPEB may be necessary for translation of CPE-containing SCP1 and SCP3 mRNAs. Recently, CPEB has been implicated in control of oocyte growth and follicular development in the mouse (56). The role of CPEB in oocyte growth and follicle development was delineated by the creation of transgenic mice expressing siRNA under the control of zona pellucida protein 3 (ZP3) promoter, which induces destruction of CPEB mRNA specifically in dictyate stage oocytes at the end of prophase I (56). Transgenic oocytes display atresia, premature oocyte maturation, parthenogenetic activation, precocious follicle activation, detached cumulus-granulosa cells, and granulosa cell apoptosis with a substantial decrease in fertility (56). Further, CPEB binds many oocyte mRNAs that encode for proteins involved in signal transduction (e.g., Smad1, Smad5), cell cycle control (e.g., spindlin, Bub1b and Mos), transcription (e.g., Obox1), maintenance of methylation patterns important for imprinting (e.g., oocyte-specific DNA methyltransferase 1) and also binds mRNA for growth differentiation factor-9 (GDF9), an oocyte-expressed growth factor critical for follicular development (56). In transgenic oocytes, GDF9 mRNA has a shortened poly (A) tail and reduced protein expression. Therefore, evidence suggests that CPEB controls the translation of GDF9, which is necessary for oocyte-follicular growth and development (56).

3.4. MicroRNA and repeat-associated small interfering RNA: Regulators of maternal mRNA degradation

Degradation of untranslated maternal mRNA is presumed to be a critical checkpoint during early embryo development. In mouse embryos, 90 percent of maternal mRNA is degraded by the two-cell stage, coincident with complete activation of the embryonic genome (1, 57, 58). Specific maternal transcripts that undergo rapid degradation following fertilization have been identified in mouse embryos (59). The majority of these mRNAs are exclusively expressed in the oocyte genome (oocyte-specific linker histone H1 [H1oo], c-mos and GDF-9) and not expressed during preimplantation development (59). Maternal mRNA degradation in mouse embryos is dependent on the 3' UTR of the mRNA transcript. For example, chimeric mRNAs composed of the c-mos coding region fused to the Hprt 3' UTR have reduced rates of degradation following microinjection into fertilized oocytes compared to transcripts composed of the Hprt coding region and c-mos 3' UTR (59). There is direct evidence that maternal mRNA clearance is critical for early development in other vertebrates. In Xenopus, oocyte-specific c-mos mRNA, which is essential for regulating meiosis, is degraded soon after fertilization. Persistence of c-mos is detrimental for embryo development, because injection of c-mos protein into two-cell embryos inhibits cleavage (60). Thus, degradation of maternal mRNAs detrimental to embryogenesis represents a conserved mechanism of vertebrate development.

Recent evidence from zebrafish embryos suggests that miRNAs may be the key regulatory molecules targeting maternal mRNA for degradation. MicroRNAs are evolutionarily conserved noncoding RNAs that regulate gene expression at the post-transcriptional level (61). In animal cells, two nucleases, Drosha in the nucleus and Dicer in the cytoplasm, are important for processing longer primary and precursor miRNAs into the ~22 nucleotide mature miRNAs (62). The mature miRNA assembles into the effector complexes called miRNPs (miRNA containing ribonucleoprotein particles), that have many features similar to RISCs (RNA-induced silencing complexes). RISCs are large ribonucleoprotein complexes that mediate the actions of small interfering RNA (siRNA) in targeting mRNA for cleavage and degradation, commonly referred to as RNA interference (RNAi). The miRNA binds to single/multiple partial complementary sequences in the 3' UTR of its target mRNA, inhibiting protein synthesis and/or targeting mRNA for destruction. A seven-nucleotide seed sequence (at positions 2 through 8 from the 5'end) in miRNAs appears to be crucial for miRNA binding and action (63). The precise mechanisms by which miRNAs silence their target mRNAs remain unclear. However, miRNAs are known to interfere with the initiation step of translation by preventing binding/blocking the action of eIF4E at the cap structure of mRNA, causing translational repression (64, 65).

Abundant evidence supports an important role for miRNAs during vertebrate embryogenesis. For example, targeted disruption of the dicer gene in mice (abolishing the generation of mature miRNAs) results in embryonic lethality (66) and *dicer* mutant embryonic stem cells fail to differentiate both *in vivo* and *in vitro* (67). Furthermore, zebrafish embryos mutant for maternal-zygotic *dicer* (*MZdicer*) activity have abnormal morphogenesis and do not process precursor miRNAs into mature miRNAs (68). The miR-430 miRNA family (miR-430a, miR-430b and miR-430c) is highly expressed during the onset of zygotic transcription in zebrafish embryos, and injection of miR-430 mature miRNAs into *dicer* mutant embryos rescues brain morphogenesis, further supporting an important role for miRNAs during development (68). It is not known whether miRNAs play a regulatory role during zebrafish oogenesis, but miR-430 targets the 3' UTR regions of maternal mRNAs at the onset of zygotic transcription, resulting in deadenylation of the poly(A) tail and degradation of the maternal mRNA pool. Less degradation of reporter mRNAs with miR-430 target sites in the 3' UTR occurs when injected into *MZdicer* mutant zebrafish embryos versus wild-type controls. Microarray analysis of RNA harvested from *MZdicer* mutant zebrafish embryos

and wild-type embryos at the onset of zygotic transcription has revealed several hundred mRNAs that are direct targets of miR-430 alone. The target mRNAs are predominantly maternally derived, and few of them are synthesized at the onset of EGA (5). The absence of miR-430 and the delayed clearing of maternal mRNAs do not interfere with EGA or embryo patterning in zebrafish embryos, but the persistence of maternal transcripts delays development and results in abnormal morphogenesis (5). An important question is whether miR-430-mediated degradation of maternal transcripts is conserved during mammalian EGA. To our knowledge, expression of miR-430 has not been reported in mammalian embryos, but several other novel miRNAs have been cloned in mouse oocytes (6). However, whether the novel miRNAs have any role in maternal mRNA degradation or inhibition of translation is not established. Although evidence from mammals is limited, it is tempting to hypothesize and likely that miRNAs are involved in degradation of maternal transcripts in mammalian embryos.

There is also evidence for distinct regulatory mechanisms mediating degradation of a specific class of RNA transcripts during oogenesis in the mouse. In mice, a significant portion of the maternal mRNA pool (approximately 13 to 14 percent of the oocyte transcriptome) is composed of transposable elements (TEs) and chimeric transcripts of TEs with host genes (52, 69). The TEs act as the first exon or first few exons of the functional chimeric mRNA transcripts and also act as stage-specific alternative promoters for a number of host genes (69). In addition, both sense and antisense transcripts of some transposable elements are co-expressed in mouse oocytes and embryos (70). Recently, a novel class of small RNAs referred to as repeat-associated siRNA (also termed retrotransposon-derived siRNAs [rasiRNA]) have been implicated in targeting retrotransposon-derived mRNA sequences for degradation in fully grown mouse oocytes (6). RasiRNAs are approximately 20- to 23-nucleotide molecules and have a preference for uridine and adenine residues at the first position, similar to miRNAs (6). RasiRNAs are mapped to both sense and antisense orientations of different retrotransposons and are derived from LINE (long interspersed nuclear elements), SINE (short interspersed nuclear elements) and LTR (long terminal repeat) elements (6). Reporter transcripts composed of the EGFP coding region with either sense or antisense retrotransposon sequences with target sites for rasiRNAs at 3' UTR are degraded rapidly in oocytes following injection (6). Therefore, it is thought that the mRNAs with sense and antisense orientations of transposable elements may form a double-stranded structure and trigger rasiRNA to degrade mRNAs via the RNAi pathway. However, it is not known whether the action of rasiRNAs is exclusive to oocytes or they have similar actions during early embryogenesis. Furthermore, the functional significance of rasiRNA-mediated maternal mRNA degradation in mouse oocytes is not understood.

Are there are any discrete loci within the cytoplasm where the maternal mRNAs are degraded? A hint about another potential mechanism of miRNA action comes from localization of miRNA-repressed mRNA complexes within or adjacent to P-bodies (63). P bodies (cytoplasmic processing bodies) are large cytoplasmic aggregates known to contain translationally masked mRNA that also serve as sites of mRNA degradation (71, 72). In addition, P bodies lack ribosomes and translation initiation factors and hence may contribute to translational repression of target mRNAs (71). It is not known whether P-bodies are sites of miRNA action or are temporary warehouses for repressed mRNAs, nor is it known how these repressed mRNAs are transported to P-bodies. Although the existence of P-bodies in vertebrate oocytes/embryos remains to be elucidated, conservation of this mechanism throughout all cell types seems likely.

4. MECHANISMS FOR TRANSLATIONAL ACTIVATION OF MATERNAL mRNA

4.1. Cytoplasmic polyadenylation: Role of embryo specific poly (A)-binding proteins

Cytoplasmic polyadenylation is a conserved molecular regulatory step in mRNA translation both in mammals and lower vertebrates during early development. During meiotic maturation, few maternal mRNAs are relieved from the repressive effects and polyadenylated initiating translation. A classical example of cytoplasmic-polyadenylation-mediated translational activation is derived from CPE-containing maternal Mos mRNA in mouse oocytes (73). Cytoplasmic polyadenylation of Mos mRNA requires three cis-elements in the 3' UTR: the polyadenylation hexanucleotide (AAUAAA) and two CPE elements located upstream of the hexanucleotide (73). Reporter mRNAs with wild-type Mos 3' UTR are translationally recruited during meiotic maturation and polyadenylated (73). Further, ablation of Mos mRNA with antisense oligonucleotides results in failure to progress to meiosis II (73), indicating that cytoplasmic polyadenylation-induced translation of Mos mRNA is necessary for normal development. The translational activation of CPE mRNAs during meiotic maturation occurs in a sequential order, but it is not clearly understood in mammalian oocytes. In Xenopus oocytes, CPEB-mediated cytoplasmic polyadenylation of CPE-containing maternal mRNAs requires the translation of RINGO/Spy mRNA. Progesterone-induced meiotic maturation dissociates the interaction of Pum2 with RINGO/Spy mRNP complex (which also includes DAZL and ePAB), releasing the mRNA from repression; as a consequence, RINGO/Spy mRNA is translated (Figure 1) (41). Whether the activation of Xenopus RINGO/Spy mRNA requires poly (A) tail elongation is not known. RINGO/Spy protein acts a cofactor for CDK activity, which presumably activates unknown downstream molecules which indirectly influence Aurora-A (a serine/threonine protein kinase) activity required for CPEB activation (41). Aurora-A activates CPEB by phosphorylation, and this correlates with cytoplasmic polyadenylation and translation of CPE-containing Mos and other maternal mRNAs in Xenopus oocytes (Figure 2) (74, 75).

Factors required for translation of CPE containing transcripts (CPEB, CPSF, Maskin, Ipl1- and aurora-related kinase 1 [IAK1, the murine homologue of

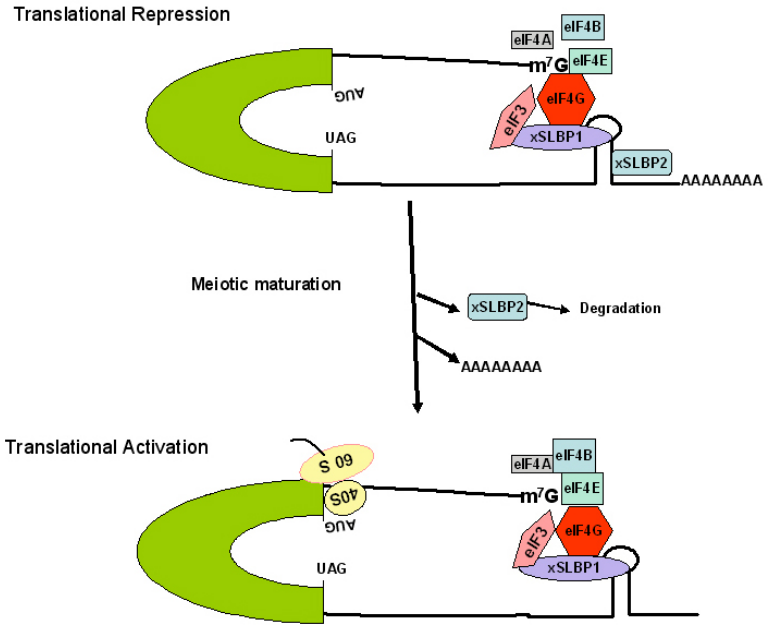

Figure 3. Schematic model for regulation of histone mRNA during oocyte maturation. In immature Xenopus oocyte, histone mRNAs have a short oligo(A) tail attached to the conserved stem-loop structure. The stem-loop sequence is bound to two stem loop binding proteins (xSLBP1 and xSLBP2). The oligo(A) tail and the binding of xSLBP2 to the stem-loop sequence inhibit translation initiation. During meiotic maturation, the oligo(A) tail is removed, the xSLBP2 is degraded by unknown mechanisms and histone mRNA is translated.

Aurora-A/Eg2]) are also found in mouse oocytes, and these mechanisms appear to be conserved among vertebrates (50). For example, inhibiting the activity of IAK1 prevents phosphorylation of CPEB and in turn blocks the progression of meiosis in mouse oocytes. Likewise, a dominant negative mutant of CPEB protein which cannot be phosphorylated by IAK1 prevents cytoplasmic polyadenylation (50). However, the intricate biochemical details of CPEB-mediated translational activation are not understood in mammalian oocytes. In Xenopus oocytes, CPEB phosphorylation leads to activation of the CPEB-associated poly (A) polymerase complex, which contains CPSF/Symplekin/GLD2, which then elongates the short poly(A) tail of CPE containing transcripts (Figure 2) (53, 76). The longer poly(A) tail binds to ePABP, which in turn recruits eIF4G to replace Maskin in the repressive Maskin-cap complex, resulting in translation of CPE mRNAs (46). Maskin undergoes differential phosphorylation by CDK1 at several residues, and these post-translational modifications appears to interfere with the repressive effects of Maskin at the 5' cap structure of the mRNA (77).

The association of poly (A)-binding protein (PABP) to the poly (A) tail of the mRNA is an important step during translation. The PABP, in turn, communicates with factors at the 5' end of the mRNA, specifically eIF4G, and regulates translational activation. Although there are structurally different groups of PABPs identified in vertebrates, this discussion will emphasize the embryonic PABPs (ePAB and ePABP2). The mouse ePAB is exclusively expressed in testes, oocytes and early embryos prior to EGA (78), but its mechanism of action is not clear. In Xenopus oocytes, ePAB protects mRNA by suppressing deadenylation and can also stimulate translation of reporter mRNAs (79, 80). The mouse ePABP2 has one RNA recognition motif, and mRNA expression is restricted to ovaries and oocytes (81, 82), but its biochemical mechanism of action is not known. Given the germ cell specific expression pattern of ePAB and ePABP2, it seems reasonable to speculate that these proteins play a functional role in the poly(A) regulation critical for expression of maternally derived transcripts during early development.

4.2. Translational regulation of maternal histone mRNA: Role of stem-loop binding proteins

In the mouse, replication-dependent histone mRNAs and proteins are stored in oocytes and embryos, and translation of these mRNAs is not coupled to DNA replication in early embryos (83, 84). Maternally derived histones are required to replace the protamines of the sperm DNA after fertilization and to assemble the newly synthesized embryonic DNA into chromatin until the embryonic genome is transcriptionally active (85). Replication-dependent histone mRNAs lack a poly(A) tail but end in a conserved stem-loop structure (86). The only known exception, histone mRNA in the Xenopus oocyte, has a short oligo(A) tail attached to the stem-loop sequence which is thought to have a role in translational repression (87). The stem-loop sequence also interacts with two stem-loop binding proteins (SLBP) that bind to the 3' end of histone mRNAs. The mammalian homologue of SLBP is xSLBP1, and xSLBP2 is specific to Xenopus oocytes. The mechanism of translational activation of histone mRNAs involves exchange of SLBPs associated with the 3' end of the mRNA. xSLBP2 inhibits translation of histone mRNAs, whereas xSLBP1 activates translation (Figure 3) (88, 89). At oocyte maturation, the oligo(A) tail is removed, the

xSLBP2 is degraded, and histone mRNAs are translated (87). But the signal or signals that mediate the detachment of the oligo(A) tail and xSLBP2 are unknown. Reporter mRNAs ending in the stem-loop sequence, with or without an oligo(A) tail, are efficiently translated in rabbit reticulocyte lysates and are active in the presence of xSLBP1 (87). However, reporter mRNAs with an oligo(A) tail are not translated in Xenopus oocytes, even in the presence of xSLBP1 (87), suggesting that the oligo(A) tail might be bound by unknown additional inhibitory factors which repress translation of histone mRNAs in the oocytes. SLBP is predicted to be the functional homologue of PABP (90, 91), directing circularization of histone mRNAs through interaction with factors at the 5' end of the mRNA. However, the precise molecular mechanism of SLBP action in translational control is not clearly understood.

In contrast to Xenopus, mouse oocytes and embryos have a single SLBP. SLBP is concentrated in the nucleus of prophase-arrested oocytes (at G2/M)(92). After entering meiosis (M-phase), the increase in CDK-1 activity mediates SLBP phosphorylation, and the protein accumulates to a high level in the cytoplasm of MII oocytes (92). SLBP is dephosphorylated following fertilization, and protein levels remain high in both the nucleus and cytoplasm during the first cell cycle of embryo development but declines by the four-cell stage (92). These post-translational modifications do not alter binding of SLBP to stem-loop RNA (92), but it is unclear whether translation of histone mRNA is altered. The accumulation of SLBP protein during oocyte maturation is definitely due to translation of existing maternal mRNA and is quite similar to other genes (e.g., tPA, spindlin and cyclin B), which are regulated by CPE elements in the 3' UTR of their mRNAs. Whether SLBP mRNA regulation requires a CPE element or any other equivalent post-transcriptional regulatory mechanism has not been resolved. Coincident with the increase in SLBP during meiotic maturation, the translation of reporter mRNAs bearing the histone 3' UTR is increased (93). Conversely, preventing SLBP accumulation by RNAi reduces translation of the reporter mRNA, as well as synthesis of endogenous histones in matured oocytes. A significant decrease in the size of the pronucleus and in the amount of acetylated histone in the chromatin of the zygotes is also observed (93). Although SLBP is the main molecular determinant of histone synthesis, its mechanism of action perhaps is slightly different in mice than in Xenopus oocytes. Unlike mice, Xenopus have two different forms of SLBPs, and an oligo (A) tail at the 3' end of histone mRNAs regulates translation. Whether mouse histone mRNAs in the oocyte have a short oligo (A) tail and whether binding of xSLBP2 homologues is a key factor in translational repression is not known.

5. TRANSLATIONAL REGULATION IN EARLY EMBRYOS: THE UNCLEAR STORY

Initiation of EGA in mouse embryos is dependent on maternally inherited proteins and/or recruitment of some maternal mRNAs for poly(A) tail elongation and translation (e.g., spindlin and cyclin-A2) following fertilization (94, 95). It is evident that new proteins are synthesized following fertilization and prior to EGA, and blocking new protein synthesis reduces transcriptional activity in embryos (96-98). Inhibiting poly(A) tail elongation by treating one-cell mouse embryos with 3'deoxyadenosine (an adenosine analog) decreases the transcriptional activity in the embryonic nucleus (96) and further supports the premise that some maternal mRNAs are polyadenylated and translated following fertilization. Cyclin-A2, in conjunction with CDK-2 activity, appears to be one such maternal mRNA recruited following fertilization, because targeting the maternal cyclin-A2 by RNAi or blocking CDK-2 with roscovitine inhibits EGA in the one-cell embryo (99). In mice, fertilization starts in a signaling cascade (e.g., phospholipase-C zeta and/or a truncated form of c-kit tyrosine kinase), which mediates a rise in intracellular calcium oscillations (100-102). The rise in calcium oscillations subsequently mobilizes maternal mRNAs for translation (103). For instance, the inhibition of mouse egg calcium release blocks fertilization-associated changes in protein synthesis (101). Further, experimental manipulation of calcium oscillations in the mouse egg is associated with changes in protein synthesis (104). But how the rise in calcium oscillation is directly or indirectly linked to translational activation of maternal mRNA is not well understood. However, it is proposed that the downstream events of calcium oscillation may activate certain kinases/phosphatases (104), which may in turn post-translationally modify RNA-binding proteins, releasing the maternal mRNA from repression.

On the contrary, mobilization of the maternal mRNA could be transcript-specific and more specific to the cis-elements and or trans-factors at the 3' UTR. Computational analysis of 3' UTRs of expressed genes from mouse oocytes and an early stage embryo cDNA library has uncovered valuable information regarding features associated with the stability of maternal transcripts during early development in mammals (52). Comparison of transcriptomes derived from full-grown mouse oocytes and two-cell stage embryos generates two sets of maternal transcripts which differ in the size of the 3' UTR (52). The stable mRNAs have a significantly longer 3' UTR than the transient ones (52), indicating that the 3' UTR is a regulatory component for the stability of maternal mRNA. A higher proportion of uracil nucleotide residue is present in the 3' UTRs of stable transcripts than in transient transcripts, which have a higher proportion of cytosines, suggesting that stability of mRNA may be due to a difference in the nucleotide content (52). Further, CPE and PBE motifs are prominent in the stable mRNA transcripts, implying the necessity of such motifs for stability (52). A classical example for the importance of cis-motif at the 3' UTR for mRNA stability during oocyte to embryo transition is derived from the spin gene. The mouse 4.1-kb spindlin transcript with an 'embryonic-type CPE (eCPE)' (approximately 1100 nucleotides of the poly(A) signal) in the 3' UTR loses its poly (A) tail during meiotic maturation but regains a poly (A) tail following fertilization, resulting in activation of translation in the embryos (95). An attractive hypothesis is that eCPE alone is sufficient to withstand global cytoplasmic polyadenylation or default degradation during meiotic maturation. Otherwise, the eCPE or transcript-specific 'translation control element' is bound with mRNA-specific trans-factors which are post-

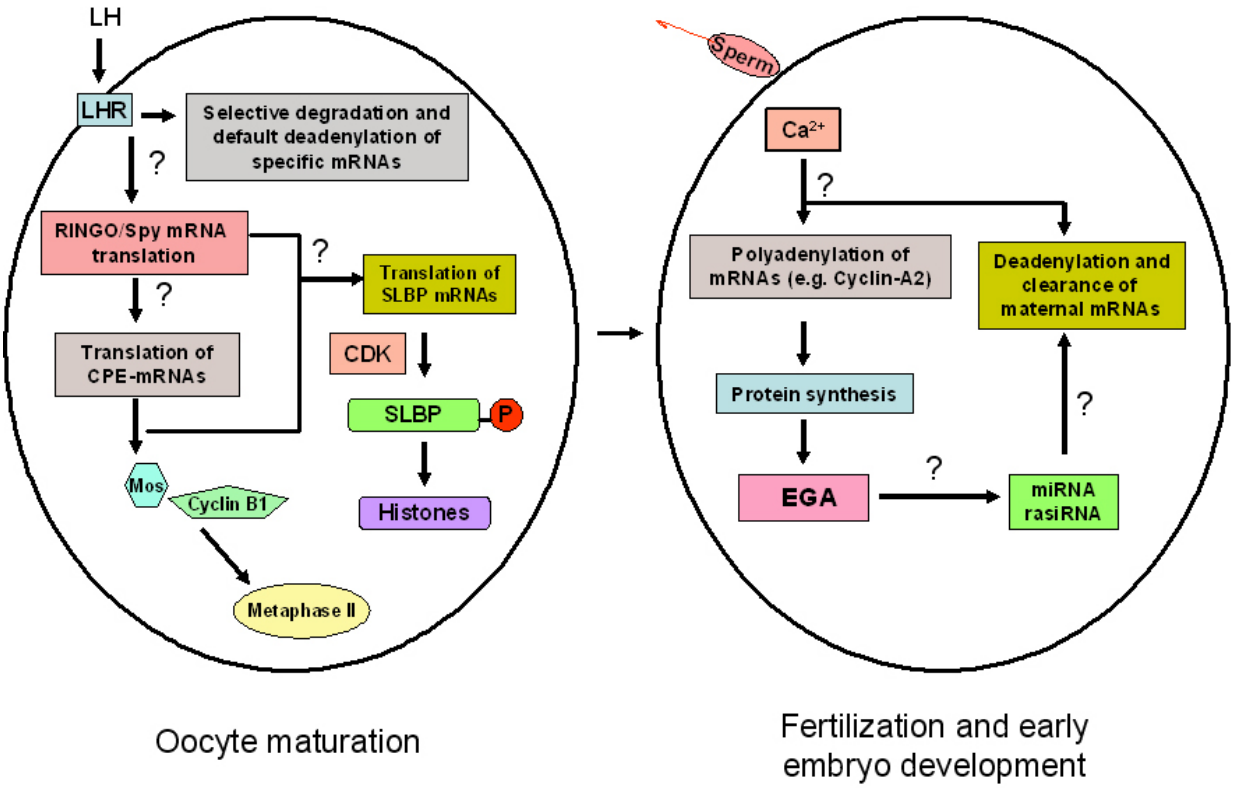

Figure 4. Salient features involved in maternal mRNA regulation during mammalian early development. The onset of meiotic maturation stimulated by the surge release of gonadotropins (Luteinizing hormone, LH) initiates a complex network of translational activation or repression of maternal mRNAs in the oocyte. RINGO/Spy appears to be the first maternal mRNA to be translationally activated. RINGO/Spy protein most likely activates translation of cytoplasmic polyadenylation element (CPE)-containing mRNAs like Mos (a serine threonine kinase) and cyclin B1. The synthesis of Mos and Cyclin B1 further activates a cascade of signaling events required for progression of meiosis and arrest at metaphase II awaiting fertilization. One other important event during meiotic maturation is the synthesis and phosphorylation of stem-loop binding protein (SLBP), which in turn is required for synthesis of endogenous histones. Selective degradation and default deadenylation of specific maternal transcripts also accompanies meiotic maturation. Fertilization triggers a rise in intracellular calcium (Ca^{2+}) oscillations which subsequently mobilizes maternal mRNA for polyadenylation and translation. The synthesis of new proteins programs embryonic genome activation (EGA) and, presumably initiates deadenylation and degradation of untranslated maternal mRNA via activation of microRNA (miRNA) and repeat-associated small interfering RNA (rasiRNA) pathways.

translationally modified following fertilization, thereby releasing the mRNA from repressive effects. This theory may not be universal across mammals, because unlike in mice, EGA occurs much later — at four to eight cells in humans and at eight to sixteen cells in cattle — which requires approximately 50 to 90 hours following fertilization. The recruitment of mRNAs and requirement of proteins may extend for two to three additional embryonic cell cycles, until the genome is activated in a stepwise manner (3). Therefore, signal(s) initiated following fertilization may not be an indicator for global recruitment and translation of maternal mRNAs in embryos of higher mammals. The best speculation is that the translation of maternal mRNA may be selective in each embryonic cycle, based on the regulatory elements present within the mRNA or the groups of RNA-binding factors with which the maternal transcripts are associated. The translational control mechanisms in higher vertebrates are not understood but may be much more complex, well controlled and vital during early embryogenesis.

6. SUMMARY AND PERSPECTIVES

From this brief review, it is clear that significant progress has been made in solving the regulatory mechanisms of translational control in mammalian oocytes and early embryos, but much remains to be learned. Some important insight about mechanisms of mRNA masking has been obtained. An ever growing list of identified trans-factors interacting with 3'-UTR elements indicates that the translation control process is intricate and may not be fully resolved. However, the translation control mechanisms have been studied for only a few maternal mRNAs, and the mechanisms appear to be diverse. Therefore, it is premature to postulate a universal model for mRNA regulation during early development. The known sequential events of maternal mRNA activation during meiotic maturation and fertilization (Figure 4) critical to mammalian development and the specific mediators involved in this process need to be resolved. In mammalian early development, whether

miRNAs and rasiRNAs are involved in translational inhibition of maternal mRNA merits significant investigation (Figure 4). In higher mammals including humans where multiple cell cycles occur before EGA, unlike in mice, perhaps there are multiple well-controlled events to stabilize maternal transcripts until the fate of the mRNA is decided following fertilization. It is possible that numerous convergent mechanisms/forces dictate the fate of a given mRNA and each mRNA may have a different predestined sum of forces.

7. ACKNOWLEDGEMENTS

The authors wish to thank the Rackham Foundation and the Michigan Agricultural Experiment Station for their generous support.

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Abbreviations: EGA: embryonic genome activation; ePABs: embryonic poly(A) binding protein; miRNA: microRNA; rasiRNA: repeat-associated small interfering RNA; tPA: tissue plasminogen activator; PARN: poly(A) specific ribonuclease; DAN: deadenylating nuclease; ZP: zona pellucida; mRNP: maternal ribonucleoprotein particles; FRGY2: frog (Xenopus) germ cell specific Y-box protein 2; MSY2: mouse Y-box protein 2; CDK: cyclin dependent kinase; MPF: maturation promoting factor; MAPK: mitogen-activated protein kinase; MOS: a serine threonine kinase; RINGO: rapid inducer of G2/M progression in oocyte; eIF: eukaryotic initiation factor; PBE: pumilio binding element; Pum-2: pumilio; FBF: Fem-3 mRNA binding factor; PUF: pumilio and FBF; DAZL: deleted in Azoospermia-like; RRM: RNA recognition motif; CPE: cytoplasmic polyadenylation element; CPEB: CPE binding protein; Eif4eloo: eukaryotic translation initiation factor 4E like; CPSF: cleavage and polyadenylation specificity factor; GLD2: germline development deficient; SCP: synaptonemal complex protein; GDF9: growth differentiation factor 9; H1OO: oocyte specific linker histone H1; UTR: untranslated region; RISC: RNA-induced silencing complex; siRNA: small interfering RNA; TE: transposable element; LINE: long interspersed nuclear elements; SINE: short interspersed nuclear elements; LTR: long terminal repeats; IAK1: the murine homologue of aurora-A related kinase 1; SLBP: stem-loop binding protein; PABP: poly(A) binding protein; eCPE: embryonic-type CPE.

Key Words: RNA-binding proteins, MSY2, CPEB, Maskin, Symplekin, ePAB, SLBP, histone mRNA, RINGO, Spy mRNA, *cis*-regulatory element, 3' untranslated region, mRNA, miRNA, rasiRNA, Review

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