Molecular Mechanisms of Metazoan Oocyte Development

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INTRODUCTION

The gametes are responsible for passing all the genetic information from one generation to another, giving rise to all the tissues in a developing animal, and ultimately guaranteeing the survival of species. The formation of germ-line stem cells occurs during early development in all animals. The differentiation of these pluripotent cells into mature gametes provides a continuous supply of sperms and eggs during adult life. Many aspects of germ-line development are conserved across species. For example, in most metazoans, female primordial germ cells (PGCs) migrate from an extragonadal site of origin to reach the somatic gonad and to produce oocytes. After a mitotic proliferative stage, the primary oocytes enter meiosis. In most animal species this process is arrested during prophase, and is completed only in response to intercellular signaling or fertilization, which trigger oocyte meiotic maturation. After the arrest, the oocyte synthesizes and stores a large amount of mRNAs that will be translated only during re-entry into the meiotic division both to promote oocyte maturation and early embryonic development. Translational control is obtained through a complex regulation carried out by different but highly conserved molecular mechanisms. Here we review the basic principles that underlie oocyte development, focusing on analogies and differences among the main model organisms.

Keywords: Mos, oogenesis, translational regulation

Abbreviations: CPE, cytoplasmic polyadenylation elements; CPEB, CPE-binding protein; CPSF, cleavage and polyadenylation specificity factor; CSF, cytostatic factor; eIF4E, eukaryotic translation initiation factor 4E; eIF4G, eukaryotic translation initiation factor 4G; GVBD, germinal vesicle breakdown; MAPK, mitogen-activated protein kinases; MPF, maturation-promoting factor; PABP, poly(A)-binding protein; PAP, poly(A) polymerase; PGCs, primordial germ cells; PRE, polyadenylation response element; RISC, RNA-induced silencing complex; UTR, untranslated region

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INTRODUCTION

In all organisms that reproduce sexually, the propagation and the maintenance of the species is ensured by the union of two types of highly specialized cells: the egg and the sperm. The cell that emerges, the zygote, inherits the genetic patrimony from both the gametes, in addition to maternal cytoplasm that supports the development of the early embryo. The formation of a mature functional gamete is therefore an essential event achieved through a series of molecular mechanisms that, at least in some aspects, are highly conserved among evolutionarily distant organisms. These mechanisms have been extensively studied in female germ cell development. The fundamental biological role played by female germ cells has in fact for years attracted the scientific interest and many evidences have confirmed that several events, ranging from specification and migration of the PGCs to programmed cellular death, are surprisingly similar in very different animals.

PGCs SPECIFICATION

Primordial germ-line stem cells (PGCs) are immortal pluripotent cells, which are generated during early development in the life of all animals. Experimental studies in diverse model organisms have shown that germ-line determination
occurs by at least two alternative mechanisms: preformation and epigenesis (Extafavour and Akam 2003) (Fig. 1). PGCs are often specified early during embryogenesis, in a defined area of the egg, named germ plasm, where maternally inherited determinants are localized: this mechanism is indicated as preformation. The germ plasm is characterized by large electron-dense particles, often referred to as “nuage”, “polar granules”, or “P granules”, containing RNAs and proteins (Santos and Lehmann 2004a). These aggregates are the most evident similarity of PGCs across phyla. *Drosophila melanogaster, Caenorhabditis elegans, and Xenopus laevis* are obvious examples of germ-line determination as preformation.

In the fruit-fly *Drosophila melanogaster*, a variety of RNAs and proteins are synthesized by the nurse cells during oogenesis and localized in the germ plasm by molecular anchoring; translational regulation is successively achieved by posterior-specific molecular mechanisms (Mahowald 2001; Vanzo and Ephrussi 2002). After fertilization, the zygote develops as a syncytium up to the stage of 6000 nuclei. When 256 nuclei have been formed, ten of them migrate to the posterior end of the embryo where germ plasm is located. Once at the posterior end of embryo, the ten nuclei become surrounded by cellular membranes, which incorporate germ plasm components to form the so-called pole cells. These cells stop to divide and are committed to the germ cell fate, while somatic nuclei continue to divide synchronously before they become incorporated into somatic cells (Matova and Cooley 2001; Santos and Lehmann 2004a) (Fig. 1).

The nematode *Caenorhabditis elegans* provides a second example where germ cells are specified by preformation principles. The zygote, in fact, contains electron-dense granules, called P granules, which are distributed asymmetrically during early embryo cleavage events. Only the cell that inherits these granules (called P4) becomes a germinal primordial cell, while the others develop into somatic founder cells. P4 then divides once during embryonic development, distributing the P granules to its daughter cells, Z2 and Z3. During post-embryonic development, Z2 and Z3 proliferate to give rise to the germ-line progeny (Strome and Wood 1982; Hirid et al. 1996; Berkowitz and Strome 2000).

In the frog *Xenopus laevis*, the germinall plasm, which is rich in mitochondria and electron-dense granulo-fibrillar material (also called mitochondrial cloud or Balbiani’s body), is localized at the vegetal pole of the oocyte. After fertilization, at the 32-cell stage, this plasm segregates into four vegetal-pole blastomeres. Next, each of these blastomeres divides asymmetrically and the few cells that specifically accumulate the vegetal plasm differentiate as PGCs (Whittington and Dixon 1975; Saffman and Lasko 1999).

In other species, including mammals, germ cells are not observed until late in development and probably arise as a result of inductive signals from surrounding tissues: this mechanism is indicated as epigenesis (Extafavour and Akam 2003).

The mouse *Mus musculus* is the best-known example of determination by epigenesis, because germ cell specification does not appear to depend on maternally localized determinants. In *Mus musculus* embryos, the first cleavage division contributes to generate blastomeres with different developmental characteristics (Plusa et al. 2005). However, only at the 8-16 cell stage blastomere fates become morphologically apparent: the internal blastomeres differentiate from the external ones, thus giving rise to embryonic and extra-embryonic tissues, respectively. The inner cell mass, derived from the internal blastomeres, develops into an epiblast that surrounds the amniotic cavity: a subpopulation of epiblast cells, proximal to the primitive streak (representing one of the first signs of gastrulation), is able to interpret the inductive signals produced by neighboring tissues and differentiate into PGCs (Fig. 1).

Several experimental evidences support the epigenetic mechanism for mouse germ-line specification: transplantation of distal epiblast cells to the proximal region of the epiblast can give rise to PGCs instead of ectodermal derivatives. On the contrary, proximal epiblast cells are unable to give rise to PGCs if transplanted to the distal region of the embryo (Tsang et al. 2001).

Despite the mechanistic differences underlying germ cell specification in different organisms, many of the genes that set germ cells apart from somatic cells are shared among species, suggesting a common germ cell identity program. For instance, the *vasa* gene, originally identified in *Drosophila* and soon after in other species, including *Xenopus, C. elegans, mouse, and humans* (Schupbach and Wieschaus 1986; Roussel and Bennett 1993; Fujisawa et al. 1994; Komiyama et al. 1994; Castrillon et al. 2000; Raz 2000), encodes a DEAD-box ATP-dependent RNA helicase whose
expression is exclusively restricted to the germ-line cells. *Vasa* gene products are present in PGCs determined by both preformation and epigenesis mechanisms, thus indicating key functions not only for specification but also for survival of these cells. Moreover, *Drosophila* Vasa is required for translation of several mRNAs, including *nanos* (Gavis et al. 1996). Nanos is a RNA binding protein, involved, together with Pumilio, in repression of specific mRNAs during embryonic germ cell formation, migration, and differentiation (Forbes and Lehmann 1998; Wreden et al. 1997; Parisi and Lin 2000; Gilboa and Lehmann 2004a; Kadyrova et al. 2007). The germ-line pathway involving the Pumilio-Nanos complex is conserved in *Diptera* and other organisms, including *C. elegans* (Subramaniam and Seydoux 1999), *Xenopus* (Nakahata et al. 2001), and humans (Jaruzelska et al. 2003), suggesting a conserved role in germ cell development and maintenance.

**PGCs migration and guidance**

Analogies across different species are also observed for mechanistic aspects of germ cell migration. Generally, germ cells emerge in an extragonadal site and then migrate through and down a variety of somatic tissues to join the somatic component of the gonad. In the gonad, the germ cells stop their mitotic divisions and eventually enter meiosis to differentiate into gametes. *Drosophila*, *Xenopus*, and mammals have similar mechanisms of germ cell migration, while *C. elegans* displays a characteristic process of gonad formation.

During *Drosophila* gastrulation, the pole cells, located at the posterior of the embryo, are first incorporated in the lumen of the posterior midgut, successively migrate through the midgut epithelium and the surrounding endodermic layer to attach to the mesoderm. Alignment with the gonadal mesoderm, pole cells condense with somatic gonadal precursor cells to form the gonad (Saffman and Lasko 1999; Santos and Lehmann 2004a). On the basis of their spherical non-motile morphology, a passive movement of the pole cells has been hypothesized during invagination of the posterior midgut. The pole cells begin to actively migrate when they pass through the posterior midgut epithelium: at this stage they form cellular extensions consisting of pseudopodia and membrane ruffling contacting each other (Callaini et al. 1995). These extensions are enhanced by hyper-activation of the JAK/STAT pathway, suggesting a major role of this signaling pathway in the regulation of cell motility during migration (Brown et al. 2006).

In *Xenopus* and mouse, the main signals of PGCs migration are thought to be those described for *Drosophila*: germ cells are incorporated passively in the gut and migrate actively toward the mesoderm to reach the somatic gonadal precursors. On the other hand, *C. elegans* has a singular way to form the gonads. During gastrulation, the ZZ and Z3 PGCs remain in their original site, extend protrusions into the gut and toward each other, and are finally reached by somatic gonadal precursors.

During migration, mitotic activity of PGCs is species-specific: *Drosophila* and *C. elegans* PGCs are mitotically inactive, *Xenopus* PGCs undergo few division cycles, while mouse PGCs actively divide (Saffman and Lasko 1999; Matova and Cooley 2001; Molyneaux and Wylie 2004).

The analysis of PGC migration in different organisms indicated that germ cells are able to reach their target site through complex interactions with somatic cells. These cells may play either a permissive (i.e. by modifying the characteristics of the midgut epithelium, thus permitting PGC migration) or an instructive role (i.e. by generating both repulsive and attractive signals) (Raz 2004). Mechanisms of PGC guidance seem to be rather conserved among species. In *Drosophila*, active migration through the gut epithelium depends on the function of trapped in endoderm-1 (Tre1), a gene expressed in PGCs and encoding a seven transmembrane domain orphan receptor related to chemokine recep-

tors. An important role for this family of receptors has been demonstrated also in mouse and zebrafish PGC migration. In *Xenopus*, there are few data available concerning molecules involved in PGC migration, since the identification of the germ-line cells within the endodermal mass is difficult and their number is limited. However, the chemokine receptor xCXCR4 is expressed in *Xenopus* presumptive PGCs (pPGCs) too, even if its involvement in PGC migration remains to be clarified (Huang et al. 2005). Moreover, the 3-Hydroxy-3-Methylglutaryl Coenzyme A (HMGC0AR/ Hmgcr), an enzyme necessary in mammals for the biosynthesis of isoprenoids and cholesterol, plays an instructive role in *Drosophila*, thus providing germ cells with attractive cues during migration toward the mesoderm (van Doren et al. 1998; Santos and Lehmann 2004b). Similar results were observed in zebrafish supporting the idea of common guidance cues for germ cells (Thorpe et al. 2004).

**CYST FORMATION**

After gonad colonization, PGCs start a distinctive program of divisions to form cysts. Cyst formation is a universal step during animal oogenesis: a founder cell, often called cytos-blast, undergoes a series of divisions followed by incomplete cytokinesis to form a syncytial cluster of 2" cells (Pep-ling and Spradling 1999). The n number of these cells is species-specific.

In *Drosophila*, the germ-line cyst is made of one oocyte and 15 nurse cells, which are interconnected by intercellular bridges called ring canals. This organization persists until the completion of oocyte maturation, a phase when nurse cells, whose function is to provide nutrients to the growing oocyte, undergo apoptosis. A syncytium is also observed in *C. elegans* germ-line cells where 8-12 nuclei arrange around a central anucleate core, called rachis: each nucleus is surrounded by an incomplete plasma membrane and joined to the rachis through a cytoplasmic bridge. However, unlike *Drosophila*, gonial cells in *C. elegans* do not form separate clusters and the formation of the syncytium is still poorly understood. Clusters of gonial cells have been nevertheless reported in different species, including *Xenopus* and mammals. In these organisms, germ cells enter meiosis only in the pre-adult gonad: this characteristic made the identification of the gonial cell clusters more difficult than in *Drosophila* where meiosis occurs during oogenesis throughout adult life. In *Xenopus*, at the beginning of meiosis, about 16 interconnected pear-shaped, highly polarized cells are found (Gard et al. 1995). In mammals, the number of germ cells in each cluster seems to be highly varied, often corresponding to 2" (Pepling and Spradling 1998).

Cyst formation appears to be a highly conserved event in early gametogenesis. Different hypotheses have been proposed to clarify the meaning of the cyst, ranging from facilitating the beginning of meiosis to germ-line sex determin-ation (Pepling and Spradling 1999). In general, even if the presence of intercellular bridges may limit the total number of gonial cell divisions, it may also allow the passage of molecules between different cells as demonstrated in the *Drosophila* cyst (Matova and Cooley 2001).

A key role in *Drosophila* cyst formation is played by the fusome, a germ-line specific organelle rich in small, endoplasmic reticulum (ER)-like vesicles (Pepling and Spradling 1999). During germ cell divisions, the fusome branches through the ring canals, extending into every cell. After cyst formation, the fusome breaks down, thus permitting cyto-plasm of molecules to transport through the ring canals (Gasken and Spradling 1998). The fusome, although indispensable for correct cyst formation in *Drosophila*, has not been identified in other organisms yet. However, cytoplasmic structures that resemble the fusome have been described in vertebrate germ cells. In *Xenopus*, for example, a structure very similar to the *Drosophila* fusome has been found based on ultra-structural criteria and biochemical composition. This structure includes numerous vesicles resembling the ER, and contains α- and β-spectrin like the *Drosophila* tu-
some (Kloc et al. 2004).

APOTOPSIS

Programmed cellular death is achieved through a highly conserved program that is present in all multi-cellular organisms. Apoptosis is involved in a multitude of biological processes, including the correct development of gametes.

In organisms like Drosophila, cell death occurs throughout oogenesis. Apoptosis is the mechanism used by nurse cells to deliver their cytoplasmic components into the growing oocyte. Cell death is also observed in the germarium (the region of the ovary where germ-line stem cells reside and divide asymmetrically and germ-line cysts are generated) and during mid-oogenesis in response to environmental stimuli (Drummond-Barbosa and Spradling 2001) possibly to avoid the additional energetic consume required to produce a mature egg (McCall 2004; Baum et al. 2005). During late oogenesis, dying nurse cells dump their cytoplasm into the growing oocyte and are then phagocytosed by adjacent follicle cells. At the entry of the egg into the lateral oviduct, epithelial cells phagocyte, in turn, apoptotic follicle cells (Cavaliere et al. 1998; Foley and Cooley 1998; Nezis et al. 2000, 2002).

Even if all dying cells in the different stages of oogenesis display the typical features of apoptotic cells (such as chromatin and cytoplasm condensation, DNA fragmentation, and cytoskeleton reorganization), during late oogenesis cell death does not seem to require the common Caspase pathway (Foley and Cooley 1998; Peterson et al. 2003; Nezis et al. 2006). It was recently reported that dying nurse cells at the end of oogenesis show no evidence of cytoplasmatic caspase activity, indicating that caspases should not play essential roles in programmed cell death of late stage egg chambers (Mazzalupo and Cooley 2006).

In C. elegans, germ cell death occurs exclusively during oogenesis of adult hermaphrodites in order to eliminate approximately half of all potential oocytes (Gumienny et al. 1999). This process is observed specifically at the end of prophase I, when germ cell nuclei are part of a large syncytium and are not mitotically synchronized. Unlike mammals and Drosophila, C. elegans has no morphologically distinct nurse cells, so apoptosis of these extra germ cells may be used to provide cytoplasmic components to the oocytes. The same apoptotic machinery acts both during somatic and germ cell death, but distinct regulatory proteins might control its activation (Gumienny et al. 1999).

In Xenopus, few apoptotic cells are detected within the ovary, even though the oocytes appear to be particularly vulnerable to cell death during yolk accumulation and apoptosis can be induced by starvation (Matova and Cooley 2001). On the contrary, no signs of apoptosis are observed within germ-line cysts, suggesting that all the cells composing a cyst could differentiate into mature oocytes (Kloc et al. 2004).

In mammals, apoptosis eliminates up to 99% of the total number of oocytes and takes place during fetal and neonatal ovarian development (a process called attrition) and between puberty and menopause (named atresia) (Morita and Tilly 1999). In particular, during fetal ovarian development in human and rat, extensive cell death occurs coincidently with two important processes: oocyte meiosis (throughout pachytene and diplotene stages of prophase I) and folliculogenesis (during the diplotene stage of prophase I) (Matova and Cooley 2001). The importance of this process for selection of those oocytes that will survive still remains to be ascertained.

OOCYTE MATURATION

The term “oocyte maturation” is used to indicate all the molecular mechanisms employed by an immature oocyte to become a fertilizable gamete. A universal characteristic of oocyte development is meiotic division. The entire process involves morphological changes and exploits molecular mechanisms that are highly conserved in all metazoans, in particular nuclear envelope breakdown (GVBD), rearrangement of the cortical cytoskeleton, and meiotic spindle assembly. Moreover, in virtually all species, meiosis includes at least one arrest phase. The cell cycle stage when meiotic arrest takes place is organism-dependent. In most animal species, the first arrest occurs at prophase I (PI; Masui and Clarke 1979; Masui 2001). During this stage, the oocyte grows while its nuclei replicate and meiotic prophase begins, therefore meiosis necessary to promote meiotic maturation and to ensure early embryonic development. Moreover, the nucleus, called germinal vesicle (GV), also enlarges in size and synthesizes mRNAs that after maturation are stored untranslated, together with ribosomal RNAs, in the cytoplasm. When meiosis resumes, stimulated by fertilization or gonadotropins secreted from the pituitary gland (in vertebrates), the large GV breaks down (GVBD) releasing its nuclei, the oocyte cytoplasm, while chromosomes condense to a metaphase state and migrate to the periphery of the cell. At telophase, an unequal cytokinesis process takes place and the first polar body, which contains little or no cytoplasm, is extruded from secondary oocytes.

In metazoans, fertilization promotes meiosis completion, but the time when fertilization occurs is species-specific (Fig. 1). In C. elegans, the oocyte arrest at the end of PI, therefore fertilization is necessary to complete both meiotic divisions. On the contrary, when oocyte maturation is hormone-dependent, a second meiotic arrest takes place before fertilization and completion of maturation. In most insects, for instance, the second arrest takes place during metaphase (MII) or anaphase (AI) of the first meiotic division, while in most vertebrates, during metaphase of the second meiotic division (MII). Furthermore, in some organisms, such as sea urchin and jelly fish, meiosis is already complete at fertilization (Masui 1991, 2001; Tunquist and Maller 2003; Greenstein 2005).

In Drosophila, the oocyte arrests transiently in PI and is loaded of RNAs and proteins by the nurse cells. Select mRNAs specifically localize within the oocyte to direct embryonic axis formation. The second meiotic arrest occurs in MI, at late stages of egg chamber development. The resumption of meiosis appears therefore to be dependent on ovulation (Heifetz et al. 2001).

Independently from the stage of arrest and the type of inducing stimuli, the resumption of the meiotic cell cycle depends upon a molecular complex: the maturation-promoting factor (MPF), which is highly conserved from yeast to human. The MPF complex is a universal regulator of the G2/M transition in the cell cycle of all eukaryotes (Yamashita 1998) and has been thoroughly studied in Xenopus laevis oocytes (Masui 2001; Dekel 2005).

MPF is a heterodimer consisting of a catalytic subunit, the p34cdc2 (Cdc2 for short) serine/threonine kinase, and a regulatory subunit, Cyclin B (Dekel 1996). The phosphorylation state of the Cdc2 kinase, when complexed to Cyclin B, modulates its activity. In some organisms, like fishes and amphibians (but not Xenopus), MPF is formed only after hormone stimulation, which induces de novo synthesis of cyclin B mRNA. The constituted factor is then activated by the cyclin-dependent kinase activating kinase (CAK) that phosphorylates Cdc2. In other organisms, such as Xenopus, MPF is present as an inactive form called pre-MPF; after hormone induction, the phosphatase Cdc25 dephosphorylates Cdc2, thus activating MPF (Yamashita 1998). A small amount of pre-MPF was found also in the mouse, where it is sufficient to induce GVBD but not to promote completion of oocyte maturation (Chesnel and Eppig 1999).

The highly conserved Mos protein, a germ cell-specific serine/threonine kinase that triggers the mitogen-activated protein kinases (MAPKs) cascade by acting as a MAPK kinase kinase (MEKK), plays a key role during oocyte maturation. The relevance of this pathway has been deeply explored, especially during Xenopus and mouse oogenesis. Numerous studies indicate that Mos performs diverse functions in different species (Gotoh et al. 1995; Abriere et al. 2007).

In Xenopus, progesterone stimulation leads to the synthesis of Mos and therefore the activation of the Mos/MEK/MAPK/p90Rsk cascade that in turn inhibits the MPF inhibitory kinase Myt1. Target of Rsk is also Bub1 that inhibits the anaphase-promoting complex APC/C preventing meiosis progression. The activation of Cdc25 by progesterone also promotes MPF activation through dephosphorylation of Cdc2. Meiosis completion in Xenopus (and in most vertebrates) is prompted by fertilization at metaphase II (c). Fertilization promotes meiosis completion in every species, however it occurs in a specific time depending on organisms; for instance, at the end of prophase I in C. elegans (a), at metaphase I in Drosophila (b), or at the end of meiosis in organisms like sea urchins (d). Some of the molecules depicted in the figure may act in a similar manner during the oocyte maturation in species other than Xenopus (see text for details).

In mammals, the interplay between MPF and the MAPK pathway seems to be different from amphibians. Data from the literature are contradictory, however recent demonstrations suggest that MAPK does not control early meiotic events but is required for metaphase II arrest. More-
over, MAPK activation is facilitated by MPF through the regulation of Mos expression (Lazar et al. 2002; Josefseberg et al. 2003).

In frog and mouse oocytes, one of the earliest biochemical events observed in response to the hormone stimulus is a decrease of cyclic adenosine 3',5' monophosphate (cAMP) levels. cAMP is thought to be generated by surrounding follicular cells and transferred to the oocyte through gap junctions; this hormone induction could conserved and interrupt cell to cell communication, thus terminating the flux of follicle cAMP into the oocyte (Dekel 2005). The negative action exerted by cAMP on meiotic maturation is achieved through the catalytic activity of protein kinase A (PKA). A model proposed by Dekel (2005) suggests that achieved through the catalytic activity of protein kinase A negative action exerted by cAMP on meiotic maturation is flux of follicle cAMP into the oocyte (Dekel 2005). The interrupted cell to cell communication, thus terminating the hormone induction could therefore be responsible for the majority of MAPK activation in early, active MAPK levels. Moreover, dMos seems to have no roles because oocytes from Rsk knockout mice maintain the ability to arrest in MI (Dumont et al. 2005). Moreover, the SAC proteins are not required for oocyte arrest at MI in the mouse (Tsurumi et al. 2004). The Mos pathway could therefore act independently from p90Rsk, probably contributing to maintenance rather than establishment of the MI arrest (Madgwick and Jones 2007).

A functional homologue of the Mos protein has been identified in Drosophila (Ivanovska et al. 2004). Injection of dmos mRNA into Xenopus embryos blocks mitosis and increases active MAPK levels. Moreover, dMos appears to be responsible for the majority of MAPK activation in Drosophila. Nevertheless, dMos seems to be dispensable for meiosis since dmos mutant flies complete meiosis and produce fertilized embryos that develop normally, although there is a reduction in female fertility (Ivanovska et al. 2004).

**TRANSLATIONAL CONTROL**

Messenger RNAs (mRNAs) and macromolecules synthesized and stored in the oocytes, during the early stages of oogenesis, are required to support their maturation and fertilization, and also early embryogenesis, since these events occur in absence of transcription. The spatio-temporal expression of mRNAs, accumulated within early oocytes, is a key control point to guarantee proper oocyte maturation and development.

Within growing oocytes, the mRNAs can have very different fates: immediate translation, storage and later recruitment for translation, or degradation only at specific stages. This different behavior depends upon mRNA association within a set of proteins that regulate mRNA availability to initiation factors and ribosomes (Eichenlaub-Ritter and Peschke 2002).

Translation initiation of the vast majority of eukaryotic mRNAs is cap-dependent, because it requires a methylated guanine residue at the 5' end of the mRNA, which is recognized by the cap binding factor eukaryotic translation initiation factor 4E (eIF4E). After being transcribed in the nucleus and before being transported into the cytoplasm, a maturing mRNA is also modified at its 3' end by the poly(A) polymerase (PAP) enzyme, which adds up to 250 adenosine residues. Properly capped and polyadenylated mRNAs are transported into the cytoplasm and efficiently translated. On the other hand, regulated mRNAs are thought to be first deadenylated and stored in a translationally silent state. Successively, the elongation of the poly(A) tail by cytoplasmic polyadenylation triggers translation of these mRNAs when protein activity is needed (Piccioni et al. 2005a). According to the model of mRNA circularization, translation initiation is promoted by the interaction between the cap structure and the poly(A) tail (Munroe and Jacobson 1990). The mRNA forms a “closed loop” complex through the poly(A) binding protein (PABP) and the scaffolding factor eIF4G (eukaryotic translation initiation factor 4E, the poly(A)-binding protein (PABP), and the scaffolding factor eIF4G (eukaryotic translation initiation factor 4E). The poly(A) polymerase (PAP) promotes the addition of adenosine residues in both nuclear and cytoplasmic compartments. The 5'- and 3'-UTRs are in cyan, while the coding region is in blue.

**Cytoplasmic polyadenylation and deadenylation**

Translational regulation by cytoplasmic polyadenylation is a conserved biological mechanism, probably occurring in all metazoans. In Xenopus and mouse, the 3’-UTRs of select mRNAs, including several cyclines, contain regulatory se-
quences called cytoplasmic polyadenylation elements (CPEs) (Barkoff et al. 2000; Mendez and Richter 2001). CPEs are AU-rich sequences capable to recruit the RNA-interacting protein CPE-binding protein (CPEB) (Huarte et al. 1992; Verrotti et al. 1996; Wickens et al. 2000; Richter 2007). CPEB is able either to engage a translational repressor called Maskin, which competes with eIF4G for eIF4E-binding, or to trigger polyadenylation-promoting translation. It is a notion that the switch from one mechanism to another (Tay et al. 2003).

A silent mRNA has a short poly(A) tail that accommodates only a few molecules of PABP not sufficient to de-repress translation or compete with Maskin. During oocyte meiotic maturation, phosphorylated CPEB is able to activate CPSF (Cleavage and Polyadenylation Specificity Factor) probably enhancing its binding to the AAUAAA sequence (named nuclear polyadenylation signal, but functional also in the cytoplasm) located downstream from the CPE; CPSF in turn attracts PAP that, in collaboration with PABP, stimulates polyadenylation. The longer poly(A) tail recruits more PABP molecules that facilitate eIF4G to displace Maskin from eIF4E-binding (Cao and Richter 2002), thus promoting efficient translation initiation.

Homologues of Xenopus CPEB are found in a number of animal species (Mendez and Richter 2001; Richter 2007). The Drosophila CPEB, called Orb, is involved in translational regulation of oskar (osk) mRNA during Drosophila oogenesis (Lantz et al. 1992, 1994; Chang et al. 1999). Oskar protein is tightly localized at the posterior pole of the growing oocyte through a localization-dependent translation mechanism of its mRNA, and promotes abdominal patterning and germ-line differentiation. The unlocalized osk mRNA is translationally repressed by the binding of the Bruno protein (Bru) to specific Bruno Response Elements (BRE) in its 3’-UTR. Orb probably enhances osk translation through the addition of a long poly(A) tail, although the stretch of adenosine residues is not sufficient to overcome Bru-mediated repression (Chang et al. 1999; Castagnetti and Ephrussi 2003). Nevertheless, Bru and Orb physically interact suggesting the existence of a multi-protein complex containing both positive and negative regulators of osk translation (Castagnetti and Ephrussi 2003).

CPEB was first discovered in Xenopus as a CPE-binding protein (Hake and Richter 1994) and subsequently cloned in the mouse (mCPEB; Gebauer and Richter 1996). The two proteins share 91% identity in the region containing the RNA-binding domains; this region is also similar to the RNA-binding domain of Orb (Lantz et al. 1992). mCPEB mRNA is mainly restricted to ovary, testis, and kidney of the mouse ovary, it is expressed in oocytes (Gebauer and Richter 1996). CPEB knockout mice show that oocyte meiotic progression is disrupted at pachytene (Tay and Richter 2001). To explore the function of CPEB after the pachytene stage, Racki and Richter (2006) generated transgenic mice expressing siRNA against CPEB mRNA after this stage and demonstrated that oocytes derived from these animals do not develop normally. CPEB is able to associate with a number of CPE-containing mRNAs, including those encoding Mos and Gdf9 (Matzuk et al. 2002; Roy and Matzuk 2006). The latter mRNA encodes for a growth factor, critical for coordinated oocyte follicle development, which is synthesized in and secreted from oocytes. In the transgenic oocyte, expressing siRNA against CPEB, the polyadenylation status of both e-mos and Gdf9 mRNAs is altered (Racki and Richter 2006), thus linking e-mos to poly(A) abundance (Racki and Richter 2006).

As previously described, the formation of a “closed loop” complex through PABP and the initiation factors eIF4A and eIF4E on a properly polyadenylated and capped mRNA is a prerequisite for efficient translation (Piccioni et al. 2005a). The adaptor protein elf4G is able to bind simultaneously the cap-binding factor elf4E, through an elf4E-binding motif (YxxxxLΦ, where Φ is an aliphatic residue) and the poly(A)-binding protein PABP. While the elf4G-elf4G interaction with the cap structure is essential for translation initiation, the elf4G-PABP binding is not strictly required for genome recruitment, but constitutes a control point to modulate translation.

A general control on elf4E is exerted by a class of inhibitors, known as elf4E-binding proteins (4E-BPs) (Karim et al. 2001), which sequester the majority of both free and cap-bound elf4E, thus preventing its binding to elf4G. Hyperphosphorylation of 4E-BPs prevents their binding to elf4E and therefore promotes translation initiation. On the contrary, specific translational repressors, all containing elf4E-binding motifs, exert their activity on select mRNAs.

Translational inactivation by masking of mRNAs

In Xenopus and mouse, most maternal mRNAs, including “housekeeping” mRNAs, undergo a default deadenylating process after GVBD, since they do not have CPE sequences. This deadenylating activity does not seem to require cis-acting specific elements (Varnum and Worman 1990) and, in Xenopus, is mediated by xPARN (Copeland and Worman 2001). Despite deadenylating, these mRNAs remain stable, but dormant, throughout oocyte maturation. On the contrary, mos belongs to a class of maternal mRNAs that contain a specific motif called EDEN (embryonic deadenylation element) within their 3’-UTR. This 17-nucleotide sequence recruits the deadenylating-promoting factor EDEN-BP that is responsible for deadenylating and, thus translational inactivation, following fertilization (Paillard and Osborne 2003). EDEN-BP homologues are CUG-BP in Caenorhabditis elegans (Paillard et al. 2003), etr-1 in C. elegans (Milne and Hodgkin 1999), and Bru-3 a paralogue of Bruno in Drosophila (Delaunay et al. 2004).
These proteins act as masking factors and can associate with the mRNA either in the nucleus or in the cytoplasm. A prototype of this group of proteins is Maskin that regulates the translation of CPE-containing mRNAs, including cyclin B1 mRNA in Xenopus oocytes. Maskin binds simultaneously CPEB and eIF4E, thus preventing the formation of the translation initiation complex (Stebbins-Boaz et al. 1999). During oocyte maturation, Maskin undergoes a differential phosphorylation necessary to disable its association from eIF4E (Barnard et al. 2005). The displacement of Maskin is concurrently promoted by the interaction between eIF4G and PABP (Cao and Richter 2002).

Similarly to Maskin, the Drosophila Cup protein plays an important role in the localization and translational repression of maternal mRNAs during oogenesis and early embryogenesis (Wilhelm et al. 2003; Nelson et al. 2004; Nakamura et al. 2004; Zappavigna et al. 2004; Piccirillo et al. 2005b). One of the mRNA targets of Cup is the above-mentioned osk mRNA. Cup is able to bind directly both Bruno and eIF4E (Nakamura et al. 2004). A simple proposed model considers the formation of a Bruno-Cup-eIF4E complex between the 5' - and 3'-UTR of osk mRNA to prevent the assembly of the eIF4E-eIF4G complex, thus inhibiting translation. Activation of osk translation is at least in part dependent on the elongation of its poly(A) tail, a process mediated by Orb. Moreover, Cup interacts with Barentsz, a plus end-directed microtubule transport factor (Wilhelm et al. 2003) suggesting a possible role in osk mRNA localization. Homologues of Cup have been identified in human (eIF4E-transporter or 4E-T; Dostie et al. 2000) and mouse (Clastr; Villaescusa et al. 2006). Claus mRNA and protein are indeed highly expressed within the cytoplasm of growing oocytes (Villaescusa et al. 2006).

Another class of proteins detected in the germ cells as specific components of mRNP particles, and shown to be involved in mRNA masking, are the Y-box proteins. All these proteins are able to interact with both DNA and RNA sequences to control transcription and translation of specific genes and mRNAs respectively (Matsumoto and Wolffe 1998). Although Y-box proteins are present in all cell types, some have been identified as germ-specific proteins implicated in the masking of stored mRNAs from translation (Sommerville and Ladomery 1996). In Xenopus oocytes, the Y-box protein FRGY2 is the major component of translationally repressed mRNPs. It binds single-stranded RNA in association with other factors such as the α-subunit of CK2 kinase and the RNA helicase Xp54. These proteins may interact with a translation repressor complex that contains CPEB and contacts the cap-binding protein eIF4E in a molecular bridge between the 5' and 3' ends of mRNAs (Winston and Sommerville 2006). Similarly, in Drosophila oocytes, the Y-box protein Ypsilon Schachtel (Yps) and the helicase Me31B are components of the osk RNP complex. Orb is also part of this complex and its function may antagonize the translational inhibition due to Yps (Nakamura et al. 2001; Mansfield et al. 2002). The above-mentioned Y-box proteins involved in translational regulation in germ cells are conserved in different organisms: homologues of FRGY2, for example, include MSY2 in mouse and CEY-2/3/4 in C. elegans.

RNA interference: siRNAs and miRNAs

Recent studies are starting to shed light on alternative mechanisms for translational control during germ-line development in particular the discovery of a growing number of stable and dormant mRNAs, associated with polyribosomes (Clark et al. 2000; Braat et al. 2004), suggests the existence of post-initiation translational repression processes based on microRNAs (miRNAs). Moreover, studies on the role played by small interfering RNAs (siRNA) during oogenesis are tending towards the integration of the regulatory mechanisms based on small antisense RNA species, with those described above.

RNA interference is an evolutionarily conserved process of post-transcriptional gene silencing, whose central players are small non-coding mRNAs: siRNAs and miRNAs, especially. Even if the two types of molecules have different origin, evolutionary conservation, and target genes, they share a similar mechanism of action, thus performing an interchangeable biochemical function (Bartel et al. 2004; Bushati and Cohen 2007). The biosynthetic pathways of miRNAs and siRNAs converge when the activity of Dicer RNAse III is induced to cleave and generate miRNAs and siRNAs from short double-stranded RNAs. This is thought to occur via RNA-induced silencing complex (RISC), which allows the antisense strand of the duplex to be bound to its target RNA on the basis of sequence complementarity. In general, siRNAs fully anneal with their target molecule and consequently promote its degradation; on the contrary, miRNAs show partial complementarity to regions of the 3' UTR of the target RNA that is in turn translationally repressed.

Several proteins associated with the RISC complex belong to a protein family containing a conserved C-terminal PIWI domain and a central PAZ domain. The founder member of this protein family is the PIWI-PAZ protein Argonaute (Ago) that is the major component of RISC complexes present in different organisms (Polonyi and Joshua-Tor 2007). The Argonaute family includes proteins that can be grouped in various sub-families. The functional differences among them are poorly understood, however their activity does not seem to be redundant. In particular, the Piwi sub-family of Argonaute proteins has been shown to associate with a novel class of small RNAs (piRNAs) mainly expressed in mammalian male germ-line, which seem to be critical for proper sperm development. Moreover, in Drosophila, Piwi proteins are involved in silencing of retrotransposons (Vagin et al. 2006), and in other organisms, including mammals, appear to be important for germline stem cell maintenance and meiosis (Girard et al. 2006; Parker and Barford 2006; Park et al. 2007).

In late-stage Drosophila oocytes, at least 4% of all expressed genes are regulated by miRNAs (Nakahara et al. 2005). Some evidences suggest that osk mRNA translation might be regulated by an RNA interference mechanism in addition to translational repression by Cup. In fact osk mRNA was found to be associated with polysomes when not localized and translationally repressed (Braat et al. 2004). Moreover, mutations in the aubergine, spindle-E, armitage, and maizestem genes (encoding a PIWI-PAZ domain protein, a DEAD-box helicase, an ATP dependent helicase, and a protein required for the localization of a subset of RNAi pathway components, respectively) all induce premature accumulation of the Oskar protein (Kennerdell et al. 2002; Cook et al. 2004; Tomari et al. 2004; Wilhelm and Smibert 2005). All these proteins are components of the RNAi pathway whose homologues were found also in other animal species.

Depending on organisms, the various components of the interference machinery are often represented by different forms. For instance some Dicer have been identified: DCR1, involved in miRNAs cleavage, acting as heterodimer with the double-stranded RNA-binding protein Loquacious, and DCR2 that excises siRNAs from long double-stranded RNAs (Lee et al. 2004; Forstemann et al. 2005). In C. elegans as well as in mammals only one Dicer has been identified. In the mouse oocyte, disruption of the Dicer gene causes growth arrest and defects in meiotic spindle organization, and chromosome congression. Moreover, a set of transcripts, whose sequences contain putative miRNA target sites, is up-regulated in Dicer knockout oocytes, thus suggesting a crucial role of Dicer in the turnover of many maternal transcripts during meiotic maturation (Murcson et al. 2007).
CONCLUSIONS

All animals emerge upon fertilization of mature oocytes that have the extraordinary ability to provide almost all the factors and components sufficient and necessary to direct the early stages of development. In the oocyte and early embryo, the chromosomes are transcriptionally silent, thus development must reside on post-transcriptional rather than transcriptional regulation of so-called maternal mRNAs and nutritional factors. Hence, it is not surprising that many players and molecular mechanisms, directing oocyte growth and maturation, appear conserved in evolutionary distant animal species. However, many of the similarities and differences observed during metazoan oogenesis still need to be studied and ascertained.

Oocytes can be easily obtained, manipulated, and cultured. In the oocyte, basic a well-established in vivo system where to study basic biological questions: regulation of mRNA stability, localization, and translation, hormonal stimuli, apoptosis, cell cycle progression, among others. What we learn in oogenesis can be transferred to more complex cell and tissue types: i.e. the CPEB protein acts not only during oogenesis, but also in neurons, thus in adult somatic cells, to modulate neuronal plasticity (Wu et al. 1998). Until recently, the basic molecular pathways have been discovered, including evolutionary conserved translational control mechanisms and machineries directing both repression and activation of dormant mRNAs. Most of these activities combine the mode of action of ubiquitous molecules (like elf4E) with specific factors (like Cup) in order to regulate translation of select mRNAs.

The studies on oogenesis are therefore far to be completed. For example, the expanding family of small RNAs, mostly siRNAs and miRNAs controlling either degradation or silencing of many RNAs, may represent another class of molecules whose mechanism of action could be studied and understood within the developing oocyte.

In an era correctly aimed at the identification of the molecular bases of genetic diseases, the egg can still function as a simplified in vivo system where to study conserved developmental pathways whose relevance to human health is increasingly becoming evident.

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