The Regulation of Oocyte Maturation

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Abbreviations

1-MA 1-methyladenine
AC adenylate cyclase
cADPr cyclic ADP-ribose
CaMKII calcium/calmodulin-dependent kinase
DHP 17α,20β-dihydro-4-pregnen-3-one
ER endoplasmic reticulum
ES cells embryonic stem cells
FF-MAS follicular fluid meiosis-activating sterol
FSH follicle-stimulating hormone
GPCR G-protein coupled receptor

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I. Concept of Oocyte Maturation

Fertilization is required for sexual reproduction, and the cells participating in this process are highly specialized. Both types of metazoan gametes—oocytes and sperm—arise from germ cells, undergo extensive differentiation, and eventually unite. Growth and maturation of both male and female gametes culminates in production of fertilization-competent eggs and sperm (Masui and Clarke, 1979). The acquisition of fertilization competence of the oocyte, the focus of this review, encompasses a number of critical events. For example, the oocyte must construct and store excessive numbers of Golgi, mitochondria, and ribosomes, make specific sperm receptors, and in oviparous animals the cell must accumulate nutritional reserves sufficient for embryonic development. The oocyte must also prepare mechanisms ensuring productive fertilization since aberrations in this process result in early death of the embryo. For instance, supernumerary sperm entering the egg bring along extra genetic material and centrosomes resulting in abnormal mitosis and death of the zygote. To avoid these harmful
consequences, the eggs have evolved several strategies, the prevailing theme of which is modification of their extracellular matrix upon fertilization to make themselves nonreceptive to additional sperm. Establishment of the permanent block to polyspermy employs exocytosis of specialized dense core secretory vesicles called cortical granules upon fertilization. Other strategies include a fast but transient membrane potential change in some marine animals that blocks additional sperm–egg fusion, and degeneration of supernumerary sperm in physiologically polyspermic yolky eggs, such as in birds.

The final development of fertilization competence of an oocyte—responsible for making it a fertilizable egg—usually occurs during the process of meiosis, and is referred to as oocyte maturation. Maturation is often segregated into nuclear and cytoplasmic events to delineate specific mechanisms and functions. Cytoplasmic maturation involves the cytoplasmic changes required to prepare the cell for fertilization, activation, and embryo development. In various model organisms, it includes acquisition by the female germ cell of competence to fuse with sperm, decondense sperm chromatin, form pronuclei, and prevent polyspermy. Nuclear maturation refers to the meiotic process of chromosomal reduction to a haploid content, so as to produce a diploid organism upon fusion with sperm. Although oocytes may be fertilized, such gamete fusions are usually nonproductive (Longo and Schuetz, 1982 and references therein).

While sperm have completed their meiotic divisions prior to fertilization in every species studied, eggs are diverse in this respect. The competence to be fertilized and start zygotic development, which defines the egg, develops at different stages of meiotic division in different animal species. Fertilization can occur at the prophase stage of meiosis (clams, marine worms), at metaphase I (MI; some insects, starfish), or at metaphase II (MII; most mammals) of meiosis. The changes induced by insemination are commonly referred to as egg activation. The conversion of G2-arrested oocytes into actively dividing zygotes involves two major processes: maturation and activation. Notably, sea urchins belong to a limited group of organisms, wherein an oocyte completes meiotic maturation forming a haploid cell before it becomes fertilizable. Therefore, the processes of maturation and activation are fully separate in this animal in contrast to many others (such as starfish, frog, or mouse).

Understanding how an immature oocyte transforms into an egg during oocyte maturation is critical for our knowledge of fertility and reproduction. The topic of oocyte maturation has been reviewed recently from multiple

1In this review, the term “maturation” is used to define the completion of meiosis by the oocytes, and production of a fertilizable egg. This is in contrast to cases where the word “maturation” refers to the entire process of oocyte development, or oogenesis.
perspectives (Kishimoto, 1999; Matova and Cooley, 2001). This review integrates these perspectives and emphasizes the regulation of oocyte maturation by cell signaling networks.

II. Life and Death of an Oocyte

A. Oocyte Origins

Gametes develop from primordial germ cells (PGC) that are set aside during early embryogenesis. The development of the egg beginning from the formation of germ cell precursors is termed oogenesis. The driving factor for specification of cells as PGCs in a variety of animals appears to be inheritance of a defined area of egg cytoplasm, called germ plasm, containing a set of localized determinants. However, it is clear that this strategy is not universal, as in certain groups of organisms including mammals and sea urchins PGCs appear to be induced de novo from other cells in the gastrulating embryo (reviewed in Matova and Cooley, 2001; McLaren, 2003; Raz, 2002; Wylie, 1999). Furthermore, ascidian embryos appear to have an ability to regenerate germ cells following removal of PGCs (Takamura et al., 2002). In animals that utilize localized cytoplasmic determinants, germ plasm can be identified morphologically by the presence of specialized organelles, which are collectively called germ granules, or specifically known as P granules in C. elegans, polar granules in Drosophila, and germinal granules in Xenopus. Determination of primordial germ cells depends on localized RNAs and proteins, especially those comprising the germ granules. Oskar, vasa, and tudor proteins were first identified in Drosophila as components of polar granules and regulators of their formation. At least one of them, vasa, appears to have a universal role and is found consistently in the germ cells of a wide variety of animals (reviewed in Matova and Cooley, 2001; Raz, 2002; Takamura et al., 2002).

In animals that rely on inductive events for primordial germ cell determination, such as mouse, key regulators of this process appear to include the newly-identified interferon-inducible transmembrane protein known as fragilis, and a nuclear protein of unknown function termed stella (Saitou et al., 2002). Coordinate with the upregulation of stella, fragilis, DAZ-like, Oct-4, and (later) vasa in primordial germ cells is a downregulation of certain homeobox genes (HoxB1, HoxA1, Lim1, and Evx1) and signaling molecules like Smad1 (McLaren, 2003). Investigators are currently attempting to determine the pathways and targets for each of these candidates by using targeted gene manipulation, microarray analysis, and other techniques.
The difference between the major mechanisms for establishing the germ line in animals (i.e., cytoplasmic localized determinants vs induction) is extreme even in closely related animals (e.g., frogs and newts). Johnson et al. (2003) have proposed that the regulative mode of germ cell specification is the primitive form, from which predetermined development by localized determinants has evolved. With this interpretation it is also easier to understand how multiple independent mechanisms may have evolved in various animal lineages (McLaren, 2003).

A hallmark of germ cells is an extensive migration from the place of their formation to the developing gonad, combined with elimination of mistargeted cells elsewhere. This migration is regulated by somatic-germ cell interactions, and several molecular participants of this process have been recently identified, which appear to be distinct between different groups of organisms. For example, in Drosophila, enzymes implicated in lipid metabolism, wunen and columbus, affect PGC migration by generating repulsive and attractive signals, respectively (reviewed in Matova and Cooley, 2001). Two wunen genes are homologues of mammalian phospholipid phosphatase type 2; they encode transmembrane proteins, whose catalytic activity is required for generation of the repellant effect (Starz-Gaiano et al., 2001). Columbus (or Hmgcr) is a homologue of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase that generates the attractant for germ cells by an unidentified mechanism. Additional genes required for germ cell migration in Drosophila act by uncharacterized mechanisms and include slam (Stein et al., 2002) and scattershot (Coffman et al., 2002). In mouse, the signaling mediated by c-kit/Steel Factor is particularly important for germ cell migration, with additional input from integrin β2 and Fgf8 (reviewed in Matova and Cooley, 2001; McLaren, 2003). Finally, in zebrafish PGC guidance is regulated through seven transmembrane G-protein coupled receptor CXCR4 [α-(CXC-) chemokine receptor type 4] expressed in the PGCs and its ligand SDF-1 chemokine (stromal cell-derived factor 1) expressed along the path of PGC migration. These molecules establish a system for the guidance of fish PGCs throughout their entire migration (Doitsidou et al., 2002; Knaut et al., 2003; Kunwar and Lehmann, 2003). Once formed, the identity and developmental potential of the germline is maintained throughout the animal’s lifetime.

B. Oocyte Development

Once the gonad is assembled from somatic and germ cells, populating germ cells differentiate and proliferate. Initially, PGCs have the potential to begin either spermatogenesis or oogenesis and this decision is directed by the
gonadal environment. In a female genital ridge, or in a nongonadal environment, PGCs differentiate as oocytes, while male gonadal somatic cells direct PGCs to a spermatogenic fate (reviewed in Adams and McLaren, 2002). In many organisms, oogonia divide several times in the gonad to form clusters of interconnected cells, often referred to as cysts. The cytoplasmic bridges left after each division form the connections between cells in such clusters allowing for continuous communication between cells and coordinated development. Alternatively, when the interconnected cells form "oocyte-nurse cell" complexes they are able to more effectively utilize biosynthetic capabilities of multiple cells to produce maternal stores of important macromolecules in the resulting egg. The best documented example of the syncytial development of oogonia is in *Drosophila*. In this animal, a single cyst consists of 16 cells, one of which differentiates into an oocyte, while the remaining 15 assume the role of nurse cells that support the oocyte's growth and differentiation throughout the rest of the oocyte's growth period. Interconnected cells are also found in *C. elegans*, *Xenopus*, zebrafish, and different mammals (reviewed in Matova and Cooley, 2001). In contrast to *Drosophila*, these cysts eventually break down, such that every cell in an individual cyst of vertebrate animals has the potential to become an oocyte.

Proliferating germ cells are referred to as oogonia or spermatogonia, and are pluripotent stem cells normally restricted to germ line differentiation (Matova and Cooley, 2001). Oogonia differentiate into primary oocytes when they begin meiosis by replicating their DNA, and arresting in prophase of first meiosis. In the ovary, transition from a mitotic to a meiotic program is regulated by the signals from somatic cells (Chuma and Nakatsuji, 2001; Seydoux and Schedl, 2001). The prophase oocyte then may spend various periods of time in this state. In the human female for example, primary oocytes are arrested from 12 to 50 years, while in frog the arrest lasts for 3 years, and most echinoderm primary oocytes are arrested for up to a year depending on the species. Somatic cells of the gonad then stimulate the primary oocyte to continue development. In most animals, the growing oocyte contains an enlarged nucleus, called a germinal vesicle (GV), and is active in transcribing an extensive array of genes whose products are necessary for oocyte development and for sustaining early embryonic development. During periods of very active RNA synthesis, especially in animals with large oocytes, the GV contains lambrush chromosomes, with extended DNA loops at the sites of RNA synthesis (Smith and Richter, 1985). During oogenesis, the oocyte accumulates an extensive collection of RNAs, proteins, and organelles, such as cortical granules, yolk vesicles, ribosomes, and mitochondria (Wessel et al., 2001).

Female germ cells interact with the somatic gonadal cells throughout their life. In some animals, such as mammals, somatic cells of the gonad closely
associate with growing oocytes to form follicles (Fig. 1), while in other animals, such as sea cucumber, individual somatic cells establish junctions with the oocyte surface without forming complete follicles. Follicles provide a specialized compartment for the growth of the oocyte, accumulate and supply nutrients for oocyte’s growth, and generate signals governing oocyte development. Formation of the follicle in many species is essential for oocyte growth and development of a mature egg, suggesting that somatic cells provide both nutritional support and developmental information for the growing gamete.

Follicle cells are intimately associated with the oocyte through numerous interdigitating processes during the growth phase and the entire follicle is coupled with gap junctions (reviewed in Matova and Cooley, 2001). In the mouse ovary, gap junctions form in advanced (secondary) follicles, but are not detected in early (primordial) follicles (Wright et al., 2001). In some reptile species (e.g., the lizard Anolis carolinensis) cytoplasmic connections may arise between an oocyte and follicle cells and form intercellular bridges (Neaves, 1971). Connections between the oocyte and somatic cells allow nutrient transfer to the developing oocyte and are essential for oocyte growth in mammals (reviewed in Carabatsos et al., 2000; Eppig et al., 1996). The oocyte loses direct physical contact with associated follicle cells just prior to the onset of maturation.

Bidirectional cell–cell communications coordinate the development of oocyte and follicle cells; these interactions take the form of autocrine, paracrine, and endocrine regulation in addition to cytoplasmic gap junctional contact mentioned above. The oocyte appears to be the dominant component of the follicle, determining the overall rate of follicular

Figure 1  Schematic representation of a late mammalian follicle (after Wright et al., 2001). The cell types comprising the follicle are shown (the oocyte and somatic granulosa cells). Also pointed out are the oocyte’s nucleus (germinal vesicle), extracellular matrix produced by the oocyte (zona pellucida), antrum, and the granulosar membrane of the follicle.
development (Eppig et al., 2002; Matzuk et al., 2002). A critical role of the oocyte was established in experiments involving the exchange of somatic and germ cell components of the follicles at different stages of development. Mouse oocytes dissociated from the midgrowth secondary follicles (12 days old) were reaggregated with the somatic cells of newborn mice and surgically implanted beneath the renal capsules of host females. Such reimplanted oocytes dramatically accelerated follicular development, resulting in formation of antral follicles with differentiated follicle cells nine days after reimplantation. This was significantly faster than normal development of antral follicles in the newborn mice, which takes 18–24 days. Thus, the oocytes appear to provide instructive signal that is able to direct the development of the somatic component of the follicle. This experimental setup provides a valuable tool for dissecting the signaling mechanisms involved in the crosstalk between the oocyte and somatic cells of the follicle during oogenesis in mammals, and perhaps other animals as well.

Embryonic stem cells are now known to differentiate into oocytes and this technology may greatly enhance our ability to experiment with the germ cell differentiation pathway. Recent work from Hubner et al. (2003) showed that ES cells can acquire the morphology and gene expression profile consistent with differentiation into oocytes. While the pluripotential nature of ES cells has been appreciated for some time, the possibility of germ line differentiation in vitro was unexpected. This result has massive technical and ethical consequences.

Key for this observation was use of a GFP reporter system driven by a promoter specifically active in oocytes that drives the transcription factor Oct4. ES cells in culture could then be examined noninvasively and followed over the culture period. The investigators found that Oct4-GFP was expressed in a subpopulation of the ES cells after a few days in culture. In fact, several different types of markers accumulated in the oocyte-like ES cells. These included the zona pellucida proteins ZP2 and ZP3, the transcription factor of germ cells Figα, a TGF-β growth factor GDF-9, and some markers of meiosis (DCM1, SCP3). Each of the markers is consistent with the ES cell becoming an oocyte, and in concert, the population of markers tested give strong support to an oocyte differentiation pathway. Coincident with this marker expression was the morphological transitions the oocyte-like cells underwent. This includes recruiting other ES cells into a follicle-like structure (able to express several markers of estrogen biosynthesis), displacement of the oocyte from the recruited cluster that resembled oocyte ovulation, and eventually even formation of structures similar to embryonic blastocysts. These findings will have great potential in dissecting mechanistic processes in oogenesis in particular, and gametogenesis in general.
C. Transformations of the Oocyte During Meiotic Maturation

1. Nuclear Changes and Chronology of Maturation

The process of oocyte maturation traditionally has been described by changes in chromosome morphology during meiosis. Before maturation starts, the oocyte contains a large germinal vesicle (GV) with a large nucleolus (Fig. 2A–C). The chromosomes in the GV are mostly decondensed, dispersed, and transcriptionally active (reviewed in Smith and Richter, 1985; Wassarman, 1983). With the initiation of maturation, transcription ceases, the chromosomes begin to condense, the GV breaks down, and nucleoli disperse (Masui and Clarke, 1979). As maturation progresses, the paired homologous chromosomes align in the middle of the cell, completing the process of meiosis.

Figure 2  Events of nuclear maturation, exemplified by sea urchin oocytes. A–C: Oocytes spend their growth period arrested in G2 phase of meiotic cell cycle, with replicated DNA (4N). After resumption of meiosis, oocytes of different species arrest for the second time at different stages of meiotic progression, such as MI (D–F), or MII (G), and are relieved from this second arrest by fertilization. Then they finish their meiotic divisions and progress into mitosis. Sea urchin oocytes are fertilized as haploid eggs after completing meiotic divisions (H–J). A, D, G, H: schematic of nuclear events of meiosis, ploidies of the nuclei are noted; B, E, I: brightfield images of sea urchin oocytes undergoing meiosis; C, F, J: corresponding fluorescent images of DNA stained with Hoechst. PB1—the first polar body; PB2—the second polar body; pn—egg pronucleus; MI—metaphase I stage of meiosis; MII—metaphase II stage of meiosis.
forming meiotic spindle during metaphase I (Fig. 2D–F). Separation of the paired homologous chromosomes is followed by the first polar body formation. Then the chromosomes remaining in the oocyte are again arranged on a meiotic spindle at metaphase II. With the second meiotic division, chromatids separate and the second polar body is formed. Finally, the chromatids remaining in the oocyte decondense and a pronucleus forms (Fig. 2H–J). The meiotic cells of different organisms become fertilizable at different stages along the process of meiosis. For example, the eggs of most vertebrate species arrest at the second metaphase, and await fertilization to release them into embryonic development, while sea urchin eggs proceed through meiosis to completion, and are stored as cells with haploid pronuclei.

How long does oocyte maturation take? The dynamics of oocyte maturation is difficult to assess for a number of reasons. First, exact observation of cellular features during in vitro oocyte maturation may not faithfully reproduce in vivo processes. Second, the progression of maturation is often not synchronous in individual members of an oocyte population, especially when the oocytes are obtained from females in different phases of their seasonal reproductive cycle (Masui and Clarke, 1979). Therefore, the timing can only be defined statistically. Finally, inaccuracy can be introduced when determining the exact time of initiation of oocyte maturation. While in certain species, the time of oocyte exposure to a hormone is known precisely (as in Xenopus or starfish for example), the oocytes of other species are able to mature in vitro without any external stimuli. Mammals and sea urchins belong to the latter group of animals, and therefore at this time one can only rely on obvious structural changes indicating the onset of maturation.

In various species, oocyte maturation proceeds at different rates, even in members of the same phylogenetic class (Masui and Clarke, 1979). For instance, oocyte maturation in starfish takes only 1 h 40 min from GVBD to completion (Kishimoto, 1999), whereas in the sea urchin, oocyte maturation from the first detected migration of GV to the cell surface and GVBD to formation of egg pronucleus takes approximately 8 h in vitro (Berg and Wessel, 1997). The extended time it takes for the sea urchin oocyte to fully mature may be explained in part by the fact that this cell needs to complete meiosis and reform a haploid pronucleus, whereas other oocytes abbreviate their maturation by arresting at MI or MII (it takes sea urchin 4 h for the first polar body to form, so MI probably occurs at ~3 h after GVBD). Other representative durations of oocyte maturation are, approximately, on average 5–6 h for fish (but up to 18 h in certain species, Thomas et al., 2002), 3 h for Xenopus (Terasaki et al., 2001), 6–7 h for mouse, rat, and hamster, and 15–20 h for human (both in vivo and in vitro, Bomsel-Helmreich et al., 1987). However, it is possible that spontaneous maturation in vitro takes
longer to proceed than the hormone-induced maturation \textit{in vivo}. In a study of brushtail possum oocytes (Glazier \textit{et al.}, 2002), the spontaneous \textit{in vitro} maturation of the oocytes took more than 24 h. However, when the oocytes were allowed to mature \textit{in vivo} and recovered at various times post gonadotropin treatment for observation, intermediate stages of meiotic maturation were not observed at all, suggesting that it is an extremely rapid process.

2. Changes of the Organelles

The process of oocyte maturation is accompanied by fundamental changes in cellular organelles. Oocytes possess a variety of organelles typical of most cells (such as Golgi apparatus, mitochondria, endoplasmic reticulum) as well as oocyte-specific (yolk granules, cortical granules, annulate lamellae, acidic granules, and pigment granules). Some of these organelles do not undergo any transformations during maturation (mitochondria, yolk), while others (such as cytoskeletal elements, cortical granules, and endoplasmic reticulum) exhibit dramatic changes—and are hallmarks of meiotic progression.

The orchestration and execution of the nuclear events of meiosis described above depends on the cell’s \textit{cytoskeleton}. The growing oocyte is characterized by elongated, labile cortical microtubules that undergo dramatic changes during oocyte maturation (Boyle and Ernst, 1989; Smiley, 1990). Microtubules progressively disassemble in the cortex, and the meiotic microtubule spindle then forms in the maturing oocyte to mediate chromosome segregation during both divisions of meiosis (a separate spindle forms in each division). Meiotic microtubule asters contributing to the spindle formation are morphologically distinct from the mitotic ones, being smaller, and not reaching distal aspects of the oocyte. In the mature sea urchin egg, microtubules can still be found at the cortex, although they are shorter and less numerous than in oocytes (Boyle and Ernst, 1989). During meiosis, eggs of most species (the most notable exception being mice) lose their microtubule organizing center, or centrosome, so that they rely on fertilization and the parental centrosome to be able to execute subsequent cytokinesis (see below). This process is particularly well documented in starfish oocytes.

The starfish oocyte begins meiosis with two centrosomes, as do somatic cells in a G2 phase of the cell cycle. During the first meiotic division the cell loses one of the centrosomes to the first polar body, which exactly mirrors the processes occurring during a mitotic division. The remaining centrosome does not replicate between two meiotic divisions (as it would have in mitosis). Instead, in an event unique to meiosis, the two individual centrioles of the remaining centrosome separate and organize the second meiotic
spindle. After the second meiotic division, one centriole is segregated into
the second polar body, and the egg is left with a single centriole (Schatten,
1994 and references therein). It appears that these centrioles are not equal,
as the second polar body centriole retains the ability to replicate during the
S-phase and can support mitotic divisions, while the egg centriole is not
functional (Tamura and Nemoto, 2001). This single centriole is targeted for
degradation during the anaphase of the second meiotic division, as detected
by the loss of focal γ-tubulin localization shortly after completion of meiosis
(Uetake et al., 2002). In contrast, the mouse oocyte has a number of
centrosomal foci (instead of well-defined centrosomes), some of which
aggregate at the poles of the meiotic spindles, and others remain spread
throughout the cytoplasm, and evade elimination during meiotic divisions
(reviewed in Schatten and Schatten, 1987).

The cortical granules accumulated in the oocytes acquire the capacity for
exocytosis during maturation. Their function is to secrete their contents at
fertilization and modify the extracellular matrix of the egg, such that it does
not support binding and fusion of extra sperm. It is critical for the cell to
rapidly exocytose its cortical granules at fertilization to block polyspermy,
but also to prevent precocious exocytosis of these cortical granules,
otherwise the extracellular matrix would be irreversibly modified and the cell
would not be receptive to sperm. The oocyte synthesizes and accumulates
large numbers of cortical granules throughout oogenesis. In oocytes where
cortical granules have been quantified, they reach 8,000 in mice and 15,000
in sea urchin (reviewed in Wessel et al., 2001). During oocyte maturation,
cortical granules move to the periphery in nearly all species examined. This
change in distribution is linked to the acquisition of the capacity of these
granules to exocytose upon fertilization. In the sea urchin, these granules
are dispersed throughout the oocytes’ cytoplasm, and translocate to
the periphery of the oocyte during maturation (reviewed in Wessel et al.,
2001). In other animals, such as starfish and frog, the cortical granules are
already in the general vicinity of the cell cortex, and are brought to the
plasma membrane and rearranged at the cell surface during oocyte
maturation. In rodents, oocytes form a cortical granule-free domain over
the region of meiotic spindle mainly by rearrangement, with some
contribution of precocious exocytosis (Deng et al., 2003). Cortical granule
rearrangements each depend on de novo formation of microfilament
networks at maturation.

Microfilaments form the scaffold for organelle movement during oocyte
maturation. In the growing echinoderm oocyte, microfilaments are predominantly found at the cortex of the cell (Boyle and Ernst,
1989; Heil-Chapdelaine and Otto, 1996). In starfish and sea urchin
oocytes, dramatic polymerization of actin takes place at the onset of
maturation (Heil-Chapdelaine and Otto, 1996; Wessel et al., 2002, and
Actin polymerization is detected at the cell cortex (where actin spikes appear transiently in starfish); however, most of the polymerized actin is detected in the nucleus. This reorganization of the actin cytoskeleton is not critical for germinal vesicle breakdown, as cytochalasins suppressing all actin polymerization do not inhibit GVBD (e.g., Connors et al., 1998).

Actin filaments in the germinal vesicle have been seen in many different oocytes, including frog, starfish, and sea urchin (Heil-Chapdelaine and Otto, 1996; Parfenov et al., 1995; Wessel et al., 2002). Although the function of the filaments is not clear, it is intriguing for several reasons: (i) they are transient, present just before GVBD and then disappear; (ii) their presence is coincident with initiation of cortical granule translocation; (iii) they are found at a time when the germinal vesicle is moving to the animal pole; (iv) and they are correlated with a change in the germinal vesicle shape and pending GVBD (Stricker and Schatten, 1989). One hypothesis of function is that they contribute to vesiculation of the nuclear envelope (Parfenov et al., 1995). In addition, several regulators of actin polymerization are also present in the nucleus or translocate to the nucleus (Randø et al., 2000), suggesting that there is some function of actin in the nucleus that is subject to regulation. Finally, Zhao et al. (1998) find actin and actin-related proteins in transcriptional complexes of mammalian cells. This has also been seen in yeast, with genetic evidence supporting actin function both in regulating transcription and chromatin remodeling (Shen et al., 2000).

In vertebrate oocytes, actin polymerization appears to be required for the translocation of the meiotic spindle from the center of the cell to an asymmetric cortical location, and as a consequence for the first polar body extrusion (Connors et al., 1998; Kim et al., 2000; Maro and Verlhac, 2002; Ryabova et al., 1986). Recent work identifies the microfilament-binding protein formin as a necessary molecular regulator of this process, as the spindle does not migrate and polar bodies are not extruded in oocytes from formin knockout mice (Leader et al., 2002). Formins, or formin-homology (FH) proteins, comprise a recently recognized protein family, which functions in regulating cell polarity by acting as effectors of Rho family small GTP-ases and remodeling actin cytoskeleton (reviewed in Alberts, 2002; Lew, 2002; Zeller et al., 1999). The other transport event that actin microfilaments are required for is translocation of cortical granules in multiple organisms, such as sea urchin, starfish, and mouse (reviewed in Wessel et al., 2001, 2002).

The scaffold formed by an extensive network of intermediate filaments containing cytokeratin is present within 1 μm of the plasma membrane in immature oocytes of the hamster, *Xenopus*, ascidians, sea urchin, and starfish (Boyle and Ernst, 1989; Schroeder and Otto, 1991, and references therein).
Upon commencement of meiotic maturation (at GVBD) in these animals, the intermediate fibers disassemble, and never reform in the mature eggs (Boyle and Ernst, 1989; Schroeder and Otto, 1991). Interestingly, an association of signaling proteins (beta-gamma subunits of heterotrimeric G-proteins) with these cytokeratin networks has been detected in starfish (Chiba et al., 1995). The subcortical network of intermediate filaments might serve as a scaffold for the assembly of the signaling protein complexes, and thus be important for signal transduction in oogenesis. The release of G-protein $\beta y$ subunits is necessary for starfish oocyte maturation to occur (see Section VA). The timing of this release is correlated with the dispersal of intermediate filaments, so the detected structural reorganization of intermediate filaments can be indicative of the release of the cell signaling mediators at maturation.

The endoplasmic reticulum (ER) of many species such as sea urchin, starfish, frog, and mouse undergoes significant changes during oocyte maturation. The changes in the ER structure are of particular interest as this organelle releases calcium at fertilization mediating egg activation, and this ability to release calcium develops during oocyte maturation. Immature oocytes of all species studied possess relatively uniform three-dimensional network of ER tubules with some individual cisternae and annulate lamellae (accumulations of cisternae) deep in the cytoplasm (Bobinnec et al., 2003; Jaffe and Terasaki, 1994; Mehlmann et al., 1995; Terasaki et al., 2001). The detected changes of the ER in the course of oocyte maturation include reorganization, or formation of circular structures around the yolk platelets in starfish (Henson et al., 1990; Jaffe and Terasaki, 1994; Mehlmann et al., 1995; Terasaki et al., 2001) and accumulation of ER clusters in an organized array immediately next to the plasma membrane (Henson et al., 1990; Mehlmann et al., 1995; Shiraishi et al., 1995; Terasaki et al., 2001).

Changes in the structure of the ER, especially dispersion of the nuclear envelope, and fragmentation of ER tubules, are due to the cell cycle progression, and have been documented for mitosis as well (reviewed in Jaffe and Terasaki, 1994; Lippincott-Schwartz, 2002). However, several features of ER behavior are unique for meiosis. In maturing oocytes, the ER is not associated with the meiotic spindle, while such association is detected in mitotic cells (Bobinnec et al., 2003; Terasaki, 2000). Furthermore, the formation of cortical clusters is specific for oocyte maturation and is ultimately required for formation of calcium release mechanisms in the egg at fertilization. These clusters disappear some time after fertilization and are not detected in mitotic embryonic cells (FitzHarris et al., 2003). In mouse and probably other animals too, the disappearance of cortical ER clusters depends on the decrease of MPF activity (FitzHarris et al., 2003 and references therein).
3. Changes in mRNA and Protein Patterns

Oocytes are actively engaged in transcription and through their growth accumulate extensive amounts of mRNA (Masui and Clarke, 1979; Smith and Richter, 1985). However, the mRNA content of the oocyte changes upon maturation. This switch in mRNA populations is brought about by both general termination of transcription at GVBD, and degradation of the select subset of transcripts (Smith and Richter, 1985). In sea urchin oocytes, the mRNAs coding for yolk and cortical granule constituents each abruptly disappear with the beginning of oocyte maturation (LaFleur et al., 1998; Laidlaw and Wessel, 1994; Wessel et al., 1998, 2000a,b). Generally, oocyte maturation does not require mRNA synthesis; the only exception found so far is sheep, where oocyte maturation is inhibited by the transcription inhibitor α-amanitin (Moor and Crosby, 1986). In most cases, mature eggs are not transcriptionally active, as they are arrested in the metaphase of the second meiotic division. The sea urchin egg, however, is an exception, in that it is arrested before fertilization with a haploid pronucleus, which is transcriptionally active and selectively accumulates histone mRNA before fertilization (Venezky et al., 1981).

Protein synthesis patterns change significantly during the transition from an oocyte to a mature egg. Certain mRNAs are translationally activated while others become repressed (reviewed in Hake and Richter, 1997; Smith and Richter, 1985). Translational repression results in part from the degradation of mRNAs, but also from selective mRNA deadenylation. In sea urchins for example, production of yolk (yp30, Wessel et al., 2000b) and cortical granule content (Laidlaw and Wessel, 1994) proteins is extremely active in the primary oocyte, but ceases at the beginning of oocyte maturation, due to mRNA degradation. In Xenopus and mouse, a specific class of maternal mRNAs is deadenylated and translationally repressed during oocyte maturation (Paynton and Bachvarova, 1994; Varnum and Wormington, 1990). Translational activation of select mRNAs during oocyte maturation is achieved by regulated elongation of their poly(A) tail (Hake and Richter, 1997), and normally leads to a several-fold increase in the rate of overall protein synthesis (Smith and Richter, 1985; Wasserman et al., 1986). Qualitative changes in the patterns of protein synthesis have been observed in many animals such as starfish, frog, and mouse. The proteins being made during oocyte maturation are mostly cell cycle regulators needed to advance the oocyte through meiosis. The prime example of such proteins is cyclin B, the regulatory component of maturation promoting factor; another one is c-mos required for two consecutive divisions of meiosis. Consistently, protein synthesis inhibitors block oocyte maturation in many (but not all) species. Some animals appear to have all the proteins necessary for maturation already produced and
stored in the oocyte (like the clam, *Spisula*, Hunt et al., 1992). Others need to synthesize cyclin proteins (mouse, fish); still others need to make different molecular cell cycle regulators, such as ringo/speedy (*Xenopus*, Ferby et al., 1999; Hochegger et al., 2001; Lenormand et al., 1999). The mature egg of the sea urchin is translationally quiescent, while in other species such as *Xenopus* or mouse the mature egg is translationally active. This may be due to the fact that these eggs are at different phases of their meiotic cycle: frog and mouse eggs are arrested at MII and are constantly replenishing their mitotic cyclin proteins, while the sea urchin egg is arrested following MII, at a “G1-like” state.

### 4. Physiological Changes

Mature eggs produce the fast block to polyspermy by changes in plasma membrane potential upon fertilization (collectively termed as “fertilization potentials”), which transiently depolarizes in ascidians and sea urchins, and hyperpolarizes in certain mammalian species (reviewed in Dale, 1994). The egg’s ability to generate this fertilization potential develops during oocyte maturation. The mature eggs of starfish as well as bovine produce fertilization currents with greater peak amplitudes and faster rise times than do immature oocytes (Miyazaki, 1979; Tosti et al., 2002). These differences reflect the changes in ionic conductance that occur during oocyte maturation, and ultimately contribute to the establishment of the fast block to polyspermy. Thus, a decrease in potassium conductance by downregulation of Na\(^+\)/K\(^+\) pumps was proposed to occur in both starfish and *Xenopus* oocytes upon exposure to maturation-inducing hormones (reviewed in Miyazaki, 1979; Smiley, 1990; Wasserman et al., 1986).

Changes in sodium currents in *Xenopus* oocyte ultimately lead to an increase in the intracellular pH after progesterone stimulation from 7.2 to 7.7. The pH change occurs due to the activity of Na\(^+\)/H\(^+\) pump in the plasma membrane of the cell (Wasserman et al., 1986). This increase in pH appears to be important for the progress of maturation, as stabilizing the pH below 7.0 completely blocks GVBD. This increase in pH in the frog oocyte is dependent upon downregulation of Na\(^+\)/K\(^+\) pumps, as a high cytoplasmic concentration of potassium may directly inhibit the Na\(^+\)/H\(^+\) pump.

### 5. Competence for Calcium Release

The capacity of the mature egg to undergo activation depends on the competence to generate a calcium transient (or transients) upon fusion with
sperm, which develops during oocyte maturation. Prophase-arrested mouse oocytes do not generate the series of calcium transients at fertilization characteristic of mature eggs (Cheung et al., 2000 and references therein). In mouse and starfish oocytes, inositoltriphosphate (IP3) receptor amounts increase during maturation (Chiba et al., 1990; Iwasaki et al., 2002), the IP3 receptor is rearranged at the cortex of the oocytes (Mehlmann et al., 1996), the endoplasmic reticulum is reorganized (reviewed in Section IIC), and intracellular calcium stores increase (Tombes et al., 1992). The maturation-associated increase in IP3 receptor protein amount results in increased sensitivity to IP3. The increase in sensitivity in turn confers to the cell the ability to generate a normal pattern of calcium oscillations and undergo normal amounts of cortical granule exocytosis (Xu et al., 2003).

D. Death

The oocyte is a terminally differentiated cell. If the oocyte does not transform into a mature egg, or if the egg is not fertilized, it dies. The demise of the germ cells throughout the animal kingdom occurs through apoptosis, or programmed cell death. Apoptosis is a conserved process of cell elimination, characterized by distinct morphological hallmarks, and requiring activation of specific intracellular programs. Examples of physiological cell death required for oogenesis include C. elegans oocytes and Drosophila nurse cells that die donating their cytoplasm to the surviving gametes (it is still unclear what prevents the spreading of death signal in these syncytial cells to the surviving oocytes), or oocyte attrition and follicular atresia in mammals. The environmental conditions such as starvation or toxic substance exposure are known to cause germ cell apoptosis as well (Matova and Cooley, 2001; Voronina and Wessel, 2001). Apoptosis induction during starvation may be a nutritional strategy in some animals with significant resources invested into egg production enabling the animals to make many eggs during the time of plenty, but allowing the option to reuse these nutrients in the time of pity. This is especially true for yolk-laden oocytes where the nutritional stockpile is great and reusable. In many species, mature eggs that have not been fertilized age rapidly, and apoptose (Morita and Tilly, 1999; Sasaki and Chiba, 2001; Yuce and Sadler, 2001). This could represent the “quality control” mechanism in the animals that ovulate their eggs arrested at the second metaphase. Such eggs could potentially damage meiotic spindles and missegregate their chromosomes upon fertilization or even activate parthenogenetically if allowed to remain in such state for a long time, and exhaust their energy supplies.
III. Maturation Promoting Factor

The cytoplasmic signaling pathways initiating the G2/M transition during meiotic resumption of the oocytes converge on activation of the maturation-promoting factor, or MPF (Taieb et al., 1997). MPF was identified in and subsequently purified from mature eggs of *Xenopus* (Lohka et al., 1988; Masui and Markert, 1971). It consists of cyclin B (or B1 and B2 in vertebrates) and cdk1 kinase, also referred to as cdc2 (Fig. 3; Labbe et al., 1989; Kobayashi, 1991, reviewed in: Maller, 1990). This complex is a universal cell cycle regulator advancing G2/M transition in both meiotic and mitotic cell cycles. Cyclins and their catalytic kinase partners (cdks, or cyclin-dependent kinases) are well recognized as regulators of general cell cycle progression (for example, reviewed in: Pines, 1999). Cyclin B is the regulatory subunit of the complex: cdk1 kinase is structurally prevented from activation as a monomer, and needs to complex with cyclin for activity (Morgan, 1997). Cyclin-dependent kinases preferentially complex with particular cyclins, and are required only at specific times during mitotic progression. Although the levels of cdks remain constant, the levels of cyclins usually fluctuate during the cell cycle due to periodic synthesis and degradation, resulting in transient kinase activities. Cyclins are classified as G1, S, or M-phase regulators based on when their activities are required in the cell cycle (Pines, 1999).

The levels of newly synthesized mitotic cyclins peak in M-phase, and mitotic cyclin/cdk1 complexes govern the transition to and progression through M-phase (reviewed in Pines, 1999). Cyclin B-cdk1 phosphorylates and regulates the function of numerous substrates, including nuclear lamins, histone H1, transcription factors, kinesin-related motors, and cytoskeletal regulators, causing structural changes in the nuclear envelope, chromatin, and cytoskeleton (reviewed in Nigg, 1995, 2001). The cdk1 itself is a subject of tight posttranslational regulation (reviewed in Nigg, 2001). In addition to the required association with a cyclin, cdk1 can be phosphorylated on multiple sites. These phosphorylation events include both stimulatory (on Thr 161) and inhibitory (on Thr 14 and Tyr 15, localized in the ATP-binding site) regulation. The kinases responsible for inhibitory phosphorylations, Wee1 and Myt1, are active during the G2 phase, and their enzymatic activity is in turn regulated by cell signaling pathways as well as by checkpoint controls (although only Myt1 is present in oocytes). The enzyme removing inhibitory phosphates from cdk1 is a dual-specificity phosphatase cdc25. The balance of cdc25 vs Wee1/Myt1 activities integrates the checkpoint and cell signaling inputs to determine the activation state on cyclin B-cdk1 complex and allow progression to the M-phase (Nigg, 2001).
The presence of multiple feedback mechanisms able to regulate cyclin B-cdk1 activity results in a switch-like, or as proposed by Ferrell, a "bistable" system (Ferrell, 2002). Such a system is characterized by the presence of two stable states, and the ability of an "all-or-none" switch from one state to the other in response to graded stimuli. In a bistable system, small stimuli have little effect on cyclin B-cdk1 activity, whereas stimuli above a certain threshold have a significant effect (Fig. 3).

The mechanisms of mitosis and meiosis regulation are quite different, despite the same cyclins being major molecular regulators of both processes. Oocytes spend prolonged periods of time arrested in the prophase of first meiotic division (see Section IIB); consistently, the control of entry into M-phase is different between mitosis and meiosis. To proceed through meiotic divisions, the oocyte accumulates and then activates MPF in response to the mitogenic signal (Fig. 4). Meiotic division itself is a modification of a mitotic one, where the cell does not undergo DNA replication between two successive rounds of division thereby decreasing the amount of DNA to a haploid state. This is a consequence of the fact that regulation of MPF during meiosis is different than that in the mitotic cell cycle, namely, MPF activity increases rapidly after the first meiotic division, and the second M-phase starts without a preceding S-phase. To gain

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**Figure 3**  Regulation of cdk1 activation. Activation of cdk1 depends on association with cyclin B and the phosphorylation of cdk1 protein within the cdk1-cyclin B complex by cdk activating kinase, CAK. Protein phosphatase 2C (PP2C) can reverse the CAK-mediated phosphorylation. Active cdk1-cyclin B can be inactivated by the Myt1 protein kinase present in the oocyte, whose effects are reversed by the activating phosphatase cdc25. Plx1—polo-like kinase 1, which phosphorylates and activates cdc25. Positive feedback loops are shown as dashed lines. The figure is adapted from Ferrell 2002 (see color plate).
insight in the molecular mechanism of meiotic M-phase initiation and progression, cell cycle dynamics of cyclin A, cyclin B, and cdk1 kinase during oocyte maturation have been studied in many organisms (reviewed in Kishimoto, 1999; Yamashita, 1998), and significant differences are found between species arguing against a unified mechanism regulating MPF activity in oocytes (Taieb et al., 1997).

One mechanism regulating MPF activity in oocytes is accumulation of cyclin B protein, the best example being fish (reviewed in Yamashita, 1998, Fig. 4). In this case, the newly synthesized cyclin B associates with the catalytic subunit (cdk1), which is not subjected to inhibitory phosphorylations, immediately forming active complexes. Other layers of regulation known for MPF include posttranslational modification of the catalytic subunit, as exemplified in *Xenopus*, mouse, and starfish (Kishimoto, 1999). In these oocytes, cyclin B is made during the growth phase of the oocytes, and newly formed cyclin B-cdk1 complexes are immediately inactivated by inhibitory phosphorylations. These are in turn removed in response to signals inducing oocyte maturation, ensuring rapid activation of the stockpile of inactive MPF. Recent data from sea urchin oocytes suggests a similar MPF activation strategy (Voronina et al., 2003). Transport of MPF

Figure 4  Schematic representation of MPF activity changes during growth and maturation of oocytes compared with levels of the regulatory subunit of MPF, cyclin B (see color plate).
between the cytoplasm and the nucleus is important for its activation and function, and the dynamic changes in MPF localization may contribute to its switch-like activation (Takizawa and Morgan, 2000). Recently, existence of additional MPF components has been proposed based on the observation that components of the germinal vesicle of oocyte are needed in addition to the activated cyclin B-cdc2 to induce GVBD in the nonstimulated oocytes in starfish (Kishimoto, 1999).

IV. Initiation of Oocyte Maturation

Hormones are the best documented stimulators of oocyte maturation. Nevertheless, stimuli that induce oocyte meiosis in various species are quite diverse, and include fertilization (in clam, *Spisula solidissima*) and even a secreted sperm protein factor (*Caenorhabditis elegans*, described further in Section IVD, Miller et al., 2001).

The somatic cells of the ovary relay the inductive signal for oocyte maturation from the hormones released into the circulation. A number of mechanisms for triggering oocyte maturation have been proposed so far that do not necessarily exclude each other. Among those are: production of a maturation-inducing substance by follicle cells that directs the oocyte to mature (starfish, fish, frog, maybe mammals); inactivation of the follicle-produced inhibitor (mammals); and inhibition of gap junction-mediated transport to prevent transfer of a follicle-produced inhibitor (fish, mammals).

A. Maturation-inducing Substance (MIS)

Steroid hormones have been found to induce oocyte maturation in a number of vertebrate species. Although traditionally such hormones are known to mediate their effects via nuclear receptors inducing transcription, it appears that signaling in the oocyte involves activation of membrane receptors, and is independent from new transcription. At this time, candidate MISs are proposed for starfish, fish, amphibians, and mammals.

**Mammals.** The meiosis-inducing activity of human follicular fluid was identified by an ability to promote maturation in mouse oocytes dissected from the follicles. Gas chromatography mass spectrometry identified the

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2These hormones could generally be referred to as “gonadotropins” (as in Masui and Clarke, 1979), but the term appears confusing, since presently it is used only for vertebrate hormones (in which capacity it will be used herein). However, the “gonad-stimulating substance” of starfish is functionally a “gonadotropin” (acting on ovary) as well.
active compound as 4,4′-dimethyl-5α-cholesta-8,14,24-trien-3β-ol, an intermediate in cholesterol biosynthesis (Table I). It was proposed to be the ovarian stimulatory factor inducing meiotic resumption of the oocytes, and it was named FF-MAS for follicular fluid meiosis-activating sterol (Table I,

### Table I  Structure of the Proposed Maturation Inducing Substances (MIS) of Various Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Name of MIS</th>
<th>Structure of MIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammals</td>
<td>4,4′-Dimethyl-5α-cholesta-8,14,24-trien-3β-ol (FF-MAS)</td>
<td><img src="image1" alt="Mammals Structure" /></td>
</tr>
<tr>
<td>Frog</td>
<td>Progesterone</td>
<td><img src="image2" alt="Frog Structure" /></td>
</tr>
<tr>
<td>Fish</td>
<td>17α,20β-Dihydro-4-pregnen-3-one (DHP) is shown in black; the extra hydroxyl of 17α,20β, 21α-trihydro-4-pregnen-3-one (THP) is shown in parentheses</td>
<td><img src="image3" alt="Fish Structure" /></td>
</tr>
<tr>
<td>Starfish</td>
<td>1-Methyladenine</td>
<td><img src="image4" alt="Starfish Structure" /></td>
</tr>
</tbody>
</table>

See also Fig. 5 for the structure of *C. elegans* MIS.
Byskov et al., 1995). This sterol was able to induce maturation in denuded or cumulus-enclosed oocytes, as well as in rat *ex-vivo* perfused ovaries (Grondahl et al., 2000; Hegele-Hartung et al., 2001; Ruan et al., 1998). The origin of FF-MAS has not yet been established, but evidence suggests that cumulus cells or follicle cells are responsible for production of this factor (Byskov et al., 1995, 2002). However, other experimental evidence argues against FF-MAS being the physiological MIS, as specific inhibitors of FF-MAS-synthesizing enzymes fail to suppress gonadotropin-induced meiosis *in vivo* in rat (Tsafirri et al., 2002), so its relevance is still a matter of debate. It would be useful to assess whether such inhibitors are indeed effective before completely ruling FF-MAS out.

For FF-MAS to be a physiological signal for meiosis its levels might increase before oocyte maturation; indeed, such increase is detected. In *vivo*, mammalian oocytes resume meiosis after a rise in gonadotropin levels in blood: follicle-stimulating hormone (FSH) followed by luteinizing hormone (LH). The amounts of FF-MAS in rabbit ovaries rapidly and significantly increase after animals were injected with FSH followed by hCG (human chorionic gonadotropin; LH activity) injection, whereas FSH on its own does not significantly increase follicular FF-MAS levels (Grondahl et al., 2003).

In *amphibians*, experimental evidence points to androgens being the physiological MIS. Progesterone (Table I) had been long suggested to be the MIS causing oocyte maturation, although other steroids such as aldosterone and testosterone are reported to be as efficient as progesterone (Masui and Clarke, 1979). Frog oocytes effectively mature in response to progesterone, and preventing conversion of pregnenolone to progesterone in the ovary by drugs (cyanoketone and eliptin) inhibits gonadotropin-induced oocyte maturation. However, measurements of steroid content of female frogs stimulated by hCG reveal that progesterone concentrations in the serum and ovaries are lower than expected to be effective (Fortune and Tsang, 1981; Lutz et al., 2001). The major constituents of steroids produced by the ovary are the androgens testosterone and androstenedione, that are very potent inducers of oocyte maturation, suggesting that androgens may be the natural MIS. These steroids are metabolites of progesterone, so inhibiting progesterone production (as in earlier experiments) would inhibit synthesis of androgens as well. Progesterone is quickly metabolized to androstenedione by isolated frog oocytes; however, this conversion is not necessary for progesterone-induced maturation, thereby resolving disparate conclusions of the earlier experiments (Lutz et al., 2001).

In *fish*, two progestin derivatives, 17α,20β-dihydro-4-pregnen-3-one (DHP) and 17α,20β,21α-trihydro-4-pregnen-3-one (THP), were identified as major active substances produced by the follicles upon stimulation with pituitary extract in multiple species (Table I, Petrino et al., 1993; Thomas
et al., 2002, and references therein). DHP is quickly and efficiently metabolized to an array of steroid compounds by the oocytes, however, none of the DHP metabolites is as active a trigger of GVBD as DHP itself, indicating that the induction of maturation is not due to metabolic conversion into a more active form. In a number of fish species, synthesis of DHP takes place in the follicle cells next to the oocyte in response to gonadotropins, and steroid accumulation occurs in the follicular layers as well as inside the oocyte such that DHP acts locally and transiently (Nagahama et al., 1994).

In starfish, the adenine derivative, 1-methyladenine (1-MA), is the natural MIS (Table I, Kanatani, 1969; Schuetz, 1971). 1-MA is produced in the follicle cells surrounding the oocyte by the transfer of a methyl group from S-adenosylmethionine onto an ATP molecule, making 1-methylATP, which is then converted into 1-methyladenine (Mita et al., 1999a,b). 1-MA is quickly metabolized in the recipient oocytes and its metabolic product is not biologically active (Toole and Schuetz, 1974). 1-MA is synthesized and secreted into the extracellular space between the follicle and the oocyte in response to a peptide released by the radial nerve, which is analogous in action to vertebrate gonadotropin (reviewed in Smiley, 1990). The gonad-stimulating substance of the starfish Asterias amurensis has been purified and identified as a polypeptide, with a molecular weight of ~2.1 kDa, although its exact amino acid sequence has not been determined (Kanatani, 1985).

1-MA is effective in inducing maturation in all starfish species; however, it seems selective for this particular group of echinoderms. Sea urchins, sand dollars, sea lilies, and sea cucumbers do not respond to this compound (reviewed in Smiley, 1990; and Wessel, personal communication). Furthermore, sea cucumbers produce an unidentified MIS, preliminary characterized as 2,8 disubstituted adenine (Smiley, 1990). Perhaps closely related adenine derivatives fulfill the role of 1-MA for echinoderms other than starfish.

B. Receptors for Maturation-inducing Substance

Work on characterizing the mammalian receptors for FF-MAS has only recently begun. The hydrophobic nature of the sterol would allow it to accumulate in the plasma membrane, or potentially permit its diffusion through the membrane, such that it could function intracellularly. However, intracellular injection of FF-MAS is ineffective in the induction of maturation (Grondahl et al., 2003). Furthermore, a potent antagonist of FF-MAS, 22-R-cholesterol, can efficiently inhibit meiotic resumption in response to extracellular FF-MAS only when it is applied from the outside,
and not injected into the oocyte, suggesting that it competes for binding to a surface receptor.

In amphibian oocytes, the controversy over the nature of the MIS receptor is still not resolved, and new developments in this area are expected considering that androgens and not progesterone are the actual MIS. Experiments attempting to distinguish whether progesterone acts on the outside or on the inside of the cells are still inconclusive, in large part due to the fact that progesterone is a hydrophobic molecule, which is able to cross biological membranes by diffusion. What appears clear, however, is that progesterone-induced as well as androgen-induced maturation does not depend on new transcription, as it is unaffected by the transcriptional inhibitor, actinomycin D (Lutz et al., 2003; Smith and Ecker, 1969). Although extracellular application of progesterone is efficient in inducing oocyte maturation, the injection of this sterol does not always lead to resumption of meiosis. Such injections were found effective by some groups (Bayaa et al., 2000; Tso et al., 1982), but not others (Godeau et al., 1978; Masui and Clarke, 1979; Smith and Ecker, 1971). Conceptually, it is possible even for the injected steroid to diffuse out of the cell, and still act on its surface. Attempts to prevent transmembrane diffusion of progesterone included covalent crosslinking to a 20 kDa polymer (polyethylene oxide), bovine serum albumin, or to polystyrene beads. These modified progesterone compounds were still active when applied to the surface of oocyte under conditions minimizing endocytic uptake (Godeau et al., 1978; Masui and Clarke, 1979), but of inconsistent activity when microinjected into the cell (Bayaa et al., 2000; Masui and Clarke, 1979). An additional confounding factor is that the preparations of crosslinked progesterone could still contain sufficient free sterol to account for the observed biological activity, which is difficult to control (Bayaa et al., 2000).

In support of the plasma membrane being the site of progesterone action, progesterone appears to bind specifically and with high affinity to frog oocyte membranes (Lutz et al., 2000). These specific high affinity binding sites can be detected on Stage V–VI (maturation-competent) oocyte membranes, but not on Stage I–III (maturation incompetent) oocyte membranes (Lutz et al., 2000). What is the nature of this membrane sterol receptor? Experimental results of Lutz et al. argue against progesterone serving as a ligand for a member of the heterotrimeric G-protein coupled receptors (GPCR). The affinity of a GPCR agonist for its receptor usually decreases upon activation of the associated Go subunits. When Xenopus oocyte membranes are incubated with the nonhydrolyzable GTP analog GTPγS locking all Go subunits in the activated state, progesterone binding stays at the same level as in untreated membranes, suggesting that progesterone does not bind to its receptor in a manner
typical for a GPCR agonist (Lutz et al., 2000). Thus, we are left with the following alternatives: the membrane progesterone receptor (mPR) is not a GPCR or, the mPR is a GPCR, but progesterone does not bind to it as a typical agonist (and may instead function as an antagonist, see Section VA).

Nuclear progesterone receptor (hormone-dependent transcription factor, nPR) may be essential for the oocyte maturation in response to progesterone (reviewed in Maller, 2001). Overexpression of nPR increased sensitivity to progesterone and accelerated the rate of progesterone-induced meiosis (Bayaa et al., 2000; Tian et al., 2000), while injection of nPR antisense oligonucleotides into the oocytes inhibited the progesterone-induced oocyte maturation after 8 days of treatment (Tian et al., 2000). The observed effects were specific for antisense but not control oligonucleotides, and could be rescued by subsequent injection of the oocyte with mRNA coding for Xenopus or human nPR. However, additional experiments are required to firmly establish the role of nPR in maturation. The antisense treatment described (Tian et al., 2000) involves culturing oocytes in vitro for 8 days without nutritional supply, which may very well change the cell’s physiology. Furthermore, even the rescue experiments could be questioned as the overexpressed nPR could initiate maturation by a nonphysiological pathway (via direct activation of a downstream kinase Rsk; Maller, 2001). Of particular concern is the fact that sterol binding specificity characteristic of conventional nuclear progesterone receptors does not match the GVBD-inducing potency profile of these sterols (reviewed in Maller, 2001). Therefore, it is important to test whether the cloned Xenopus nPR exhibits an unusual sterol-binding profile.

Following the suggestion that androgens may be the in vivo signal inducing meiotic maturation in frog, the role of nuclear androgen receptor (nAR) in Xenopus oocytes maturation was tested (Lutz et al., 2003). Treatment of the oocytes with dsRNA for 2.5 days was able to significantly inhibit oocyte maturation in response to androgen without altering the responsiveness to progesterone. nAR protein was found expressed throughout the Xenopus oocyte, and a fraction of the protein was associated with the plasma membrane. A number of sterol ligands demonstrated the ability to induce nAR-dependent transcription in a heterologous system. Unexpectedly, when assayed for the ability to induce oocyte maturation, two of these sterols (dihydrotestosterone and R1881) appeared to be potent antagonists of testosterone-induced, but not progesterone-induced oocyte maturation. This may cast a doubt on the nAR function as a MIS receptor; however, at the same time it afforded an opportunity to test the importance of androgen-mediated signaling in an ovarian explant. Both R1881 and dihydrotestosterone were able to inhibit maturation of oocytes in hCG-treated Xenopus ovaries, despite efficient production of testosterone by the
ovaries under these conditions, arguing that the testosterone-induced signaling is indeed the predominant \textit{in vivo} pathway for induction of oocyte maturation in frog (Lutz \textit{et al.}, 2003).

Since progesterone- and androgen-dependent maturation does not require new transcription, the mechanism of transcription-independent nuclear receptor effect may be through activation of Src family tyrosine kinases. Recently, a proline-rich motif unique to nPR was uncovered in the N-terminal domain of the protein, which appeared to mediate direct hormone-dependent binding of nPR to SH3 domains of various cytoplasmic proteins. This motif appeared necessary and sufficient for Src activation by a SH3 domain-displacement mechanism, and presence of this domain in protein overexpressed in \textit{Xenopus} oocytes was required for the enhancement of the response to progesterone (Boonyaratanakornkit \textit{et al.}, 2001). However, overexpression of a fusion of nPR N-terminal DNA binding domain with estrogen-binding domain in \textit{Xenopus} oocytes did not make these oocytes responsive to estrogen (Bayaa \textit{et al.}, 2000). Nevertheless, this result could be explained by a requirement for the presence of the nPR ligand-binding domain for the N-terminal part to bind to and activate Src efficiently (Boonyaratanakornkit \textit{et al.}, 2001).

In fish the plasma membrane of the oocyte possesses specific high affinity binding sites for MIS. Maturation-inducing substance (DHP, Table I) acts at the cell surface and is ineffective in inducing maturation when injected into oocytes. Investigation of the binding affinity of plasma membrane and nuclear MIS binding sites for a range of sterols demonstrates that they are characterized by markedly different binding affinities for a number of steroid compounds. Thus, it is unlikely that a nuclear hormone receptor participates in the MIS signaling at the plasma membrane. Furthermore, the affinity of plasma membrane sites for MIS decreases with incubation of the membranes with GTP\(_\gamma\)S, consistent with the hypothesis of a GPCR being an MIS receptor, since activation of the associated G\(\alpha\) subunits leads to a decrease in the affinity of a typical GPCR for its ligand (reviewed in Thomas \textit{et al.}, 2002).

Recently, the fish MIS candidate receptor was cloned by an innovative approach. Monoclonal antibodies were produced against a partially purified, solubilized oocyte plasma membrane fraction. They were then screened for the ability to bind to the solubilized membrane receptor using a hormone receptor capture protocol, where the efficiency of immunoprecipitation of a receptor selectively binding the radiolabeled ligand is assessed. This assay was optimized in a way to maintain specific ligand binding in the solubilized preparations of the membranes, which is not always possible for GPCRs. Three monoclonal antibodies were selected, and expression screening of the cDNA library identified a protein with no similarity to known receptors (Thomas \textit{et al.}, 2002; Zhu \textit{et al.}, 2003b). Computer analysis
of the amino acid sequence of this protein predicts seven transmembrane
domains, consistent with a GPCR structure, but suggestive of representing
a separate subclass of these receptors. In line with this, a number of proteins
homologous to the suggested fish membrane progestin receptor (mPR)
have been isolated from several vertebrate species (Zhu et al., 2003a).
Immunolocalizations, as well as subcellular fractionations, consistently
detect mPR localized on the plasma membrane of the oocyte. Heterologously expressed mPR confers sensitivity to DHP in transfected
mammalian tissue culture cells. In these cells, DHP application reduces
cAMP production and activates MAP kinase; these effects are consistent
with proposed signaling through this receptor in the oocyte at resumption of
meiosis. The protein profile shows that mPR is low in previtellogenic
oocytes, increases with oocyte growth, and disappears upon oocyte
maturation, consistent with what would be expected for a receptor, whose
function is specific for oocyte maturation. Depletion of the mPR in
the zebrafish oocytes by morpholino antisense oligonucleotides blocks
maturation in vitro in response to DHP, consistent with this protein being
the MIS receptor (Zhu et al., 2003b). However, further experiments would
be helpful to definitely establish the proposed role for mPR in oocyte
maturation. For example, bacterially overexpressed protein displays an
unexpected sterol-binding profile, which does not match the steroid
specificity of maturation stimulation. Furthermore, a rescue of the antisense
treatment experiments in zebrafish would be useful, accompanied by
immunological detection methods of the mPR protein.

Thus, it is suggested that fish oocytes utilize a GPCR-mediated induction
of oocyte maturation, in general contradiction to signaling described for
frog. Are these differences to be reconciled in the future? It is possible that
nuclear and plasma membrane sterol receptors participate at different stages
of oocyte maturation in the MIS signaling. The homologues of the fish mPR
have been recently isolated in a number of vertebrates including frogs (Zhu
et al., 2003a). Future experiments will test the involvement of this GPCR
family in the signaling at oocyte maturation in different species, and define
the contribution of nPRs, if any.

In starfish, a variety of evidence indicates that 1-MA acts at the oocyte
surface. For example, intracellularly injected 1-MA does not cause
reinitiation of meiosis (reviewed in Kanatani, 1985). Furthermore, 1-MA
specifically binds to the oocyte surface: binding of a radiolabeled 1-MA is
inhibited in a dose-dependent manner by unlabeled 1-MA and biologically
active analogs (1-benzyladenine and 1-ethyladenine), but not by biologically
inactive analogs (1,9-dimethyladenine or 1-methylhypoxanthine) (Mita
et al., 2001; Yoshikuni et al., 1988).

It is hypothesized that a seven-transmembrane GPCR in the plasma
membrane binds 1-MA (Kishimoto, 1999). G-protein coupled receptors
(GPCRs) are known to have different affinities for receptor agonists depending on their association with G-proteins. The binding affinity of the starfish 1-MA receptor changes consistent with it being a GPCR: the affinity of starfish oocyte cortices for 1-MA decreased after in vitro treatment with GTPγS that dissociates G-proteins from their receptors (Chiba and Hoshi, 1995). Induction of oocyte maturation by treatment with reducing agents (reviewed in Kanatani, 1985) most likely does not act on the receptor, since pertussis toxin blocks the induction of maturation by 1-MA (see Section IVA), but not by dithiothreitol (Chiba et al., 1992; Chiba and Hoshi, 1995). The exact nature of the 1-MA receptor has not been determined yet, despite many efforts to biochemically characterize or clone it (e.g., Kalinowski et al., 2003).

C. Follicle Cells and Gap Junctions

Oocytes of many species develop in close contact with the somatic cells of the ovary. For example, in mammals, a multilayered complex of somatic cells surrounds the oocyte in a specialized structure called a follicle (Fig. 1). The follicle cells adjacent to the oocyte form numerous specialized contacts (gap junctions) with the oocyte (reviewed in Matova and Cooley, 2001). These structures are important for supplying the oocyte with nutrients, and for communication between somatic cells and the oocyte.

Gap junctional communication plays an important regulatory role for oocyte maturation. In mammals, hormones targeting oocyte development exert their action on somatic cells of the follicle, which then relay the signal to the oocyte. The pituitary gland releases the gonadotropins FSH and LH. FSH causes the follicle cells to proliferate and differentiate, and LH initiates the oocyte’s progression through meiosis and ovulation. The follicle cells mediate this initiation of oocyte maturation in response to hormones.

The oocyte is usually incapable of maturation during its growth period (Masui and Clarke, 1979) but once meiotic competence is achieved in a full grown oocyte, the granulosa cells become the major suppressor of meiotic progression. Mammalian and fish defolliculated oocytes undergo spontaneous maturation in culture, while they do not when they are cultured enclosed in follicles (reviewed in Conti et al., 2002; Patino and Purkiss, 1993). As follicle cells significantly influence the state of the oocyte it was hypothesized that the oocyte is maintained in the immature state by a follicular “arrester” produced by granulosa cells. How do the follicle cells communicate with the oocyte to keep the cell cycle blocked?
Numerous gap junctions exist between the cells of the ovarian follicle, providing connections among somatic cells, and between follicle cells and the oocyte. These intercellular membrane channels can be detected by transfer of metabolites or small fluorescent dyes. Gap junctions are essential for oocyte growth and development and were proposed to provide the way for a small molecule “arrester” to diffuse into the oocyte. The abundance of gap junctions is regulated by the amounts of their constituent proteins, connexins, in the cell. “Coupling efficiency” of existing gap junctions is further regulated by their subunit composition and posttranslational modifications of connexins by intracellular signaling mediators (reviewed in Kidder and Mhawi, 2002). Regulation of follicle gap junctions within the follicle is hypothesized to be involved in the induction of oocyte maturation. In certain animals, like the Syrian hamster, it was determined that downregulation (decrease in number and density) of follicle cells’ gap junctions is correlated with an irreversible commitment of the oocytes to maturation (Racowsky et al., 1989). On the other hand, in the mouse, the oocyte–cumulus cells coupling index remained constant throughout the GVBD period in hCG-stimulated animals (Eppig and Downs, 1988) and in the explanted cumulus cell–oocyte complexes induced to mature with gonadotropin (Downs, 2001). The molecular mechanism for downregulation of gap junction coupling efficiency is suggested to act at several levels including phosphorylation of the protein and decrease in net levels of protein and mRNA. In rats, the gap junction protein connexin-43 is rapidly phosphorylated in response to exposure to LH (Granot and Dekel, 1994). Furthermore, prolonged exposure to LH causes complete elimination of connexin-43 protein correlated with a decrease in its mRNA level. The experimental manipulation of gap junctions can also influence the meiotic state of the oocyte. For example, application of chemicals such as 1-octanol that suppresses gap junction-mediated cell–cell coupling appears to initiate oocyte maturation in follicles of the fish Fundulus heteroclitus as well as in mouse cumulus-enclosed oocytes (Cerda et al., 1993; Downs, 1995). However, Xenopus follicle-enclosed oocytes cannot be stimulated to mature in this way (Patino and Purkiss, 1993), suggesting that the input of a gap junction-delivered arrester in mediating prophase arrest of the oocyte varies in different animals.

Gap junctions appear to transmit both inhibitory and stimulatory signals between follicle cells and the oocyte. If prophase I arrested, follicle-enclosed fish or mouse oocytes are treated with stimulatory ligands, GVBD is triggered at greater frequencies than those achieved by just removing the follicle cells (Cerda et al., 1993; Downs, 1995, and references therein). Furthermore, induction of meiotic maturation in cumulus-enclosed oocytes depends significantly on the presence of functional gap junctions between the oocyte and cumulus cells, as assessed by either inhibiting gap junctions
in intact follicles chemically, or by dissociating cumulus cells from the oocyte, and subsequently coculturing them together (Downs, 1995, 2001). Experiments in *Xenopus* oocytes produce comparable results: while oocyte maturation of the follicle-enclosed oocytes can be induced by gonadotropin treatment, gap junction-inhibiting reagents suppress this induction (Patino and Purkiss, 1993). Thus, even though in *Xenopus* no inhibitory signal is transmitted through gap junctions, there appears to be an activating input instead. This effect appears to result specifically from disruption of gap junctional communication, and not from detrimental effects of the 1-octanol on the follicle cells or the oocyte itself. This conclusion is based on the result that treatment with 1-octanol does not decrease the amounts of steroids produced by the follicle cells, and does not inhibit maturation of follicle-free oocytes in response to external progesterone. It is hypothesized that gap junctional coupling of follicle cells and oocyte in *Xenopus* either facilitates delivery of the hydrophobic sterol to the oocyte surface, or provides a cytoplasmic route of progesterone delivery to the oocyte.

D. A Whole New Can of Worms

A strategy of oocyte maturation regulation unique to the worm *C. elegans* has been recently described. Maturation of the oocytes in this animal is dependent on the presence of sperm in the spermatheca (but not fertilization). Major sperm protein, MSP (Fig. 5), secreted by sperm was found to be the active component inducing oocyte maturation (Miller et al., 2001). This mechanism is quite unusual in that no other known meiosis-inducing hormone is a peptide (Table I). As the MSP gene family in *C. elegans* consists of 40 genes whose protein products differ by one to four amino acids, knockout experiments do not appear feasible. However, the evidence for MSP being the maturation-inducing activity is strong, since recombinant MSP is able to induce oocyte maturation in the absence of sperm, while injection of antibodies to MSP into the uterus of the adult nematode leads to a reduction in the oocyte maturation rate (Miller et al., 2001). A screen for the receptors of MSP was carried out by a combination of microarray-based identification of oocyte-enriched genes, and RNAi-mediated ablation of the candidates followed by assay for MSP binding. These efforts identified a Vab-1 Eph (ephrin) receptor protein tyrosine kinase (RPTK) as the only definite candidate (Kuwabara, 2003; Miller et al., 2003). However, the phenotype of *vab-1* mutants is inconsistent with this protein positively regulating oocyte maturation. Instead, Vab-1 is a suppressor of oocyte meiosis, and MSP induces maturation by antagonizing Vab-1 activity. It appears that Vab-1 expressed on the oocyte surface
receives a constitutive signal from the Efn-2 ephrin also expressed in the germ line to keep up the inhibitory activity. This system thus functions as a sperm-sensing checkpoint mechanism that inhibits oocyte meiotic maturation until sperm is available for fertilization. Potentially, this model of “inhibition relief” may be defined by the hermaphrodite reproductive strategy of *C. elegans*, and appears unique to this animal. However, other organisms could utilize hormone-mediated relief of constitutive inhibition of cell cycle progression as a means to induce oocyte maturation as well (see Section VA).

V. Cytoplasmic Control of Maturation

The ultimate target of signal transduction pathways initiated at oocyte maturation is activation of MPF kinase and release of the oocyte from the block in meiotic progression. Among candidate second messengers linking the MIS receptor to MPF activation are cAMP and calcium; these and more upstream components of signaling machinery will be examined in this section.
A. Signal Transduction through G-proteins: Ignition or Parking Brake?

How is the presence of hormones interpreted by an oocyte to achieve release of the cell from the prophase block? The most detailed analysis of an intracellular signaling pathway activated during oocyte maturation comes from the studies of starfish. Heterotrimeric G-proteins are universal signaling molecules present in most of the tissues. As their name suggests, the complex consists of three subunit types: alpha (GTPase), beta, and gamma. The inactive state is a heterotrimeric complex with the $\alpha$ subunit in a GDP-bound form. Upon activation, the GDP of the $\alpha$ subunit is exchanged for GTP, and $\alpha$ dissociates from the $\beta\gamma$ complex. Free GTP-$\alpha$ and free $\beta\gamma$ diffuse in the plane of membrane and regulate the activity of their respective effectors. Upon hydrolysis of GTP by the $\alpha$ subunit, it returns to the inactive GDP-conformation and reassociates with $\beta\gamma$, thus concluding the activity cycle. Different $\alpha$ subunits have distinct sets of intracellular targets and some $\alpha$ proteins have opposing effects on the same targets. For example, $\alpha$s and $\alpha$i both target adenylate cyclase, however $\alpha$s activates this enzyme while $\alpha$i inhibits it.

Mouse oocytes can be maintained in the prophase arrest in culture indefinitely when they are enclosed in their follicles. However, when the follicle cells are removed, mouse oocytes enter maturation spontaneously. It was documented that such cumulus-free mouse oocytes could be maintained in meiotic arrest in a transient and dose-dependent manner when microinjected with GTP$\gamma$S, an activator of G-protein signaling (Downs et al., 1992). This data led to a model whereby spontaneous maturation of the oocyte is induced by removal of inhibitory signal normally present in the ovary and transduced via the heterotrimeric G-proteins (summarized in Fig. 6A). This model was recently supported by work of Mehlmann and colleagues (Mehlmann et al., 2002) suggesting that the inhibitory ovarian signal is mediated by the activity of $\alpha$s. Injection of anti-$\alpha$s inhibitory antibody induced oocyte maturation in follicle-enclosed oocytes, while injection of anti-$\alpha$i inhibitory antibody did not have any effect on spontaneous oocyte maturation. Furthermore, injection of cholera toxin, which hyperactivates $\alpha$s-mediated signaling, into mouse oocytes was able to significantly inhibit both spontaneous and FF-MAS-induced maturation suggesting that activation of $\alpha$s signaling is able to sustain meiotic arrest of the oocyte (Downs et al., 1992; Grondahl et al., 2000). The most likely target activated by $\alpha$s in this cell is adenylate cyclase. It produces cAMP, a second messenger that can sustain meiotic arrest of the oocyte (see Section VC). Consistently, the effect of cholera toxin on spontaneous maturation is more pronounced when the oocyte is cultured in the presence of hypoxanthine, inhibiting cAMP degradation (Downs et al., 1992).
This model of G-protein function in mouse oocytes is supported in *Xenopus* oocytes, which can be stimulated to mature by injection of an antibody inhibiting Gs activity. Such injected oocytes proceed through GVBD with the same time course as progesterone-stimulated ones, and the resulting eggs are fertilizable (Gallo *et al.*, 1995). Conversely, GTPγS and cholera toxin antagonize the action of progesterone on *Xenopus* oocytes, while GDPβS has no effect (Cork *et al.*, 1990; Schorderet-Slatkine *et al.*, 1982). Increasing the amounts of Gαs in *Xenopus* oocytes blocks progesterone-induced oocyte maturation as well. This effect is even more pronounced if the mutant constitutively activated form of the protein is expressed (Romo *et al.*, 2002). Furthermore, depletion of endogenous Gαs by injection of antisense oligonucleotides induced early events of oocyte maturation independent of progesterone addition (Romo *et al.*, 2002). The activation of adenylate cyclase in response to Gs signaling can be mediated by both Gαs and Gβγ complex interaction with the enzyme (Simonds, 1999). In the *Xenopus* oocyte, βγ subunits released upon Gs activation are also thought to play a significant role in the signaling pathway further activating adenylate cyclase. Experiments by multiple research groups demonstrate that sequestration of endogenous βγ enhances, while overexpression of exogenous βγ inhibits progesterone-induced as well as androgen-induced maturation in a number of studies (Lutz *et al.*, 2000, 2003; Sheng *et al.*, 2001; but not Gallo *et al.*, 1995). The observation that the amount of βγ subunits in mouse oocytes decreases with oocyte maturation is consistent with the model that a relative decline in βγ is involved in the commitment of the oocyte to meiotic maturation (Allworth *et al.*, 1990).

**Figure 6** G-protein signaling and oocyte maturation. **A.** Maintaining vertebrate oocyte prophase arrest by G-protein signaling. Depicted is a summary of experimental data in mouse and *Xenopus* oocytes (see text for details and references). **7TM GPCR**—seven-transmembrane G-protein-coupled receptor; **AdC**—adenylate cyclase; **GRK**—G-protein coupled receptor kinase. Blue color: reagents decreasing cAMP levels in oocytes and promoting oocyte maturation, red color: reagents found or expected to increase cAMP levels in oocytes. **B.** Induction of starfish (and possibly fish) oocyte maturation by G-protein signaling (see text for details and references) (see color plate).
The evidence in support of a constitutively active G-protein-coupled receptor upstream of this inhibitory Gs signaling was recently reported (Wang and Liu, 2003). Reagents known to promote classic GPCR desensitization, such as G-protein receptor kinase (GRK) and β-arrestin, were found to induce Xenopus oocyte maturation independent of progesterone, presumably by downregulating the Gs signaling originated from an upstream GPCR (Wang and Liu, 2003).

These results are summarized in a common model of sustaining prophase arrest in a vertebrate oocyte by a combination of signaling by Gas itself and βγ subunits generated through Gs activation (Fig. 6A). The adenylate cyclase enzymes requiring both Gas and βγ subunits for activation have been previously described in mammals (Simonds, 1999).

However, it is still unclear how the effects of Gs activation are reversed in vivo by hormone action on a mouse follicle or a Xenopus oocyte. Hormone-dependent resumption of maturation in both mouse and Xenopus oocytes includes a rapid decrease of cellular cAMP. Could this effect be mediated through activation of Gi family of heterotrimeric G-proteins (which is known to antagonize Gs by inhibiting adenylate cyclase)?

It is possible to experimentally detect Gi activation during mouse oocyte maturation. The amounts of Gai proteins somewhat decrease during mouse oocyte maturation as determined by western blotting (Jones and Schultz, 1990). However, dissociation of the Gai from the βγ subunits is detected by a substantial decrease in the amounts of pertussis toxin ribosylation substrate in the mature egg in comparison to the oocyte, that cannot be accounted for by the decrease in Gai protein levels (Allworth et al., 1990; Jones and Schultz, 1990). The reduction in the PTX substrate amount occurs as well in vitro, and can be detected after 3 h of in vitro culture, coinciding with GVBD when mouse oocytes mature spontaneously (Jones and Schultz, 1990). This suggests that activation of Gi may take place upon removal of the mouse oocyte from the follicle.

Involvement of Gi-mediated signaling in the response of mouse and frog oocytes to maturation-inducing hormones was tested by using its specific inhibitors (Faerge et al., 2001; Goodhardt et al., 1984; Grondahl et al., 2000; Lutz et al., 2000). Pertussis toxin inhibits certain members of the Gai family of heterotrimeric G-proteins by ADP-ribosylation and blocks their respective signaling. Gi inhibition by pertussis toxin does not consistently affect resumption of meiosis in response to MIS in both vertebrate species suggesting that suppression of Gs signaling might not occur through induction of “opposing” Gi signaling. The injected pertussis toxin A-subunit was found effective (Pellaz and Schorderet-Slatkine, 1989), while incubation of oocytes with the whole pertussis toxin is generally not effective (Downs et al., 1992; Faerge et al., 2001; Goodhardt et al., 1984; Kline
et al., 1991; Sadler et al., 1984). It appears that even though the Gi signaling might be activated during the oocyte maturation, it does not contribute directly to eventual resumption of meiosis.

Activation of all G-proteins in the mouse oocyte by GTPγS injection does not significantly interfere with FF-MAS-induced maturation, nor does it have a stimulatory effect (Grondahl et al., 2003). In contrast, when GDPβS is injected, FF-MAS-induced meiosis resumption is significantly decreased. These effects are markedly different from the spontaneous maturation, which is inhibited by GTPγS injection (Downs et al., 1992). It appears, that for FF-MAS to exert its action, inhibition of G-protein signaling is not required; instead, a (pertussis toxin-insensitive) G-protein must be activated.

Overexpression or injection of constitutively activated Ga1 in frog has minimal effects on either spontaneous or progesterone-induced maturation, which indicates that Gi activity is not important for the Xenopus maturation response (Kroll et al., 1991; Lutz et al., 2000). Participation of another type of G-protein was suggested, as injection of constitutively active Go1 is able to stimulate Xenopus GVBD (Kroll et al., 1991). However, the Go1-mediated maturation depends on PKC activation while progesterone-mediated maturation does not. Finally, expression and activation of heterologous Gi-coupled receptors does not cause Xenopus oocyte maturation (Kalinowski et al., 2003). It is conceivable then that the function of G-proteins is to maintain the oocyte’s cell cycle arrest rather than to induce oocyte maturation. The action of progesterone thus might be in relieving the Gs/βγ mediated inhibition. Possible mechanisms include functioning as an antagonist of a constitutively active GPCR; in this light, the fact that progesterone does not bind to the oocyte membranes in the manner typical of GPCR ligands becomes less puzzling (see Section IVB). An alternative mechanism could be hypothesized to include a novel effect of cytoplasmic progesterone receptor on Gαs, βγ, adenylate cyclase, or phosphodiesterase potentially through activation of Src (see Section IVB).

Probing the signal transduction pathway leading to oocyte maturation in fish uncovered that—consistent with other vertebrates—hyperactivation of Gαs by cholera toxin inhibits the oocyte’s response to MIS. The involvement of Gi signaling in MIS-induced maturation is uncertain as microinjection of activated pertussis toxin into oocytes blocks the MIS-induced maturation only in select species (Thomas et al., 2002). Recent experiments identified a candidate fish MIS receptor, which is capable to signal through Gi when expressed in mammalian tissue culture cells (Zhu et al., 2003b). In the context of other oocytes, these contradictory results need to be further evaluated, as they suggest that there may be a diversity in oocyte maturation signal transduction pathways within vertebrates.
The 1-MA signal inducing starfish oocyte maturation proceeds through a G-protein pathway (Fig. 6B). The only G-protein involved in regulation of 1-MA-induced maturation in starfish appears to be G\textsubscript{i}, since the 1-MA-initiated signal is inhibited by pertussis toxin (Shilling et al., 1989). The G\textsubscript{ai} subunit modified by pertussis toxin was biochemically purified from starfish oocytes and subsequently cloned (Chiba and Hoshi, 1995; Tadenuma et al., 1991). Furthermore, expression of mammalian G\textsubscript{i}-family-linked receptors, such as human adenosine A1 receptor and rat ADP receptor, causes the starfish oocyte to resume meiosis in response to corresponding agonists (Kalinowski et al., 2003). G\textsubscript{as} protein is present in starfish oocytes as well, as detected by ADP-ribozylation of the protein by cholera toxin (Chiba and Hoshi, 1995). Nevertheless, injections of the anti-G\textsubscript{as} inhibiting antibody in starfish oocytes neither stimulated GVBD, nor inhibited GVBD in response to 1-MA stimulus (Gallo et al., 1995).

The \(G_i\) subunits of G\textsubscript{i} appear to be the key for the initiation of oocyte maturation. Starfish G\textsubscript{i} activation, especially release of \(G_i\beta\gamma\) subunits, is necessary and sufficient to initiate maturation, while signaling through the activated G\textsubscript{as} subunit appears less significant (Chiba and Hoshi, 1995; Jaffe et al., 1993). The free \(G_i\beta\gamma\) complexes relay the signaling further along the pathway, including phosphatidylinositol 3-kinase (PI3K) as a likely downstream component (Section VD, Sadler and Ruderman, 1998).

**B. Calcium Ions**

Calcium is a universal signaling molecule involved in many important processes such as muscle contraction, synaptic transmission, and fertilization. Recent research suggests that calcium also governs certain transitions of cell division cycle, such as nuclear envelope breakdown and separation of chromosomes at the metaphase–anaphase transition (reviewed in Whitaker and Larman, 2001). It is therefore possible that calcium signaling contributes to the release of the oocyte from the meiotic prophase arrest.

A transient increase in the intracellular calcium concentration has long been known to be required for the induction of oocyte maturation, or GVBD, as it is blocked by injection of calcium chelators into the cell (Masui and Clarke, 1979; Pesty et al., 1998). Hormone addition to mammalian, Xenopus, and starfish oocytes leads to the increase in cytoplasmic calcium concentration, under certain conditions in the form of a pronounced spike (Su and Eppig, 2002; Wasserman et al., 1986; Witchel and Steinhardt, 1990, and references therein). Mouse oocytes dissected from the follicles and spontaneously resuming meiosis *in vitro* display repetitive calcium transients prior to GVBD as well (Carroll et al., 1994). These maturation-associated...
calcium transients are generally smaller in amplitude than fertilization-associated calcium transients (reviewed in Schultz and Kopf, 1995). These results are comparable to calcium signaling in mitosis documented for embryonic cells (in a host of organisms: sea urchin, starfish, *Xenopus*, mouse), which exhibit calcium rises associated with nuclear envelope breakdown (Whitaker and Larman, 2001 and references therein).

Could simply introducing calcium ions into the cell induce oocyte maturation? Most reports conclude that calcium on its own is insufficient for induction of oocyte maturation (see below), although the evidence in *Xenopus* is still controversial (Han and Lee, 1995; Noh and Han, 1998; Wasserman *et al.*, 1986 and references therein). The calcium ionophore A23187 causes oocyte maturation in a very narrow group of species (some of them, like *Spisula*, resume meiosis in response to fertilization, and the calcium transient in this species is normally induced by egg activation). For most species, including starfish, A23187 is ineffective, and it simply kills sea urchin oocytes (reviewed in Kanatani, 1985). This suggests that the calcium release is not sufficient for the initiation of oocyte maturation, although calcium ionophore treatment is likely to cause a super-physiological increase in cytoplasmic calcium concentration, and thus cause nonphysiological effects. Still, cytoplasmic calcium transients are necessary for the execution of certain aspects of oocyte meiosis.

The stages of oocyte maturation that can be regulated by calcium include nuclear envelope breakdown (in starfish, but not in mouse), extrusion of the first polar body, and cortical granule translocation to the cell surface (Santella and Kyoizuka, 1994; Santella *et al.*, 1999; Su and Eppig, 2002; Tombes *et al.*, 1992 and references therein). Starfish oocytes injected with heparin (which interferes with IP3-mediated calcium release) delay the GVBD in response to 1-MA, but more importantly, do not undergo normal transformations of the cytoskeleton and do not redistribute cortical granules at the plasma membrane (Santella *et al.*, 1999).

The mechanism of calcium action at meiosis is not yet fully understood. The strongest possibility, based on comparison with the active mitotic players, is action through the calcium/calmodulin-dependent kinase, CaMKII. Activation of calmodulin and CaMKII has been reported during mitosis in a number of organisms and cultured cell types (reviewed in Whitaker and Larman, 2001). The suggested role of CaMKII at the time of mitosis entry is activation of cdc25 phosphatase, an activator of maturation promoting factor (Patel *et al.*, 1999). In support of meiotic function of this enzyme, CaMKII was found active during the response to progesterone in *Xenopus* oocytes (Stevens *et al.*, 1999). Expression of a constitutively activated mutant CaMKII is sufficient for induction of partial *Xenopus* oocyte maturation (movement of GV, but not GVBD) independent of hormonal signal (Waldmann *et al.*, 1990). However, the role of CaMKII in
mouse meiotic resumption is unclear, since the CaMKII inhibitors, KN-93, myristoylated AIP (autocamptide-2-related inhibitory peptide), and specific calmodulin antagonist W-7, do not prevent mouse oocytes from spontaneously resuming maturation when cultured in vitro (Su and Eppig, 2002). Instead, CaMKII appears to fulfill a later function in mouse meiosis. All the mentioned CaMKII inhibitors inhibit extrusion of the first polar body in a dose-dependent fashion, apparently blocking the metaphase to anaphase transition.

Another potential downstream target of calcium is protein kinase C (PKC). Artificial activation of PKC in *Xenopus* oocytes can cause GVBD in the absence of hormone (Kroll et al., 1991; Stith and Maller, 1987). However, it is probably not a physiological mediator of progesterone signaling, since inhibition of PKC by injection of inhibitory peptide does not interfere with progesterone-induced maturation (Kroll et al., 1991). Furthermore, artificial PKC activation in mouse oocytes actually prevents GVBD (Bornslaeger et al., 1986; Downs et al., 2001 and references therein). Thus, PKC does not normally mediate calcium signaling in the maturing oocyte.

The pathway of calcium increase during meiosis is still under investigation. This ion can be released from the internal stores via two main types of calcium channel receptors: the inositol 1,4,5-triphosphate receptor (IP3R), and the ryanodine receptor (RyR) insensitive to IP3, but stimulated by various agents such as calcium, caffeine, nicotinic acid adenine nucleotide phosphate, or cyclic ADP-ribose (cADPr). The prevailing route for calcium release during mitosis is through generation of IP3 produced by PLCγ (reviewed in Whitaker and Larman, 2001). IP3 is a strong candidate for the meiotic mediator of calcium release since its levels increase in *Xenopus* oocytes upon progesterone stimulation (Noh and Han, 1998 and references therein). It appears that the release of calcium is mostly IP3-mediated, since injection of reagents preventing IP3 generation or binding to the receptor delays (in starfish) or prevents (in *Xenopus*) the onset of normal maturation, while introduction of 8NH2-cADPr (inactive analog of cADPr), ruthenium red, or procaine (specific inhibitors of RyR channel) does not (Han and Lee, 1995; Iwasaki et al., 2002; Noh and Han, 1998; Santella et al., 1999). However, RyR-mediated calcium release is important (at least, in starfish) since a combination of heparin and 8NH2-cADPr is more efficient than either reagent on its own, and prevents GVBD of starfish oocytes completely (Santella et al., 1999). Producing IP3 artificially in *Xenopus* oocyte by activation of heterologously-expressed serotonin receptor does not result in induction of oocyte maturation, which is consistent with the conclusion that calcium release, by itself, is insufficient for the induction of maturation (Noh and Han, 1998). However, the amounts of IP3 produced by this artificial mechanism are less than those of
the control oocytes responding to progesterone, making the conclusions somewhat questionable.

C. cAMP

Cyclic AMP is a well-known second messenger, whose regulatory targets include kinase and GTP exchange factor activities. In somatic cells, cAMP functions both as a positive and a negative regulator of the mitotic cell cycle progression (depending on cell type), exerting its effects mainly in the G1 part of the cell cycle. In contrast, progression of the oocyte through the meiotic prophase block appears to be negatively regulated by the cAMP levels (reviewed in Conti et al., 2002).

Cyclic AMP has been repeatedly proposed to be the mysterious inhibitor of oocyte maturation, being made in follicle cells and transferred into the follicle-enclosed oocytes in mammals (see Section IIIC, reviewed in Webb et al., 2002). In support of this concept, a number of reports point to inhibition of oocyte maturation by high cytoplasmic cAMP levels (see below). cAMP is produced by adenylate cyclases and degraded by phosphodiesterases. In starfish, phosphodiesterase inhibitors such as IBMX (3-isobutyl-1-methylxanthine) or other xanthine derivatives have an inhibitory effect on the maturation of 1-MA-treated oocytes (Doree et al., 1976), potentially by interfering with cAMP degradation, but their effects on cAMP levels have not been tested. Furthermore, in frog, forskolin (activator of adenylate cyclase) either delays Xenopus oocyte maturation or blocks maturation completely in a concentration-dependent manner (Noh and Han, 1998). Forskolin inhibits spontaneous oocyte maturation in mouse and fish as well (Schultz et al., 1983; Thomas et al., 2002). In the sea urchin, artificially elevating levels of cAMP in isolated oocytes also maintains the G2 arrest (Wessel et al., 2002).

Do cAMP levels decrease naturally upon MIS exposure? In hCG-stimulated mice, oocytes exhibit a 30% decrease in cAMP, as detected by radioimmuno assay 1.5 h after gonadotropin administration (Schultz et al., 1983). However, the concentration of cAMP in the whole follicle actually increases up to and throughout GVBD (Eppig and Downs, 1988; Schultz et al., 1983). To explain these dynamics, a regionalized increase in cAMP confined to the follicle cells is proposed resulting either from blocked gap junctional communication between the oocyte and somatic cells, or from selective downregulation of gap junction permeability for cAMP while maintaining permeability for other molecules (Qu and Dahl, 2002). In explants of oocytes enclosed in cumulus cells, stimulation of cumulus cells by FSH leads to a transient increase in cAMP levels of the oocyte as detected by the in situ fluorescent indicator FlCRhR (Webb et al., 2002).
Key here is that the oocyte increase is dependent on the presence of functional gap junctions between cumulus cells and the oocyte. Consistently, oocyte maturation in FSH-treated complexes is less than in control groups early on after FSH treatment, at the time of detected cAMP increase, and is significantly induced later (by 10 h post-FSH), when cAMP levels return to baseline (Downs et al., 1988). These data suggest that the changes in cAMP levels occurring during oocyte maturation in mouse depend on the mechanism of maturation induction.

The evidence in starfish oocytes is less clear. Some reports indicate that maturation-inducing 1-MA causes a 10–30% decrease in cAMP, starting within 2 min after hormone addition (Meijer and Zarutskie, 1987), but other evidence suggests that cAMP amounts do not change significantly during oocyte maturation (reviewed in Kanatani, 1985). Even though starfish oocyte maturation depends on activation of Gi protein, signaling through the Gai (adenylate cyclase-inhibiting) subunit is not required for the induction of maturation, while it is the release of βγ complex that ultimately stimulates meiotic resumption.

The cAMP levels appear to decrease in frog and fish oocytes upon stimulation of meiosis resumption. In Xenopus, the cAMP concentration decreases (10–15%) upon progesterone stimulation within 15 min of treatment (Cork et al., 1990; Schorderet-Slatkine et al., 1982). Induction of oocyte maturation in fish is correlated with a 20–60% decline in the cAMP concentration of the follicle upon treatment with DHP (reviewed in Haider, 2003; Thomas et al., 2002). Even though these are subtle changes in cAMP concentration, they could still be important for the oocytes’ meiotic transitions after being amplified via changes in the activity of the cAMP-dependent kinase, and exhibit a threshold effect.

The role of cAMP in the regulation of maturation appears to be mediated by its effects on cAMP-dependent protein kinase A (PKA). Microinjections of PKA inhibitors, such as the regulatory subunit of PKA (rPKA), the PKA inhibitory protein (PKI), membrane-permeable cAMP antagonist (rp-cAMP), or small molecule inhibitor H-89 are able to induce oocyte maturation (or at least accelerate hormone-induced maturation) in some species, such as Xenopus or mouse (Leonardsen et al., 2000; Noh and Han, 1998), but not others, such as starfish (reviewed in Kanatani, 1985). On the other hand, stimulating the signaling output of PKA by injection of the catalytic subunit of PKA (cPKA) inhibits the response to hormone in both Xenopus and starfish (reviewed in Kanatani, 1985; Masui and Clarke, 1979). Interestingly, the total PKA activity in Xenopus oocytes does not change after exposure to progesterone (Cicirelli et al., 1988). One proposed mechanism of PKA action is by phosphorylation and inhibition of cdc25, a cyclin B-cdk1 activating phosphatase (Duckworth et al., 2002). Unexpectedly, the kinase-inactive cPKA blocks progesterone-induced
maturation in *Xenopus* as effectively as wild-type cPKA (Schmitt and Nebreda, 2002). Apparently, this inhibition is not a result of sequestering known regulatory proteins that inhibit endogenous cPKA activity (such as PKI) and represents either a new signaling mechanism or sequestration of cdc25 by the excessive kinase dead cPKA. Interestingly, while microinjection of cPKA into mouse oocytes inhibited spontaneous oocyte maturation, it failed to suppress FF-MAS-induced maturation, suggesting that a decrease in cAMP levels and PKA activity is more critical for initiation of spontaneous maturation than for the response to FF-MAS (Faerge et al., 2001).

Oocytes may be able to overcome the inhibitory effects of cAMP when they are stimulated with maturation-inducing substance. For example, isolated mouse oocytes cultured in the presence of the phosphodiesterase inhibitor hypoxanthine do not experience significant changes in their cAMP levels, yet they are able to efficiently respond to FF-MAS (Grondahl et al., 2003). Furthermore, cumulus cell-enclosed mouse oocytes cultured in the presence of cell permeable dbcAMP and stimulated by FSH still enter meiosis (Downs, 1995). Similarly, the inhibitory influence of xanthine derivatives on the starfish oocytes’ response to 1-MA can also be overcome by increasing 1-MA concentration (Doree et al., 1976). Furthermore, raising cAMP in starfish or mouse oocytes by applying forskolin delays GVBD in response to 1-MA or FF-MAS respectively but does not block it completely (Hegele-Hartung et al., 1999; Meijer and Zarutskie, 1987). Finally, injection of cholera toxin into starfish oocyte is effective in increasing the levels of cytoplasmic cAMP; still, it does not block 1-MA-induced maturation (reviewed in Kanatani, 1985).

Artificially decreasing the levels of cAMP in the oocyte is useful to test whether this in itself would be sufficient to cause oocyte maturation. Culture of mouse oocytes outside of their follicular environment leads to a decrease in cAMP levels. For spontaneous maturation of mouse oocytes to occur, the drop in cytoplasmic cAMP concentration needs to take place to 50–60% of initial levels (Schultz et al., 1983). Another way to decrease the levels of cAMP is by stimulation of heterologous Gi-coupled serotonin receptor overexpressed in the oocyte. In *Xenopus*, stimulation of 5-HT1aR by serotonin lowers cytoplasmic cAMP concentration, but never induces GVBD (Noh and Han, 1998). However, this experiment is flawed in that the resting values of cAMP in HT1aR-expressing oocytes appear to be twice that of controls, and serotonin treatment only serves to bring cAMP closer to the control levels, but never below those. Finally, expression of cAMP-degrading enzymes such as mouse phosphodiesterase 3 or rat phosphodiesterase 4 in *Xenopus* oocytes induces GVBD to the same extent as progesterone (Andersen et al., 1998; Conti et al., 2002). Thus, it appears that a physiological decrease in cAMP is sufficient by itself to induce a reentry of oocyte into meiosis.
The physiological mechanisms regulating cAMP levels in the oocytes are currently unknown. In different animals, evidence favors cAMP regulation at the level of synthesis (by regulating adenylate cyclase), as well as at the level of degradation (by regulating phosphodiesterase, PDE). In starfish, *Xenopus*, and fish, a decrease in adenylate cyclase activity was proposed to be responsible for the decrease in cAMP (Meijer and Zarutskie, 1987; Schorderet-Slatkine et al., 1982; Thomas et al., 2002). The candidate regulators of adenylate cyclase are G-proteins, and generally Ga subunits activate adenylate cyclase, while Gi subunits inhibit its function. However, this simplistic view has been altered during the last decade with the identification of multiple different types of adenylate cyclases that are responsive to multiple regulators in addition to Ga subunits, e.g., Gβγ subunits, calcium levels, and protein phosphorylation (Simonds, 1999). Thus, characterization of adenylate cyclases present in the oocyte is necessary to gain understanding in regulation of oocyte maturation. Recently, expression of type 3 adenylate cyclase (AC3) was detected in the oocytes of rat and mouse (Horner et al., 2003). Furthermore, the AC3 knockout mice are characterized by a significant percentage of ovarian oocytes escaping meiotic arrest. AC3 is activated by Ga protein and is inhibited by an increase in intracellular calcium through phosphorylation by CaMKII (Simonds, 1999). Indeed, AC3 activity is detected in rodent oocytes, raising intracellular calcium can inhibit it, and this inhibition can be rescued by in turn inhibiting CaMKII (Horner et al., 2003). These observations suggest a possible mechanism for the contribution of calcium signaling to the regulation of meiotic resumption through its effects on cAMP levels.

The degradation of cAMP is also important for the induction of oocyte maturation. Type 3 PDE appears to be active in mouse oocytes, but its regulation has not been extensively explored (Conti et al., 2002; Webb et al., 2002). This activity is required both in vitro and in vivo, as mice injected with PDE3 inhibitors ovulate GV-arrested oocytes (reviewed in Conti et al., 2002). Inhibition of oocyte maturation by phosphodiesterase inhibitors does not distinguish between the impact of constitutive and regulated PDE activity, making important the analysis of PDE3 activity profile during maturation. A significant increase in the PDE3 activity is detected in rat oocytes preceding both spontaneous and LH-induced meiosis (Richard et al., 2001). PDE activity increases as well in fish oocytes upon stimulation of oocyte maturation with DHP (Haider, 2003). However, in *Xenopus*, the basal oocyte level of PDE activity does not change after progesterone treatment (Sadler and Maller, 1987). It appears that animals can utilize a decrease in cAMP production, or increase in cAMP degradation, or a combination thereof to stimulate oocytes’ meiotic resumption.
Thus, cAMP is clearly not the only intracellular messenger involved in the initiation of meiotic divisions, but lowering its concentration is important for oocyte maturation to occur. The hormonal action on the oocytes might have the effect of altering the oocytes’ sensitivity to cAMP levels in addition to decreasing these levels. The data reviewed here are consistent with the hypothesis that meiotic reinitiation in response to hormonal treatment is triggered by a stimulatory signal that can be effective despite sustained inhibitory influence of cAMP, while decreasing cAMP levels is important for spontaneous oocyte maturation. Maturation may be influenced by a threshold effect of cytoplasmic cAMP concentration, such that supraphysiological levels of cAMP are inhibitory for the meiotic progression. If, however, cAMP is only slightly elevated, the hormone-induced signaling is able to overcome the inhibition and promote maturation. In this case, normally physiological conditions reflect a balance of cAMP and hormonal activation. Such a threshold effect would impart on the oocyte an all-or-nothing property for meiotic progression, analogous to MPF activation.

D. PI3K

Phosphatidylinositol 3-kinase (PI3K) is another proposed mediator of hormone-induced maturation. This kinase converts the plasma membrane lipid phosphatidylinositol-4,5-bisphosphate \( \{\text{PI(4,5)P}_2\} \) to phosphatidylinositol-3,4,5-trisphosphate \( \{\text{PI(3,4,5)P}_3\} \) (reviewed in Cantley, 2002). Signaling proteins with plekstrin-homology domains accumulate at the sites of PI3K activation by directly binding to its product PI(3,4,5)P\(_3\). Particularly important binding partners are two serine–threonine kinases, Akt/PKB and PDK1 (phosphoinositide-dependent kinase 1). Association with PI(3,4,5)P\(_3\) at the membrane brings these two kinases in proximity, and results in phosphorylation and activation of Akt/PKB by PDK1. Akt/PKB activity then leads to multiple cellular changes, including cell polarization, cell cycle entry, cell survival, and oocyte maturation (Cantley, 2002).

In *Xenopus*, activated forms of PI3K can induce maturation, and PI3K activity increases about 2-fold in progesterone-treated oocytes (reviewed in Ferrell, 1999). Moreover, microinjection of a dominant-negative fragment of PI3K inhibits progesterone-induced maturation. Potentially PI3K could contribute to oocyte activation through the downstream Akt/PKB kinase, which stimulates PDE3. Consistent with this notion, expression of constitutively active Akt/PKB kinase is sufficient to induce *Xenopus* oocyte maturation through PDE3 activation (Andersen et al., 1998). However, an inhibitor of PI3K, wortmannin, does not inhibit progesterone-induced maturation in frog, so the role of PI3K in this species is not altogether clear (Ferrell, 1999). On the other hand, PI3K contribution appears more definite
in starfish oocyte maturation. It is thought to be activated by the heterotrimeric G-protein \( \beta\gamma \) subunits, and PI3K inhibitors, wortmannin and LY294002, inhibit 1-MA-induced maturation (Sadler and Ruderman, 1998). The downstream kinase, Akt/PKB is activated in 1-MA stimulated oocytes, and this activity is necessary for 1-MA-induced maturation (Okumura et al., 2002). Accordingly, constitutively active Akt/PKB protein is able to induce 1-MA-independent oocyte maturation. The proposed target of Akt/PKB is Myt1, the kinase that phosphorylates and inhibits cyclin B-associated cdk1. Myt1 activity decreases upon phosphorylation by Akt/PKB, thereby shifting the equilibrium towards production of active cyclin B-cdk1 complexes and resumption of meiosis (Fig. 7).

E. MAPK

Activation of the MAPK (mitogen-activated protein kinase) cascade is associated with resumption of oocyte meiosis in many different species. Mos codes for a serine/threonine kinase that functions upstream of MAP kinase, and its normal expression is confined to germ cells. Oocytes accumulate mos mRNA, which is translated into protein during meiotic maturation and causes activation of downstream MAPK. For a number of years, it was inferred that the signal inducing meiotic resumption is transduced through

![Figure 7](image-url)  
*Figure 7*  Signaling pathways regulating the state of MPF activation in the maturing oocyte (details in text). In red: protein kinase A activity is inhibitory to MPF activation. In green: PI3K activity resulting in Akt/PKB activation leads to activation of MPF (see color plate).
MAPK and requires mos protein accumulation, based on the ability of mos and its downstream targets to induce hormone-independent maturation (reviewed in Dupre et al., 2002). Furthermore, initial experiments in Xenopus suggested that mos synthesis is required for GVBD. However, later data obtained in various animals and with many techniques such as specific MAPK inhibitors (starfish, Sadler and Ruderman, 1998), transgenic mos RNAi (mouse, Stein et al., 2003), mos gene knockouts, mos DNA antisense (mouse, O’Keefe et al., 1989), and morpholino antisense treatments (Xenopus, Dupre et al., 2002) suggest that MAPK activation is not required for the resumption of meiosis (or GVBD) in any animal. Instead, MAPK activity plays a role in acceleration of MPF activation in the oocyte at meiotic resumption (Dupre et al., 2002), and is needed for suppression of interphase (DNA replication) between two divisions of meiosis and subsequent arrest at the second metaphase of meiosis (Dupre et al., 2002; O’Keefe et al., 1989; Stein et al., 2003).

VI. Concluding Remarks

In this review of the signaling networks bringing about oocyte maturation of different species, we encountered significant variability among animals at almost every level. The maturation-inducing hormones range from a nucleotide (starfish) and complex sterols (vertebrates) to a protein (nematode), or even to the uncertainty of whether such physiological inducers even exist (mouse). The receptors of these hormones are mostly unknown, but the evidence for them ranges from a receptor tyrosine kinase (nematode), to a seven-transmembrane G-protein-coupled receptor (fish and starfish), to a cytoplasmic transcription factor hormone receptor, or an unidentified membrane receptor (Xenopus). Consequently, the identity of the signaling mediators varies, and the heterotrimeric G-proteins, which are strongly implicated in the induction of oocyte maturation in certain species (starfish), appear inhibitory to maturation in other species (mouse, Xenopus), or not involved in regulation of oocyte maturation in yet other species (nematode). Significant discrepancies are encountered even in the involvement of the particular second messengers such as levels of cAMP, calcium, or phosphatidylinositol-3,4,5-trisphosphate (product of PI3K activity) in the regulation of meiotic resumption. The common thread to all these regulatory mechanisms appears to be the final downstream requirement to activate the cyclin B-cdk1 complex (MPF).

To identify more common principles of oocyte maturation regulation, it would be useful to compare and contrast the regulatory mechanisms present in evolutionary close organisms, which nevertheless exhibit noticeable differences in the progression of oocyte maturation. An example of a pair
suitable for comparative studies is starfish and sea urchin. Since they are both members of the echinoderm phyla, we would expect to find similarities in their oocytes.

However, several aspects are divergent. For example, the starfish stores its gametes as oocytes, and resumption of oocyte meiosis coincides (and is caused by the same hormone, 1-MA) with ovulation and release of gametes by the animal. In contrast, the sea urchin stores its gametes as mature eggs, which have already resumed and completed meiotic division within the gonad. Accordingly, fertilization occurs at different meiotic stages in these animals. Furthermore, meiotic divisions are relatively quick in starfish (90 min), while in sea urchin meiosis takes considerably longer (9 h).

Sea urchin eggs have been an extremely popular model for studies of fertilization and early development. Nevertheless, the regulation of sea urchin oocyte maturation has not been thoroughly scrutinized despite providing an important advantage of “divorcing” oocyte maturation and egg activation. We find it very strange that something as fundamental as meiotic maturation and fertilization appears to have diverged in mechanisms so greatly between animals, when other key developmental events are far better conserved. Perhaps this divergence in germ cells reflects the positive selection pressure on the genes involved in reproduction relative to the rest of the genome (Swanson and Vacquier, 2002). Finding other highly divergent genes in the future may be suggestive of their involvement in this process. In addition, with recent advances in genomics, development of databases comparing gene expression patterns in the oocytes of various animals might help in identifying new important molecular regulators of oogenesis on the basis of evolutionarily conserved pattern of expression (Schlecht and Primig, 2003).

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References


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