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Molecular Mechanisms of Metazoan Oocyte Development

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ABSTRACT

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, al 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

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Invited Review

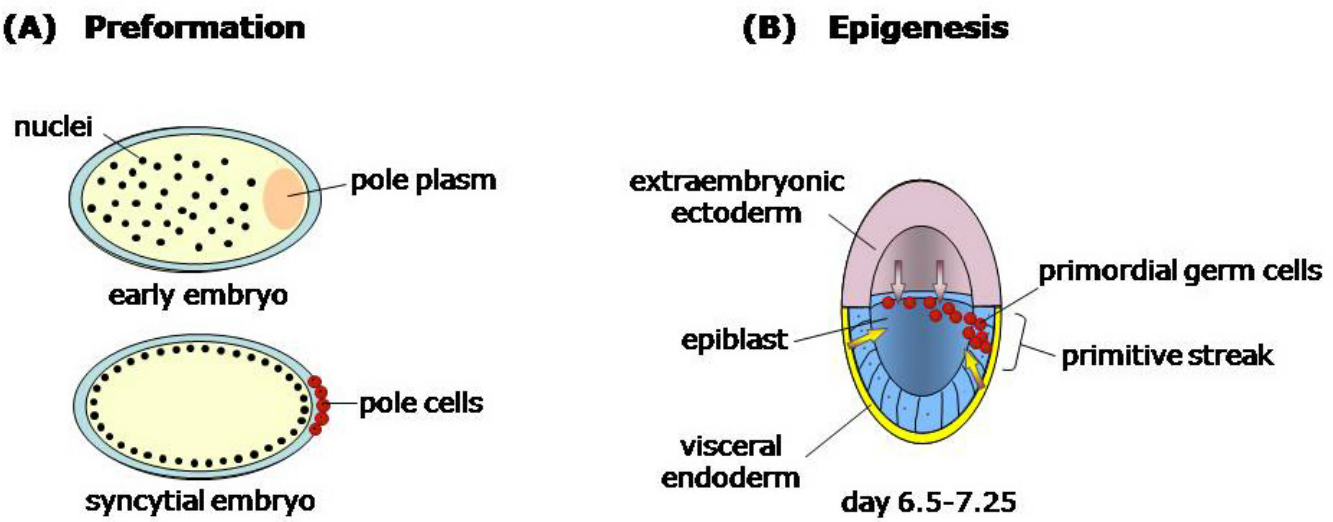

Fig. 1 Mechanisms of germ cell specification: preformation and epigenesis. (**A**) The pole plasm, localized at the posterior end of the *Drosophila* mature egg, contains mRNAs and proteins previously synthesized by the nurse cells and then transported to the oocyte. During early embryogenesis, some nuclei of the syncytium migrate posteriorly to the periphery and incorporate the pole plasm to become pole cells (the precursors of primordial germ cells). (**B**) In *Mus musculus*, no preexisting germ plasm has been observed in oocytes. Primordial germ cells (PGCs) appear only around day 7 postfertilization, after differentiation of embryonic and extraembryonic tissues, in the area called primitive streak, has occurred. These cells arise from a subpopolation of epiblast cells that are able to receive inductive signals from the extraembryonic ectoderm (pink arrows) and visceral endoderm (yellow arrows). PGCs and their precursors are depicted in red in both panels.

occurs by at least two alternative mechanisms: preformation and epigenesis (Extavour 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; Hird *et al*. 1996; Berkowitz and Strome 2000).

In the frog *Xenopus laevis*, the germinal 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 (Whitington 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 (Extavour 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 differentiates 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; Roussell and Bennett 1993; Fujiwara *et al*. 1994; Komiya *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 embryo patterning and 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. After 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 steps of PGCs migration closely resemble 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 Z2 and Z3 PGCs remain in their original site, extend protrusions into the gut and toward each other, and are finally reached by somatic gonadal cells.

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 receptors. 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 ascertained (Nishiumi *et al*. 2005). Moreover, the 3-Hydroxy 3-Methylglutaryl Coenzyme A (HMGCoAR/ *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 cystoblast, undergoes a series of divisions followed by incomeplete cytokinesis to form a syncytial cluster of 2^n cells (Pepling 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 then 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 unfixed, often corresponding to 2^n (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 determination (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 cell types 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 cytoplasm transport through the ring canals (de Cuevas 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* fu-

some (Kloc *et al*. 2004).

APOPTOSIS

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*, where cyst formation 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 cytoplasmic 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 coincidentally with two important processes: oocyte meiosis (throughout pachitene 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, thus accumulating yolk and molecules 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 nucleoplasm into the 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 metazoa, fertilization promotes meiosis completion, but the time when fertilization occurs is species-specific (**Fig. 2**). In *C. elegans*, the oocyte arrests 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 (MI) 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 broadly 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 1995).

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; Abrieu *et al*.

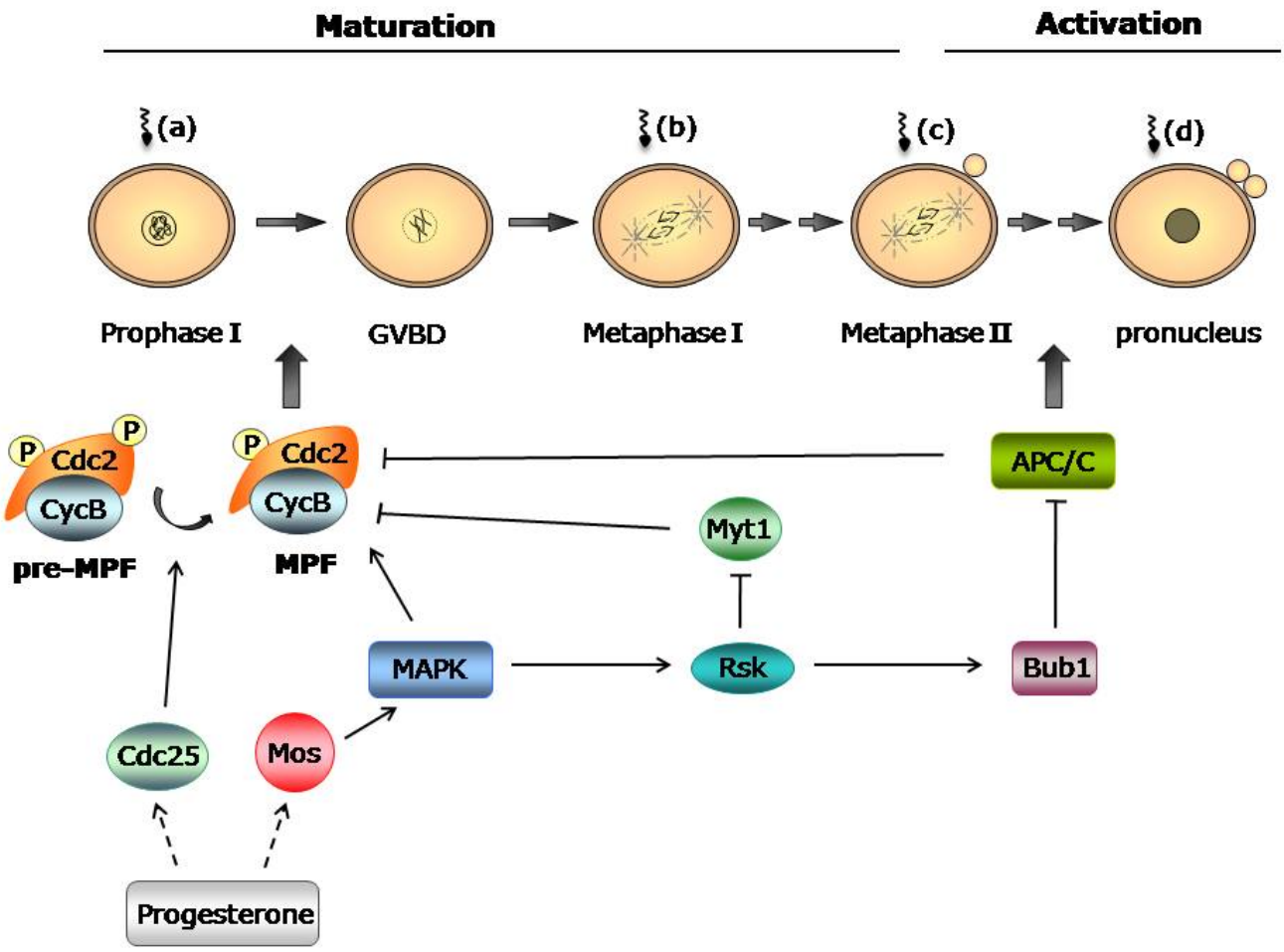

Fig. 2 Summary of molecular events triggering maturation of *Xenopus* oocytes. Resumption of meiosis from primary arrest during prophase I is induced by maturation-promoting factor (MPF) activity triggered by progesterone. This process involves a series of molecular activities and morphological changes, including nuclear envelope breakdown (GVBD) and meiotic spindle assembly. Mos protein represents the key player involved both in MPF activation and cytostatic factor (CSF) establishment at metaphase II. Progesterone stimulation promotes the activation of Mos/MAPK/Rsk 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 oocyte maturation in species other that *Xenopus* (see text for details).

2001; Josefsberg *et al*. 2003; Lazar *et al*. 2004).

In *Xenopus*, progesterone stimulation leads to the synthesis of Mos and therefore the activation of the Mos/MEK/MAPK/$p90_{Rsk}$ cascade that in turn inhibits the MPF inhibitory kinase Myt1. Moreover, MAPK activation may also contribute to stabilize MPF nuclear localization through phosphorylation of Cyclin B. Therefore, Mos signaling directly takes part to the activation and stabilization of MPF during the G2/meiosis I transition (Tunquist and Maller 2003) (**Fig. 2**). The MPF and Mos pathways, promoted by progesterone, are both required for meiotic maturation induction (Haccard and Jessus 2006). However, despite the numerous available data, the precise roles played by MAPK and MPF in oocyte maturation are not fully understood yet. Kotani and Yamashita (2002) demonstrated that, at least in *Rana* oocytes, MAPK reorganizes the microtubule array, surrounding the GV, at the onset of GVBD, without any MPF activity, while MPF alone, in absence of MAPK activity, induces GVBD. However, MAPK and MPF collaborate to accomplish other morphological events, including chromosome condensation, necessary for meiotic progression.

Xenopus oocytes, after GVBD and chromosome condensation to the metaphase state, arrest their cycle in metaphase II. At this stage, the activity of the cytostatic factor (CSF) is important to establish and maintain the arrest. The molecular composition of CSF is still unknown, but many evidences suggest that its major component is likely to be a protein kinase. In any case, CSF is not intended as a single molecule but as an activity that arises during oocyte maturation, induces metaphase arrest, is maintained until oocyte fertilization/activation, and then is inactivated (Masui 2000; Tunquist and Maller 2003). Mos is one of the proteins that satisfies all these criteria; some evidences suggest that the MAPK pathway, stimulated by Mos, activates Bub1, a conserved protein involved in the spindle assembly checkpoint (SAC) pathway in somatic cells. The final target of this pathway is the anaphase-promoting complex or cyclosome (APC/C), an E3 ubiquitin ligase that directly ubiquitinates Cyclin B and other proteins to promote entry into anaphase. During CSF arrest, Mos activates Bub1 through the MAPK pathway, thus causing inhibition of the APC/C (Schwab *et al*. 2001; Tunquist *et al*. 2002; Tunquist and Maller 2003). Consequently, in *Xenopus*, the protein Mos is involved in a large pathway important for both activation and stabilization of MPF activity during the G2/meiosis I transition and the establishment of CSF arrest during meiosis II (**Fig. 2**).

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; Josefsberg *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: hormone induction could therefore 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 cAMP prevents pre-MPF activation and represses *de novo* synthesis of Cyclin B1. The reduction of cAMP allows MPF activation that, besides promoting GVBD and chromosome condensation, induces polyadenylation of the *mos* mRNA, thus its translation (Lazar *et al*. 2002; Josefsberg *et al*. 2003; Lazar *et al*. 2004). cAMP reduction has been described also in other organisms, such as rat and fish, however in some species like rabbit, pig and sheep, oocyte maturation is associated with a transient increase of cAMP levels (Schmitt and Nebreda 2002).

In mammals, another unanswered question is the role played by Mos/MEK/MAPK/p90$_{Rsk}$/SAC proteins in CSF activity. While in frog this pathway is important for the establishment of CSF arrest during meiosis II, in mouse, p90$_{Rsk}$ seems to have no roles because oocytes from Rsk knockout mice maintain the ability to arrest in MII (Dumont *et al*. 2005). Moreover, the SAC proteins are not required for oocyte arrest at MII in the mouse (Tsurumi *et al*. 2004). The Mos pathway could therefore act independently from p90$_{Rsk}$, probably contributing to maintenance rather than establishment of the MII arrest (Madgwick and Jones 2007).

A functional hortologue of the Mos protein has been identified in *Drosophila* (Ivanovska *et al*. 2004). Injection of d*mos* 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 d*mos* mutant flies complete meiosis and produce fertilized embryos that develop normally, although there is a reduction in female fertility (Ivanovska *et al*. 2004).

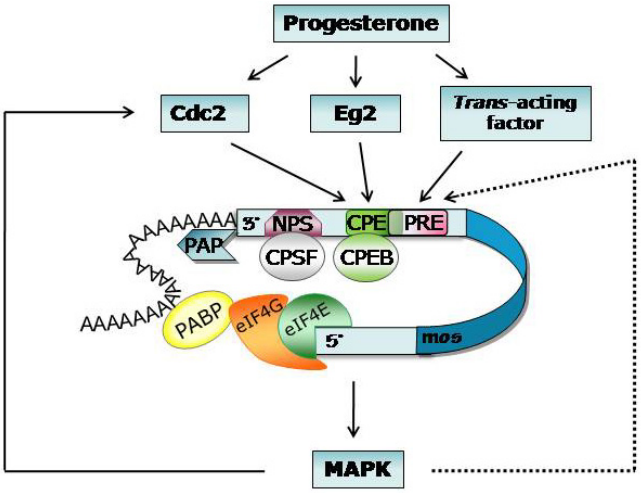

Fig. 3 Scheme of *cis*-acting sequences and *trans*-acting factors directing cytoplasmic polyadenylation-dependent translation of *mos* mRNA. The 3' untranslated region (3'-UTR) of *mos* mRNA contains a nuclear polyadenylation signal (NPS), a cytoplasmic polyadenylation element (CPE, that is bound to CPE-binding protein, CPEB), and a polyadenylation response element (PRE). All these sequences modulate polyadenylation events in the cytoplasm of *Xenopus* oocytes. Progesterone stimulation appears to activate *mos* translation in two temporally distinct phases: an early PRE-dependent translation, through a *trans*-acting factor (Charlesworth *et al*. 2006), and a CPE-dependent translation, stimulated by Aurora/Eg2 kinase activation (Mendez *et al*. 2000). PRE-dependent *mos* mRNA polyadenylation promotes its initial translation, which leads to the establishment of a Mos/MAP kinase positive feedback loop (dotted line). In turn, MAPK activation stimulates Cdc2 to further enhance CPE-dependent *mos* mRNA translation. The NPS sequence is able to direct both nuclear, one of the maturation events of eukaryotic pre-mRNAs (Piccioni *et al*. 2005a), and cytoplasmic polyadenylation by binding to cleavage and polyadenylation specificity factor (CPSF). The circularization of the mRNA is simplified in the figure by highlighting the cap-binding protein eIF4E (eukaryotic translation initiation factor 4E), the poly(A)-binding protein (PABP), and the scaffolding factor eIF4G (eukaryotic translation initiation factor 4G). 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.

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 with 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 guanosine 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 maturating 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 initiation factors eIF4G and eIF4E (Wells *et al*. 1998) (**Fig. 3**). It has been proposed that the "closed loop" complex both stabilizes the association of the cap-binding initiation factors and facilitates the recovery of ribosomes for a new cycle of translation (Eichenlaub-Ritter and Peschke 2002; de Moor *et al*. 2005; Piccioni *et al*. 2005a). Several proteins can interact with *cis*-acting sequences embedded in the 5' or 3' untranslated regions (UTRs) of the mRNAs, thus regulating the length of the poly(A) tail and/or competing for the assembly of a translationally active eIF4E-eIF4G complex. If some aspects of these processes appear to be species-specific, most of the molecules involved are conserved in developmentally distant organisms, thus suggesting a common mode of action.

Cytoplasmic polyadenylation and deadenylation

Translational regulation by cytoplasmic polyadenylation is a conserved biological mechanism, probably occurring in all metazoa. In *Xenopus* and mouse, the 3'-UTRs of select mRNAs, including several *cyclins*, 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 CPEB phosphorylation that induces the switch from a 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 derepress 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% of 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 and, within the ovary, it is exclusively present 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 c-*mos* and *Gdf9* mRNAs is altered (Racki and Richter 2006), thus linking CPEB function to poly(A) elongation and consequent translation of select mRNAs during the dictyate stage.

The CPE pathway is highly networked since the products of CPE-containing messages may target themselves CPE sequences. The Aurora A/Eg2 kinase, for instance, which phosphorylates CPEB in *Xenopus* oocytes is itself encoded by a CPE-containing mRNA (Vasudevan *et al*. 2006).

Additional components of the cytoplasmic polyadenylation machinery have been recently found. Kim and Richter (2006) reported a mechanism by which the poly(A) tail length of CPE-containing mRNAs is regulated. They demonstrated that the *cyclin B1* mRNA, upon acquisition of a long poly(A) tail in the nucleus of *Xenopus* oocytes, undergoes CPE-dependent deadenylation in the cytoplasm by the deadenylase PARN. In particular, they showed that GLD-2, a factor that has a poly(A) polymerase activity and is an atypical member of the DNA nucleotydil-transferase superfamily, and the deadenylase PARN are enzymes of the same CPEB-containing RNP complex. The enzymatic activity of PARN appears to be greater than GLD-2, so the poly(A) tail is kept short until oocyte maturation, when Aurora A phosphorylates CPEB, thus causing PARN expulsion from the complex and allowing GLD-2 activity. GLD-2 homologs were identified also in yeast (Saitoh *et al*. 2002; Read *et al*. 2002), *C. elegans* (Wang *et al* 2002), mouse, and human (Kwak *et al*. 2004).

One of the most studied mRNAs, known to be regulated by cytoplasmic polyadenylation, is *mos*. In *Xenopus*, the synthesis of the Mos protein begins shortly after progesterone stimulation and prior to GVBD. In vertebrates, *mos* translation is CPE- and CPEB-dependent (Gebauer *et al.* 1994; Mendez *et al*. 2000; Piccioni *et al*. 2005a). In addition, Charlesworth *et al*. (2002, 2006) demonstrated that the 3'-UTR of *mos* mRNA contains a polyadenylation response element (PRE) that, bound by a trans-acting factor, directs its early cytoplasmic polyadenylation and translational activation in a progesterone-triggered and CPE-independent manner. In summary, *mos* mRNA translation occurs after progesterone stimulation in two temporally and mechanistically distinct phases: an early PRE-directed and a later CPE-dependent Mos protein synthesis, which in turn activates the MAP kinase cascade to assure proper oocyte maturation (**Fig. 3**).

In *Xenopus* and mouse, most maternal mRNAs, includeing "housekeeping" mRNAs, undergo a default deadenylation process after GVBD, since they do not have CPE sequences. This deadenylating activity does not seem to require *cis*-acting specific elements (Varnum and Wormington 1990) and, in *Xenopus*, is mediated by xPARN (Copeland and Wormington 2001). Despite deadenylation, 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-nuclotide sequence recruits the deadenylation-promoting factor EDEN-BP that is responsible for deadenylation, and thus translational inactivation, following fertilization (Paillard and Osborne 2003). EDEN-BP homologues are CUG-BP in somatic human cells (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).

Translational inactivation by masking of mRNAs

As previously described, the formation of a "closed loop" complex through PABP and the initiation factors eIF4G and eIF4E on a properly polyadenylated and capped mRNA is a prerequisite for efficient translation (Piccioni *et al*. 2005a). The adaptor protein eIF4G is able to bind simultaneously the cap-binding factor eIF4E, through an eIF4E-binding motif (YxxxxLΦ, where Φ is an aliphatic residue) and the poly(A)-binding protein PABP. While the eIF4E-eIF4G interaction with the cap structure is essential for translation initiation, the eIF4G-PABP binding is not strictly required for ribosome recruitment, but constitutes a control point to modulate translation.

A general control on eIF4E is exerted by a class of inhibitors, known as eIF4E-binding proteins (4E-BPs) (Karim *et al*. 2001), which sequester the majority of both free and cap-bound eIF4E, thus preventing its binding to eIF4G. Hyperphosphorylation of 4E-BPs prevents their binding to eIF4E and therefore promotes translation initiation. On the contrary, specific translational repressors, all containing eIF4E-binding motifs, exert their activity on select mRNAs.

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 induce its dissociation 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; Piccioni *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 (Clast4; Villaescusa *et al*. 2006). *Clast4* 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 (Weston 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 the RNase III endonuclease Dicer generates RNA duplexes of 21-23 nucleotides. However, while Dicer cleaves miRNAs from a hairpin RNA, siRNAs originate by Dicer cleavage of a long double-stranded RNA. Successively, the RNA-induced silencing complex RISC allows the antisense strand of the duplex to recognize its RNA target 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 mRNA 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 (Tolia 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 subfamily 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 germ-line 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 *maelstrom* 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, in *Drosophila* two 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 (Murchison *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. They represent 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).

In the oocyte, 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 eIF4E) 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 deseases, 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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