Chapter 1
Post-transcriptional Control of Gene Expression During Mouse Oogenesis

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Abstract Post-transcriptional mechanisms play a central role in regulating gene expression during oogenesis and early embryogenesis. Growing oocytes accumulate an enormous quantity of messenger RNAs (mRNAs), but transcription decreases dramatically near the end of growth and is undetectable during meiotic maturation. Following fertilization, the embryo is initially transcriptionally inactive and then becomes active at a species-specific stage of early cleavage. Meanwhile, beginning during maturation and continuing after fertilization, the oocyte mRNAs are eliminated, allowing the embryonic genome to assume control of development. How the mammalian oocyte manages the storage, translation, and degradation of the huge quantity and diversity of mRNAs that it harbours has been the focus of enormous research effort and is the subject of this review. We discuss the roles of sequences within the 3′-untranslated region of certain mRNAs and the proteins that bind to them, sequence-non-specific RNA-binding proteins, and recent studies implicating ribonucleoprotein processing (P-) bodies and cytoplasmic lattices. We also discuss mechanisms that may control the temporally regulated translational activation of different mRNAs during meiotic maturation, as well as the signals that trigger silencing and degradation of the oocyte mRNAs. We close by highlighting areas for future research including the potential key role of small RNAs in regulating gene expression in oocytes.

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1.1 Introduction

Translational control of mRNA plays a central role in regulating gene expression during oogenesis and early embryogenesis. This reflects the developmentally regulated nature of transcriptional activity in oocytes and after fertilization. During their growth phase, mammalian oocytes are transcriptionally active (Fig. 1.1). Near the time that oocytes reach full size, transcription drops to very low or undetectable levels, and it remains so throughout the terminal stage of oogenesis, termed meiotic maturation. Transcription remains low after fertilization until it resumes at a species-specific stage of early embryogenesis—for example, at the late 2-cell stage in mice, 4-cell stage in humans, and 8- to 16-cell stage in the cow. This means that the period of development encompassing late growth of the oocyte until early/mid-cleavage of the embryo depends on mRNAs synthesized during oocyte growth. This poses an enormous regulatory challenge to the growing oocyte, which at full size has accumulated almost twice as much mRNA as is present in a blastocyst. Some newly synthesized mRNAs must be translated to support growth of the oocyte, whereas others must be safely stored and then translationally activated at the appropriate stage of late oogenesis or early embryogenesis. In addition, these oocyte (maternal) mRNAs must subsequently be degraded to permit the newly transcribed embryonic mRNAs to assume control of development. How the mammalian oocyte manages the storage, translation, and degradation of the huge quantity and diversity of mRNAs that it harbours has been the focus of enormous research effort and is the subject of this review.

1.2 A Brief Primer on Translational Control

Newly transcribed mRNAs typically undergo three major processing events within the nucleus—splicing, capping, and polyadenylation (some mRNAs, notably those encoding the replication-dependent histones, are neither spliced nor polyadenylated). The latter two are particularly relevant to translation. Capping describes the addition of a 7-methylguanosine structure (m_7GpppN) to the 5′-end of an mRNA during transcription (Cowling 2009). Polyadenylation, by contrast, occurs at the 3′-end. A polyadenylation signal (AAUAAA) in the 3′-untranslated region of the primary mRNA signals recruitment of a protein termed cleavage and polyadenylation specificity factor (CPSF) to the 3′-end. CPSF in turn both triggers cleavage of the transcript at a site 3′ to the polyadenylation sequence and recruits polyA polymerases (PAPs) that catalyse the addition of ~250 adenosine nucleotides to the 3′-end of the mRNA (Millevoi and Vagner 2010). Six mammalian PAPs have been identified: PAPα (*Papola*) and PAPγ (*Papolg*) in the nucleus, GLD2 (*Papd4*) and GLD4 (*Papd5*) in the cytoplasm at least in some cell types, and two that are restricted to round spermatids and mitochondria, respectively (Burns et al. 2011;

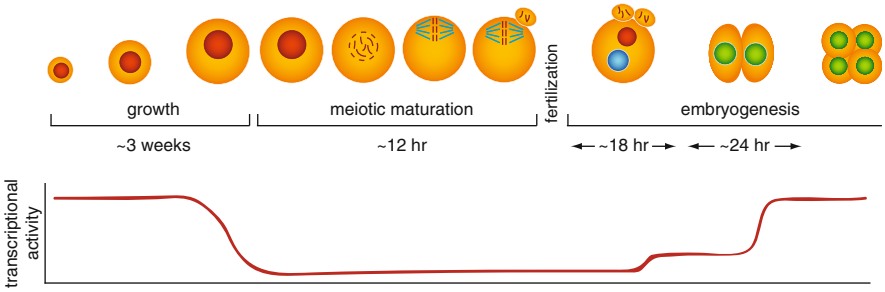

Fig. 1.1 Ooogenesis, early embryogenesis, and transcriptional activity. Time periods indicated correspond to the mouse. During oocyte growth, which requires about 3 weeks, the oocyte is at late prophase I of the cell cycle and is transcriptionally active. Transcription drops substantially near or at the end of the growth phase. During meiotic maturation, which spans about 12 h, the oocyte enters M-phase, completes the first meiotic division, and then arrests at metaphase II. Transcription is not detectable during this time. Following fertilization, weak transcription can be detected during the first cell cycle, which spans about 18 h. The major activation of transcription occurs at G2 of the 2-cell stage. Hence, late oogenesis and early embryogenesis depend on mRNAs that have been transcribed during oocyte growth

Nakanishi et al. 2006; Schmid et al. 2009). Capped and polyadenylated mRNA is exported to the cytoplasm.

In the cytoplasm, the 5′-end of the RNA interacts with the cap-binding protein, eIF4E (Abaza and Gebauer 2008; Groppo and Richter 2009; Jackson et al. 2010; Richter and Sonenberg 2005). At the other end, polyA-binding protein (PABP) associates both with the poly(A) tail and, through its N-terminal region, with eIF4G. eIF4G and eIF4E together with eIF4A form the cap-binding complex, eIF4F, which in turn binds via eIF3 to the 40S ribosomal subunit. Through the association of eIF4G with PABP, the mRNA becomes pseudo-circularized, which renders its translation substantially more efficient (Fig. 1.2). Thus, long or short poly(A) tails are generally correlated with high or low translational activity, respectively. Nonetheless, certain mRNAs have been identified whose translation is associated with deadenylation or is independent of polyadenylation (Kleene et al. 1984; Tadros et al. 2007; Vardy and Orr-Weaver 2007a).

Translational inactivation and subsequent degradation of an mRNA comprises three steps. First, deadenylases shorten the poly(A) tail. Three families of deadenylases have been identified: the CNOT (also known as CCR4-NOT) family, the PAN2-PAN3 heterodimer, and the poly(A)-specific ribonuclease (PARN) (Goldstrohm and Wickens 2008). It has been proposed that PAN2–PAN3 shortens the tail from several hundred to about 100 nt, after which CCR4-NOT removes most or all of the remaining tail (Yamashita et al. 2005). PARN is mainly nuclear, but could also contribute to cytoplasmic deadenylation. Numerous regulatory proteins as well as short RNAs can influence the activity of these deadenylases (Goldstrohm and Wickens 2008; Standart and Jackson 2007; von Roretz and Gallouzi 2008). Deadenylation is followed by, and may be required for, removal of the 5′-cap by decapping enzymes (Franks and Lykke-Andersen 2008; Garneau

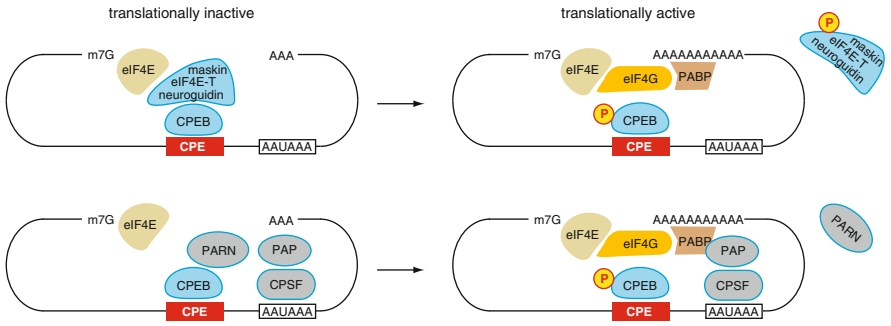

Fig. 1.2 Two models of CPE-dependent translational regulation. *Upper*: In translationally inactive mRNA, an adaptor protein such as maskin, eIF4-T, or neuroguidin associates with both CPEB and eIF4E, thus preventing interaction between eIF4E and eIF4G. During meiotic maturation, CPEB becomes phosphorylated and the adaptor protein no longer inhibits eIF4E–eIF4G interaction, permitting translational activation of the mRNA. *Lower*: In translationally inactive mRNA, both deadenylases (PARN) and adenylases (PAP) are associated directly or indirectly with CPEB and the mRNA carries a short poly(A) tail. During maturation, the deadenylase is 'ejected' from the complex, permitting elongation of the poly(A) tail and translational activation of the mRNA. Other factors in the complexes are not shown for clarity. These models are not necessarily mutually exclusive and other models of CPE-dependent regulation exist. Modified from Radford et al. (2008)

et al. 2007). The deadenylated and decapped RNA is then degraded by nucleases that digest it from both the 5′- and 3′-ends. Although the signals that target a translationally active mRNA for degradation are not fully understood, PABP can attract PAN deadenylases to the mRNA. This suggests two points. First, the length of the poly(A) tail of an mRNA, and hence its translational efficiency, may be determined by the relative strength of competing PAPs and deadenylases that act simultaneously on it. Second, translation of an mRNA may promote its deadenylation and subsequent degradation, thus providing a translation-coupled pathway of mRNA turnover.

Complementing these studies of the biochemical basis of mRNA metabolism, recent work has addressed where in the cell these events occur and specifically the potential role of cytoplasmic ribonucleoprotein particles (RNPs) (Balagopal and Parker 2009; Eulalio et al. 2007; Kulkarni et al. 2010; Parker and Sheth 2007). Microscopically visible RNPs have been observed in a variety of cell types and given different names; here, they will be termed (processing) P-bodies. Although the molecular composition of these large RNPs likely varies depending on cell type, they typically harbour many of the enzymes involved in mRNA metabolism, including deadenylases, decapping factors, and exonucleases, as well as factors that modulate these activities. miRNAs and proteins involved in miRNA-mediated RNA silencing, such as Argonaute proteins and GW182 (TNRC6A), have also been detected in P-bodies and recently discovered to interact with PABPs and with deadenylases (Tritschler et al. 2010) and with both PAN2-PAN3 and CCR4-NOT (Braun et al. 2011; Chekulaeva et al. 2011; Fabian et al. 2011). Based on their composition and several lines of experimental evidence, it has been proposed that

P-bodies can act as sites of mRNA degradation. mRNAs localized in P-bodies can also return to the translationally active pool, however, suggesting a more general function as sites to which (some) mRNAs are transported for subsequent storage or degradation. Nevertheless, it remains unresolved whether P-bodies, by providing locally high concentrations of enzymes and co-factors, are the sites of processes such as deadenylation and decapping or, alternatively, are aggregates that serve no regulatory function but simply become detectable when a large quantity of non-polysomal mRNA is present.

1.3 Translational Control During Oocyte Growth

Although non-growing oocytes are transcriptionally active, entry into the growth phase is accompanied by dramatic changes in the pattern of gene expression (Pan et al. 2005). The growing oocyte remains transcriptionally active until near the end of growth when activity drops considerably. Transcriptional arrest is correlated with a change in the nuclear distribution of the chromatin, termed the non-surrounded nucleolus (NSN) to surrounded nucleolus (SN) configuration (De La Fuente 2006). Many of the mRNAs synthesized in growing oocytes are immediately translated; however, a significant and developmentally crucial fraction—estimates run as high as 30 % (Piqué et al. 2008)—are translationally repressed until meiotic maturation or after fertilization. Notably, these repressed mRNAs are not degraded but instead are stored in a stable form; bulk oocyte mRNA in mouse oocytes has a remarkably long half-life estimated at 8–12 days (Brower et al. 1981). Considerable work, both in mammals and non-mammalian organisms, has focused on the mechanisms by which these silent mRNAs are translationally repressed and stored.

The best-known mechanism of translational repression operates through the cytoplasmic polyadenylation element (CPE, also known as the adenylation control element [ACE]) (Brook et al. 2009; Kang and Han 2011; Radford et al. 2008). This U-rich element (consensus: UUUUA(A)U) is located in the 3′-utr within about 200 nt of the polyadenylation signal and was first identified in mRNAs known to be translationally repressed in growing oocytes and activated during meiotic maturation (Fox et al. 1989; Huarte et al. 1992; McGrew et al. 1989). Its role has been clearly demonstrated using reporter mRNAs carrying the 3′-utr of the mRNA of interest—insertion of a CPE represses translation whereas mutation of existing CPE activates translation (Huarte et al. 1992; Tay et al. 2000). Although these experiments were carried out using fully grown immature oocytes, it is believed that the CPE functions in the same way in growing oocytes. The CPE is bound by the CPE-binding protein (CPEB), a 62-kDa protein first identified in oocytes of *Xenopus* and having homologues in a wide range of species. CPEB is highly conserved, particularly in the C-terminal portion that contains two RNA recognition motifs and a Zn-finger domain (Gebauer and Richter 1996; Welk et al. 2001). Vertebrates also contain related proteins, CPEB-2, -3, and -4 that are expressed in

different cell types including oocytes and show different RNA-binding preferences (Standart and Minshall 2008). The crucial role of CPEB-dependent translational repression in maintaining the normal programme of oocyte development is illustrated by the severe abnormalities in growing oocytes that lack CPEB (Racki and Richter 2006).

Many lines of evidence support the link between a CPE-dependent short poly(A) tail and translational repression. First, although mRNAs containing a CPE are initially provided with a long poly(A) tail in the nucleus, this is rapidly removed following their export to the cytoplasm leaving a short tail of 20–40 bases (Huarte et al. 1992; Kim and Richter 2006). Second, mRNAs provided with a long poly(A) tail in vitro, but not a short tail, are translated following injection into immature oocytes. Third, overexpression of *Gld2* in immature oocytes of *Xenopus* lengthens the poly(A) tail of mRNAs including *Ccnb1* and *Mos,* both of which contain CPE sequences (Nakanishi et al. 2006), consistent with the model that competing polymerase and deadenylase activities determine the steady-state length of the poly(A) tail. Intriguingly, when a competitor RNA containing a CPE was injected into immature oocytes, endogenous mRNAs containing a CPE became translationally activated even though their mRNA did not become polyadenylated (Stutz et al. 1998). Although the large excess of competitor that was required means that the results should be interpreted with caution, this suggests that translational repression may depend on more than shortening of the poly(A) tail.

While the role of CPEB in mediating translational repression is firmly established, its mechanism remains to be fully elucidated (Fig. 1.2). *Xenopus* oocytes contain a protein termed maskin that is able to bind both CPEB and eIF4E. This prevents eIF4E from binding to eIF4G, and translation is consequently repressed. Although the mammalian maskin homologue, TACC4, is expressed in oocytes (Yang et al. 2010), the site to which eIF4E binds is not conserved, implying that this mechanism may not operate in mammals. Neuroguidin, originally identified in cells of the nervous system, is also able to bind CPEB and eIF4E and to repress translation (Jung et al. 2006), and *Ngd* is expressed in mouse oocytes (http://www.ncbi.nlm.nih.gov/geoprofiles), suggesting that it could serve the same function as maskin. In addition, at least two other mechanisms through which CPEB may repress translation have been identified. CPEB in amphibians can bind to the PARN deadenylase, suggesting a simple model by which it represses translation. Although most PARN appears to be nuclear in amphibian oocytes (Kim and Richter 2006; Standart and Minshall 2008) and in mammalian somatic cells and oocytes (K.-F. Vieux & H.C., unpublished) it is possible that a quantitatively small fraction in the cytoplasm is sufficient to serve this function. CPEB can also interact with 4E-T (eIF4E-transporter), a protein that in turn binds to eIF4E (eIF4E1b in oocytes (Evsikov et al. 2006)) and prevents the latter from interacting with eIF4G (Richter and Sonenberg 2005; Standart and Minshall 2008). This would inhibit pseudocircularization of the mRNA thus repressing translation, although it does not seem to account for the shortening of the poly(A) tail. These interactions have been established biochemically in *Xenopus* and *Drosophila*, whose 4E-T homologue is termed Cup, and 4E-T is expressed in mouse oocytes (Villaescusa et al. 2006).

The central role of CPEB in translational repression in immature oocytes does not preclude a role for other mechanisms. The PUmilio-Fem-3-binding factor (PUF) family of RNA-binding proteins are found throughout the plant and animal kingdoms (Wickens et al. 2002). They are characterized by the presence of eight repeats of a ~40-amino acid sequence, termed the Pumilio-homology domain, that are located in the C-terminal region of the proteins. Each of the eight repeats contacts a different RNA base in the PUF-binding element (PBE; consensus UGUANUAU) that is found in many translationally repressed mRNAs. A clue to their mechanism of repression may lie in the observation that they can bind directly to the CNOT8 deadenylase in vitro (Goldstrohm et al. 2006; Morris et al. 2008). Intriguingly, through their conserved C-terminal region, PUF proteins can also bind to CPEB (Nakahata et al. 2001). Moreover, many mRNAs that contain CPEs also contain putative PBEs (Piqué et al. 2008; Radford et al. 2008). Thus, PUF proteins might repress translation of PBE-containing mRNAs either independently or in co-operation with CPEB (Piqué et al. 2008). Indeed this cooperativity has been demonstrated in *Xenopus* for cyclin B1 mRNA (Nakahata et al. 2003).

Two PUF proteins exist in mammals, PUM1 and PUM2. PUM1 is expressed in a wide variety of cell types, whereas PUM2 is expressed only in ES cells, testis, and the ovary where it is restricted to the oocyte (Moore et al. 2003). Although mice bearing a mutation in *Pum2* are fertile (Xu et al. 2007), these observations are consistent with a potential role for PUM proteins in translational control in mammalian oocytes.

The factors discussed so far repress translation through binding to specific sequences in the 3′-utr of specific target mRNAs. A different paradigm of translational control may be illustrated by YBX2 (formerly MSY2). This protein is very abundant in oocytes but rapidly degraded during early embryogenesis, implying that its function is restricted to oogenesis (Yu et al. 2001). Mice lacking YBX2 are hypo-fertile, and their oocytes manifest a variety of abnormalities during growth (Medvedev et al. 2011; Yu et al. 2004). Although the function of YBX2 is not yet fully understood, its Y-box contains a conserved cold-shock domain that includes RNA-binding motifs and Y-box proteins in amphibian oocytes associate with and repress translation of numerous mRNAs. Mammalian YBX2 displays some sequence preference in RNA binding, but also binds to a wide range of mRNAs in vitro, suggesting that it may act as a sequence-independent RNA-binding protein (Yu et al. 2002). The observations that oocytes lacking YBX2 have a reduced quantity of mRNA and that YBX2 is associated with the detergent-insoluble fraction of oocytes suggest that it may be part of a complex that sequesters and stabilizes translationally repressed mRNAs. It would be interesting to test whether mRNAs that are normally repressed in growing oocytes become translationally activated in the absence of YBX2.

The potential role of P-bodies in translational control in somatic cells raises the possibility that they play important roles in oocytes as well. Supporting this idea is the presence of RNP complexes in the oocytes of a variety of non-mammalian species. Recent work in *C. elegans* has identified structures termed storage bodies that may be derived from the well-known germinal (P-) granules and are defined by their position in the oocyte, mechanism of assembly, and presence of an RNA

helicase, CGH-1 (Boag et al. 2008). CGH-1 associates with a subset of oocyte mRNAs and, in *cgh-1* mutants, these mRNAs are depleted. These observations suggest that CGH-1 in the storage bodies may protect this subset of mRNAs—perhaps those that will be translationally activated later during development—from degradation. Oocytes of *Xenopus* contain RNPs whose protein composition shows a remarkable resemblance to P-bodies. Intriguingly, these oocyte P-bodies also contain CPEB, providing circumstantial evidence that they also may store translationally silent mRNA.

Two recent studies have addressed P-bodies in mammalian oocytes. In the first, GFP-tagged DCP1A was expressed in fully grown immature oocytes, where it became localized in many small and a few large cytoplasmic foci (Swetloff et al. 2009). Staining using anti-DCP1A revealed only the large foci. A subset of the GFP-DCP1A foci were also stained by antibodies recognizing the P-body components DDX6, an RNA helicase that is orthologous to CGH-1, and RAP55, an RNA-binding protein. CPEB, however, could not be immunologically detected. In the second study (Flemr et al. 2010), several P-body components (AGO2, DCP1A, DDX6, GW182) were found to co-localize in cytoplasmic foci in growing oocytes. YBX2, an RNA-binding protein discussed earlier, was also present in these foci. In oocytes larger than 50 μm diameter, however, these foci were not detected. Instead, at least for DDX6, GW182, and YBX2, staining was enriched in the periphery of the oocyte, in a narrow band that the authors term the subcortical RNP domain (SCRD). Interestingly, c-*mos* mRNA, which is translationally repressed in immature oocytes, also localized at the oocyte periphery, although technical limitations mean that co-localization with specific SCRD components could not be tested.

Both studies indicate that mammalian oocytes contain structures that biochemically and functionally resemble P-bodies, but obviously differ with respect to the dynamics of their assembly and localization during oocyte growth. It is interesting to note that in *Xenopus*, maskin and PARN are not detectable until late in oogenesis, whereas P-bodies may be more abundant at earlier stages (Minshall et al. 2007). Hence, there is precedent for the notion that oocytes at different stages of growth may employ different mechanisms to silence and store mRNAs. Nonetheless, especially in view of the similarities between P-bodies and stress granules and the crucial supporting role of the granulosa cells in oogenesis, it will be important to verify that oocytes growing in vivo also contain P-bodies.

Mouse oocytes also contain RNA- and protein-containing fibrillar matrices, termed cytoplasmic lattices (CPLs) (Capco et al. 1993; Lehtonen et al. 1983). When PADI6, a peptidyl arginine deiminase, was found to co-localize with the lattices (Wright et al. 2003), this opened the way to experimental analysis of their function. In oocytes of *Padi6*−/− mice, the lattices cannot be detected and ribosomal components—specifically rRNA and protein S6—display increased solubility (Yurttas et al. 2008). This has led to the proposal that the CPLs may be storage sites for the large fraction (~70%) of oocyte ribosomes that are not in polysomes (Bachvarova and De Leon 1977). Very recent studies have identified PADI6 within a large protein complex that includes FLOPED, MATER, TLE6, and Filia (Li et al. 2008). Oocytes lacking any of these genes give rise to embryos that arrest during

early cleavage, consistent with the proposal that the CPL-associated ribosomes are required for protein synthesis in the early embryo. Although protein synthesis in the oocyte is not quantitatively affected in the absence of PADI6 (Yurttas et al. 2008), it is conceivable that the CPLs play a role in storing silent mRNAs that will be translationally activated following fertilization. Finally, it should be noted that a sub-population of mRNAs is preferentially associated with the spindle region in mature eggs, validating the concept that mRNAs can be differentially distributed within the cytoplasm of mammalian germ cells (VerMilyea et al. 2011) as also demonstrated in amphibians (Eliscovich et al. 2008).

What about the very large population of oocyte mRNAs that are actively translated in growing oocytes? It is generally assumed that this represents a default pathway—an mRNA will be translated unless some structural element such as a CPE acts to repress translation—and mRNAs known to be translated typically do not possess such sequences in the region of the 3′-utr close to the polyadenylation signal. Despite its repressive activity, however, not all mRNAs that contain a CPE are completely silent in immature oocytes. For example, mouse immature oocytes contain a small quantity of cyclin B1, indicating that even though *Ccnb1* is activated during maturation, it is nonetheless weakly translated in immature oocytes (Holt et al. 2010; Marangos and Carroll 2008). Since keeping cyclin B1 at a low level is essential to prevent precocious initiation of maturation, this weak translation likely reflects incomplete repression. A more telling example is the stem-loop-binding protein (SLBP). Growing oocytes absolutely require SLBP to accumulate mRNAs encoding the histones that will be used to assemble chromatin during the embryonic cell cycles (Arnold et al. 2008). Yet, *Slbp* contains a functional CPE that represses its translation in growing oocytes (Yang et al. 2010). How are immature oocytes able to overcome the repressive activity of the CPE and produce the SLBP that they need?

One explanation may be that *Slbp* contains other sequences that enable a sufficient level of expression to be achieved in spite of the repressive effects of the CPE. We have found that the 3′-utr of *Slbp* confers a higher translational activity to a reporter RNA than does the 3′-utr of *Ccnb1* (Q. Yang and H.J.C., unpublished). Within the *Slbp* 3′-utr is a highly conserved U-rich sequence that is not present in the *Ccnb1* 3′-utr. Mutation of the sequence reduces the activity conferred by the *Slbp* 3′-utr, whereas its insertion in to the *Ccnb1* 3′-utr increases activity. This suggests that the translation of an mRNA is determined by net activity of positive and negative regulatory elements. This could provide a mechanism for the oocyte to regulate the supply of proteins whose steady-state quantity needs to be restricted within a narrow range.

1.4 Translational Control During Oocyte Maturation

Meiotic maturation is the final stage of oocyte development before fertilization (Fig. 1.2). During maturation the nuclear envelope becomes disassembled (germinal vesicle breakdown, GVBD), releasing the contents of the nucleus into the cytoplasm.

The chromosomes condense, become aligned on the first meiotic spindle, and the oocyte undergoes the first meiotic division. The oocyte chromosomes then become aligned on the second meiotic spindle, at which point meiosis becomes arrested until the egg is activated by fertilization. In contrast to oocyte growth, which is a protracted process requiring several weeks or months, depending on the species, oocyte maturation is relatively rapid, spanning ~12 h in mouse and ~36 h in humans. Importantly for this discussion, it is characterized by significant changes in translational activity.

About 25 years ago, a series of papers appeared demonstrating that, during oocyte maturation in mice, the mRNA encoding tissue plasminogen activator (tPA) becomes elongated and translationally activated (Huarte et al. 1987; Strickland et al. 1988). Further work demonstrated that the lengthening is due to an increase in the length of the poly(A) tail and that this increase depends on a sequence in the 3′-utr subsequently identified as the CPE (Stutz et al. 1997, 1998; Vassalli et al. 1989). Since then, numerous mRNAs have been identified that become translationally activated during maturation, and these consistently carry a CPE that is necessary for activation (Dai et al. 2005; Gebauer et al. 1994; Gershon et al. 2006; Murai et al. 2010; Oh et al. 2000; Salles et al. 1992; Tay et al. 2000; Tremblay et al. 2005; Yang et al. 2010). In addition to the CPE, polyadenylation also requires the nuclear polyadenylation signal. Conversely, mRNAs such as *Actb* that do not carry a CPE near the polyadenylation signal become deadenylated and translationally silenced (Bachvarova et al. 1985; Yang et al. 2010; Chen et al. 2011). These results suggested a simple 'binary-switch' model in which mRNAs bearing a CPE are silent in growing and fully grown immature oocytes and then activated in maturing oocytes, whereas mRNAs lacking a CPE show the opposite behaviour.

Recent work comparing the mRNAs associated with polysomes, as a marker of translational activity, in immature (GV-stage) and mature oocytes has extended and refined this model (Chen et al. 2011). Of the ~7,600 mRNAs analysed, about two-thirds showed a less than twofold change and were defined as stable. The remaining one-third were about equally divided between those becoming more abundant (translationally activated) or less abundant (translationally repressed) during maturation. Comparison of the 3′-utr of the entire mRNA population revealed that several motifs, including the CPE and a sequence resembling the consensus DAZL-binding sequence (see below), were enriched in those recruited to polysomes. Conversely, mRNAs whose 3′-utr lacked either of these sequences became less abundant on the polysomes. These studies extend the role of the CPE in translational activation to a population-wide basis and implicate other sequences in the 3′-utr, but also highlight that many mRNAs are associated with polysomes in both immature and mature oocytes.

The mechanism by which the CPE switches from repressing to activating translation has been carefully studied in amphibians (Radford et al. 2008). In one model, maskin or an analogous protein becomes phosphorylated, weakening its interaction with eIF4E and thus permitting assembly of a translationally active complex (Fig. 1.2). A non-exclusive second model focuses on a shift in the balance of power within the protein complex bound via CPEB to the CPE from the deadenylase PARN to the cytoplasmic PAP, GLD-2. The first event is phosphorylation of CPEB

on Ser-174 (Thr-171 in mice). Although there is not yet consensus regarding the responsible kinase—both Aurora A (Hodgman et al. 2001; Mendez et al. 2000a) and calmodulin kinase II (Radford et al. 2008) have been proposed—this event occurs well before the activation of cyclin-dependent kinase-1 (CDK1) and thus precedes GVBD. Phosphorylation of CPEB increases the strength of its binding to CPSF and to GLD-2, which leads to 'ejection' of PARN from the protein complex at the 3′-end of the mRNA, thus permitting GLD-2-mediated elongation of the poly(A) tail (Barnard et al. 2005; Mendez et al. 2000a, b). The longer poly(A) tail attracts the embryonic poly(A)-binding protein (ePAB) that interacts with eIF4G and thereby increases the affinity of the eIF4F complex for the cap structure and hence the efficiency of translation. CPEB phosphorylation may serve a second function through its effect to decrease its affinity for Pumilio proteins, which would be predicted to relieve translational repression. Expression of a mutant CPEB lacking Ser-174 acts in a dominant-negative manner to inhibit polyadenylation (and maturation), confirming the essential role of CPEB phosphorylation in translational activation. After CDK1 has become activated and GVBD has occurred, CPEB becomes phosphorylated by a CDK-dependent mechanism. This leads to its ubiquitination and degradation (Reverte et al. 2001; Setoyama et al. 2007), which is required for certain mRNAs to become polyadenylated during the later stages of maturation (Mendez et al. 2002).

Although less well studied in mammals owing to the small amount of material that can be obtained, the available evidence (references cited earlier) suggests that CPEB plays a similar role during the translational activation of silent mRNAs in maturing oocytes. Despite the broad similarity, certain differences between mammals and amphibians are worth noting. Unlike amphibians, where the crucial Ser-174 phosphorylation precedes activation of CDK1, there is no detectable phosphorylation of CPEB in mammalian oocytes until after GVBD (Chen et al. 2011; Uzbekova et al. 2008; Yang et al. 2010). This relatively late phosphorylation of CPEB does not, however, challenge the importance of this event in driving polyadenylation and translational activation, as no mRNAs have yet been demonstrated to become translationally activated before or independently of CPEB phosphorylation. In addition, although CPEB becomes degraded during maturation in mammals as in amphibians (Chen et al. 2011; Uzbekova et al. 2008; Yang et al. 2010), it is unknown whether this is required to activate translation of specific mRNAs. Finally, although *Gld2* mRNA is present in oocytes, it is translationally repressed until maturation (Nakanishi et al. 2006) and, more importantly, deletion of the gene does not inhibit polyadenylation during maturation (Nakanishi et al. 2007). These results seem to rule out GLD2 as the PAP responsible for mRNA polyadenylation during maturation, at least in the mouse. Although canonical PAPα, which would be released from the nucleus at GVBD, might provide the activity, in vitro studies indicate that it is inactivated by phosphorylation during maturation (Colgan et al. 1996) and experiments in *Xenopus* show that prior removal of the nucleus does not prevent polyadenylation during maturation (Fox et al. 1989). The recently identified GLD4 (see above) is an alternative candidate.

Comparative analysis of different mRNAs has revealed that they do not become polyadenylated at the same time during maturation. In *Xenopus*, for example, the

mRNA encoding Mos becomes polyadenylated before GVBD but those encoding different cyclin B proteins not until metaphase I (Piqué et al. 2008). What determines the difference in timing? Piqué et al (2008) synthesized 3′-utr sequences that contained different combinations and spacings of CPE, PBE, and polyadenylation signals and injected mRNAs carrying each 3′-utr into *Xenopus* oocytes. They then determined when each became polyadenylated and translated. Based on the results, they derived a code according to which the specific arrangement of these three elements determines at what stage of maturation an mRNA will become translationally activated.

Analysis of mRNAs recruited to polysomes during oocyte maturation in the mouse identified several motifs including the CPE that were enriched in these mRNAs, but did not reveal a specific code analogous to that in the frog (Chen et al. 2011). Instead, another mechanism by which different mRNA species are sequentially activated was uncovered. Among the enriched motifs was the consensus binding site (UUU[C/G]UUU) for the DAZ family of proteins. *Dazl* contains a putative CPE and is translationally activated during maturation, leading to an increase in the amount of DAZL (see also (Liu et al. 2009)). Next, it was shown that DAZL immunoprecipitates contained numerous mRNAs (including *Dazl* itself) possessing putative DAZL-binding elements, and in the case of a known DAZL target, *Tex19.1*, these were confirmed to be functional. Finally, when translational activation of *Dazl* was inhibited, oocyte maturation was disrupted as illustrated by an absent or grossly abnormal metaphase II spindle. The authors propose that *Dazl* becomes translationally activated during early maturation by a classical CPEB-regulated mechanism, leading to the accumulation of DAZL, which subsequently activates ‘late’ translation of DAZL-responsive mRNAs. This provides an elegant mechanism by which activation of different mRNAs can be temporally regulated during maturation.

1.5 Translational Silencing and Degradation of Oocyte mRNAs

Although the continuing transcriptional silence following fertilization means that early embryogenesis relies on mRNAs synthesized in growing oocytes, and some stored mRNAs are not translationally activated until after fertilization, the elimination of this maternal legacy in preparation for the transition to embryonic control of development begins during meiotic maturation. Early studies showed that as much as half of the mRNA in fully grown oocytes becomes either deadenylated or degraded during maturation (Bachvarova et al. 1985). More recent microarray and polysomal analyses (Chen et al. 2011; Su et al. 2007) suggest that when the number of mRNA species rather than bulk quantity of mRNA is assessed, approximately ¼ are affected. These results clearly establish that a large subset of mRNAs, which presumably have remained stable and polyadenylated throughout the extended period of oocyte growth, become rapidly targeted for deadenylation and/or degradation when maturation begins. What triggers these processes and why some mRNAs are targeted and others spared remain largely mysterious.

Some recent studies, however, have begun to shed light on the details and the underlying molecular control.

YBX2 (MSY2) was discussed earlier in the context of its potential role in stabilizing mRNA in growing oocytes. During maturation, YBX2 becomes phosphorylated by a CDK1-dependent mechanism and this is associated with a decrease in the amount of YBX2 and mRNA present in the detergent-insoluble cytoplasmic fraction (Medvedev et al. 2008). Expression of a non-phosphorylatable YBX2 in maturing oocytes prevents the decrease in some mRNAs that normally occurs during maturation. Conversely, insertion of acidic residues into putative phosphorylation sites triggers a decrease in some mRNAs in immature oocytes. These results suggest that upon phosphorylation, YBX2 might lose its ability to protect mRNAs from degrading activities present in the cytoplasm. It would be interesting to learn whether mRNAs that are translationally active during growth or maturation, respectively, are differentially sensitive to the loss of YBX2 activity.

As discussed earlier, mRNA degradation in somatic cells is preceded by and tightly linked to deadenylation. A simple model is that mRNAs lacking a CPE become deadenylated and degraded during oocyte maturation, whereas those bearing a CPE are polyadenylated and stable (at least during maturation). We have observed three patterns of polyadenylation during maturation (Yang et al. 2010; Q.Y. and H.J.C., unpublished). As expected, mRNAs that become translationally silenced during maturation, such as *Actb*, become deadenylated. Some mRNAs that become translationally activated during maturation, such as *Ccnb1*, become continuously polyadenylated during maturation. Some of these will be deadenylated after fertilization, a process mediated in amphibians by the embryonic deadenylation element (EDEN) (Paillard et al. 1998) and its associated protein, EDEN-BP, in a cell cycle-dependent manner (Detivaud et al. 2003). In contrast, others such as *Slbp* and *Orc6l* become polyadenylated and activated during early maturation and then deadenylated during late maturation. Importantly, those mRNAs that become deadenylated are not degraded to a greater extent than those that remain polyadenylated. Consistent with these results, deadenylated actin mRNA was previously reported to remain stable in mature oocytes (Bachvarova et al. 1985; Paynton et al. 1988) and a substantial fraction of the mRNAs removed from polysomes during maturation are not degraded (Chen et al. 2011). Thus, deadenylation of mRNAs during maturation does not necessarily target them for immediate degradation as also observed in *Xenopus* (Audic et al. 1997).

Work in amphibians indicates that PARN, released into the cytoplasm at GVBD, is responsible for most of the mRNA deadenylation that occurs during maturation (Copeland and Wormington 2001; Korner et al. 1998). In mammals, analysis of a small set of mRNAs revealed that these were not deadenylated until after metaphase I of maturation (Yang et al. 2010). Similarly, analysis of mRNAs released from polysomes indicates that although some are degraded before metaphase I, most appear to remain stable until after metaphase I (Chen et al. 2011). This suggests that the activities responsible for deadenylation and degradation do not appear until late maturation. Recent work in amphibians offers a potential clue to understanding this temporal control. The tristetraprolins are Zn-finger proteins that

can bind AU-rich elements (ARE) found in many short-lived mRNAs. C3H-4, a tristetraprolin in *Xenopus* that is encoded by a CPE-containing mRNA, is synthesized during oocyte maturation and is required, likely by recruiting deadenylases, for deadenylation of specific oocyte mRNAs (Belloc and Mendez 2008). By analogy, perhaps a functionally similar protein that is newly synthesized during early maturation leads to deadenylation and or degradation of specific mRNAs during late maturation. According to this model, mRNAs carrying a binding site for the protein (analogous to the ARE site recognized by C3H-4) would be selectively targeted through this mechanism. It should be noted, however, that ARE sites have not been linked to mRNA degradation during maturation in mammals (Thelie et al. 2007). Another candidate is the decapping protein, DCP1A, which accumulates during maturation owing to translational activation of its mRNA (Flemr et al. 2010) and could be a limiting factor that controls mRNA degradation during late maturation.

1.6 What's Next for Translational Control?

What does the future hold for studies of translational control in oocytes? An immediate challenge is to determine whether silent mRNAs are stored in specific cytoplasmic structures and, if so, whether their stability depends on these structures. The localization of *mos* mRNA in a sub-cortical domain in mouse oocytes is consistent with this possibility, and the co-localization of CPEB with P-bodies in somatic cells and amphibian oocytes suggests a mechanism by which the mRNAs could be recruited. The possibility that the mechanisms regulating mRNA storage and activity differ in oocytes at different stages of growth, evoked both by the P-body studies described earlier and by the observation that neither maskin nor PARN is present in mid-growth stage amphibian oocytes (Flemr et al. 2010; Minshall et al. 2007), needs to be explored. Although the role of the CPE and CPEB is long established, much remains to be learned about how they work both in immature and maturing oocytes. For example, KHDC1B is a newly identified RNA-binding protein that can interact with CPEB and whose oocyte-specific expression suggests a function in translational control (Cai et al. 2010). The expression of PUM1 and PUM2 in oocytes suggests a role for these proteins also. In addition, numerous other CPE-independent mechanisms of translational control that have been identified in non-mammalian oocytes may play key roles in regulating translation in growing, fully grown, or maturing mammalian oocytes (Charlesworth et al. 2006; Sugimura and Lilly 2006; Vardy and Orr-Weaver 2007b).

Finally, might there be a role for small RNAs? In the zebrafish, a specific micro (mi) RNA regulates deadenylation and degradation in embryos of a specific subset of maternal mRNAs (Giraldez et al. 2006). Similarly, embryonically expressed Piwi-interacting (pi) RNA is required in *Drosophila* for deadenylation and degradation of maternal *nos* mRNA (Rouget et al. 2010). It appears to act through a complex that includes an Argonaute protein, the CCR4 (CNOT 6) deadenylase, and

Smaug, a protein previously implicated in the degradation of maternal mRNA in early embryos (Tadros and Lipshitz 2009). Moreover, the recent discovery that GW182 can interact with PABP (Tritschler et al. 2010) and with both PAN2-PAN3 and CCR4-NOT (Braun et al. 2011; Chekulaeva et al. 2011; Fabian et al. 2011) also suggests a link between miRNAs, P-bodies, and mRNA metabolism. Different classes of small RNAs, including miRNAs and endogenous small interfering (endo-si) RNAs, are present in mammalian oocytes (Suh and Blelloch 2011; Tam et al. 2008; Tang 2010; Watanabe et al. 2008). Loss of either AGO2 or Dicer in oocytes triggers changes in the abundance of a large number of mRNAs and prevents their normal development (Kaneda et al. 2009; Murchison et al. 2007; Tang et al. 2007). In contrast, oocytes lacking *Dgcr*, which is required for the production of miRNAs but not of endo-siRNAs, develop normally (Ma et al. 2010; Suh et al. 2010). This suggests that endo-siRNAs regulate mRNA levels in oocytes. The availability particularly in the mouse of genetic tools and RNAi technologies will enable us to further dissect this and other mechanisms of translational control.

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