REPRODUCTION
REVIEW

Focus on Meiosis

Stops and starts in mammalian oocytes: recent advances in understanding the regulation of meiotic arrest and oocyte maturation

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Abstract

Mammalian oocytes grow and undergo meiosis within ovarian follicles. Oocytes are arrested at the first meiotic prophase, held in meiotic arrest by the surrounding follicle cells until a surge of LH from the pituitary stimulates the immature oocyte to resume meiosis. Meiotic arrest depends on a high level of cAMP within the oocyte. This cAMP is generated by the oocyte, through the stimulation of the G_s G-protein by the G-protein-coupled receptor, GPR3. Stimulation of meiotic maturation by LH occurs via its action on the surrounding somatic cells rather than on the oocyte itself. LH induces the expression of epidermal growth factor-like proteins in the mural granulosa cells that act on the cumulus cells to trigger oocyte maturation. The signaling pathway between the cumulus cells and the oocyte, however, remains unknown. This review focuses on recent studies highlighting the importance of the oocyte in producing cAMP to maintain arrest, and discusses possible targets at the level of the oocyte on which LH could act to stimulate meiotic resumption.
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Introduction

Meiosis is the process by which diploid germ cells – oogonia or spermatogonia – reduce their number of chromosomes in half in preparation for combining with a haploid cell of the opposite sex to create a genetically new, diploid individual. In female mammals, meiosis occurs over a prolonged period of time; oogonia enter meiosis but become arrested at the diplotene stage of the first prophase (Eppig *et al.* 2004). Meiosis resumes in response to a surge of luteinizing hormone (LH) from the pituitary gland during the estrous or menstrual cycle, shortly before ovulation. The process by which the oocyte completes the first meiotic division and undergoes other cytoplasmic changes, and progresses to metaphase II is called oocyte maturation. Because the mature, fertilizable oocyte has a relatively short lifespan in the female reproductive tract, the timing of oocyte meiotic arrest, as well as maturation, must be tightly regulated. This review will highlight recent studies examining how the oocyte maintains arrest, and will discuss potential mechanisms whereby LH acts to stimulate meiotic resumption.

The functional unit within the ovary is the follicle, which is comprised of one or more layers of granulosa cells surrounding the oocyte (Fig. 1) (Gougeon 1996, Zeleznik 2004). Ovarian follicles form during embryonic development (Gougeon 1996, Eppig *et al.* 2004). During follicular growth, the somatic cells divide to form several layers, the oocyte enlarges, and a fluid-filled antrum begins to form. Some follicles at the early antral stage are 'recruited' to continue growing; this growth is dependent on the pituitary gonadotropin, follicle-stimulating hormone (FSH) (Gougeon 1996, Zeleznik 2004). During this phase, the antrum divides the granulosa cells into two separate compartments: mural granulosa cells form the outer layers, while the cumulus cells surround the oocyte. The oocyte grows to its full size (~75 μm diameter in the mouse, ~100 μm in the human), but remains arrested in prophase I. If an oocyte is removed from an antral follicle, it spontaneously resumes meiosis and progresses to second metaphase (Pincus & Enzmann 1935). This indicates that the follicle cells hold the oocyte in prophase arrest. Recent progress in clarifying the nature of this arrest is discussed below.

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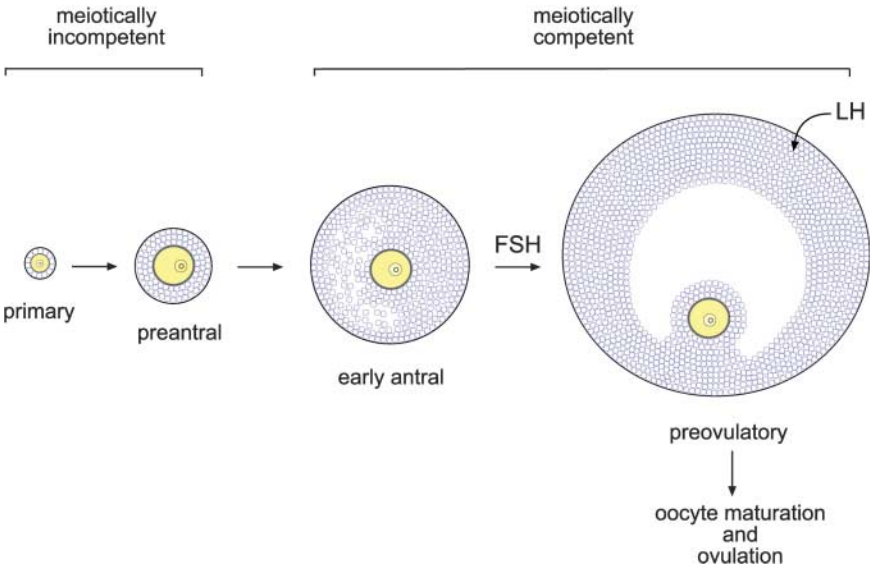

Figure 1 Development of the mammalian ovarian follicle. Follicles, consisting of somatic cells (blue) surrounding an oocyte (yellow), grow within the ovary. Oocytes within the smallest (primary and preantral) follicles are meiotically incompetent and will not mature spontaneously if removed from their follicles. At the early antral stage, the oocytes acquire meiotic competence and are able to mature if isolated from their follicles. At this stage, follicles are recruited for further growth by FSH. The follicle enlarges and develops LH receptors on the outermost, mural granulosa cells. A surge of LH stimulates the oocyte to resume meiosis, as well as ovulation of the mature oocyte. For more details, see Gougeon (1996) and Zeleznik (2004).

Meiosis resumes in response to a preovulatory surge of LH. LH receptors are located on the mural granulosa cells but not on the cumulus cells or the oocyte (Peng *et al.* 1991, Eppig *et al.* 1997), so the mechanism(s) by which LH stimulates oocyte maturation is indirect. Although much is known about LH signaling in the mural granulosa cells (Richards *et al.* 2002), how the LH signal is passed on to the oocyte is incompletely understood. The mechanism by which LH triggers oocyte maturation is currently being studied, and hypotheses of how LH causes the oocyte to resume meiosis are discussed below. Ultimately, LH action on the mural granulosa cells translates to a change in signaling molecules within the oocyte to initiate meiotic resumption.

Because the entire follicle surrounding the oocyte must remain intact in order to preserve its normal function, the oocyte has been largely inaccessible to biochemical studies of its function in a physiological environment. The mechanisms that maintain meiotic arrest of the oocyte, as well as the mechanisms by which LH triggers resumption of meiosis, have therefore been technically difficult to study. Many researchers have utilized oocytes or cumulus–oocyte complexes that have been removed from their follicles and maintained in meiotic arrest artificially. While such experiments have been useful for identifying some of the major components involved in the maintenance of meiotic arrest, they are difficult to interpret in the context of what happens *in vivo*, and in terms of elucidating the earliest steps in the process of oocyte maturation. Recently, new methods for microinjecting the mammalian oocyte within its follicle have provided a means to directly test hypotheses pertaining to meiotic arrest and resumption (Mehlmann *et al.* 2002, 2004, Kalinowski *et al.* 2004). This microinjection method has been used to clarify the important role of the G_s G-protein, as well as the necessity for the G_s-linked receptor, GPR3, in the maintenance of arrest in the mouse oocyte (see below).

Maintenance of meiotic arrest

Prior to the midcycle surge of LH, the growing oocyte acquires the ability to undergo oocyte maturation. The acquisition of meiotic competence occurs around the time of antrum formation (Erickson & Sorensen 1974, Sorensen & Wassarman 1976, Mehlmann *et al.* 2004) and corresponds to a point at which the oocyte achieves a threshold level of maturation-promoting proteins, such as CDK1 (cyclin-dependant kinase) and cyclin (de Vantéry *et al.* 1996, 1997, Kanatsu-Shinohara *et al.* 2000). Despite the ability of the fully grown oocyte to mature, it remains arrested in prophase I until the LH surge. It is well established that meiotic arrest is regulated by cAMP levels within the oocyte (Conti *et al.* 2002, Eppig *et al.* 2004). Spontaneous maturation of oocytes isolated from their follicles can be prevented by including membrane permeant cAMP analogs or cAMP phosphodiesterase inhibitors, such as hypoxanthine or 3-isobutyl-1-methylxanthine (IBMX), in the culture medium (Cho *et al.* 1974, Dekel & Beers 1978, Conti *et al.* 2002). Moreover, cAMP levels decrease in oocytes following removal from their follicles (Törnell *et al.* 1990), as well as in isolated oocytes after removal of IBMX (Schultz *et al.* 1983*a*, Vivarelli *et al.* 1983). The decrease in oocyte cAMP occurs within 2 h after washing out IBMX, a time during which the oocyte becomes committed to resuming meiosis (Schultz *et al.* 1983*a*, Vivarelli *et al.* 1983).

The downstream pathway(s) by which high cAMP levels prevent meiotic maturation is incompletely understood, and a detailed discussion is beyond the scope of this review (Fig. 2 and see Eppig *et al.* 2004). Ultimately, the cAMP level within the oocyte affects the activity of the CDK/cyclin B (CYB) protein complex, also known as maturation, meiosis or mitosis promoting factor (MPF). High cAMP levels within the oocyte result in the phosphorylation of CDK1 on Thr14 and Tyr15, rendering it

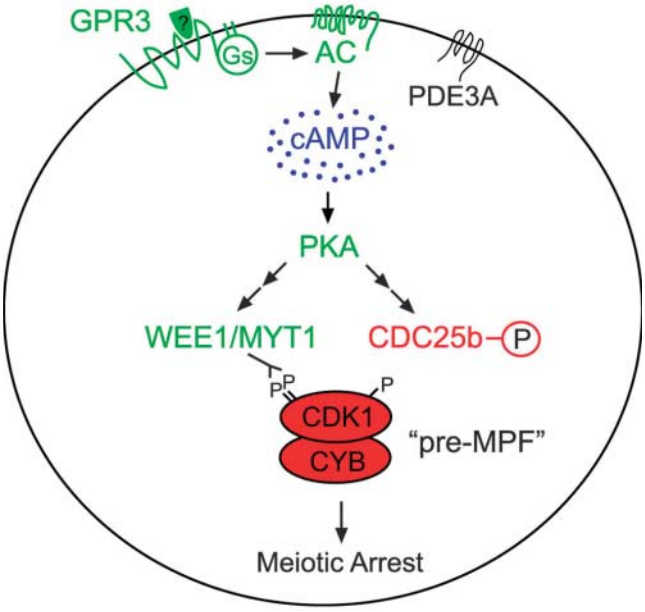

Figure 2 Cell signaling leading to the maintenance of meiotic arrest. GPR3, activated either constitutively or by an unknown ligand from the follicle cells, activates G_s, which stimulates AC to cause an elevation of cAMP. cAMP activates protein kinase A (PKA), which ultimately causes the cell cycle regulatory complex, CDK1/cyclin B (CYB), to be phosphorylated (P) and thereby inactivated. This results becaused PKA leads (directly or indirectly) to the phosphorylation of the phosphatase CDC25b (CDC25b-P), which inactivates it. PKA may also stimulate the activity of the WEE1/MYT1 kinase that phosphorylates CDK1 to keep it inactive and therefore prevent meiotic resumption. The activity of the cAMP phosphodiesterase, PDE3A, is thought to be kept low in the immature oocyte, thus preventing the breakdown of cAMP and maintaining high levels of cAMP within the oocyte.

inactive (Duckworth *et al.* 2002). A decrease in oocyte cAMP early in oocyte maturation leads to the dephosphorylation of CDK1 on Thr14 and Tyr15, and the MPF complex becomes active such that the oocyte can re-enter meiosis. The discrete set of steps through which cAMP activates or inactivates MPF are still under investigation. The major players are protein kinase A (PKA), which, through an undetermined number of steps, regulates the activities of the phosphatase CDC25 and the kinase WEE1/MYT1 (Eppig *et al.* 2004). CDC25 dephosphorylates CDK1, while WEE1/MYT1 phosphorylates it. Oocytes from mice lacking the *Cdc25b* gene are unable to activate MPF and cannot undergo meiotic resumption, highlighting the importance of this phosphatase (Lincoln *et al.* 2002). Similar knockout studies have not yet been done to examine the importance of WEE1 or MYT1. Future studies are needed to clarify the entire pathway by which cAMP levels affect the activity of MPF.

cAMP could be produced either by the oocyte or by the follicle cells that surround it. One long-standing hypothesis is that cAMP is produced by follicle cells and diffuses through gap junctions to the oocyte (Anderson & Albertini 1976, Bornslaeger & Schultz 1985, Piontkewitz & Dekel 1993, Webb *et al.* 2002*b*). Gap junctions are present between the cumulus cells and the oocyte (Albertini & Anderson 1974, Anderson & Albertini 1976). However, the lack of specific inhibitors against gap junctions in the oocyte has complicated efforts to clarify their possible role in the maintenance of meiotic arrest. For further discussion, see Piontkewitz & Dekel (1993), Webb *et al.* (2002*b*) and Eppig *et al.* (2004).

An alternative hypothesis for how high levels of cAMP are maintained in competent, fully grown oocytes is that the oocyte produces its own cAMP through a G-protein-linked receptor in the oocyte plasma membrane that stimulates G_s and, subsequently, adenylyl cyclase (AC) (Fig. 2). Several lines of evidence support this hypothesis. (1) Mouse oocytes contain all of the components necessary to produce cAMP, including the G_s G-protein (Mehlmann *et al.* 2002), a G_s-coupled G-protein receptor, GPR3 (see below) (Mehlmann *et al.* 2004), and AC (Horner *et al.* 2003). (2) Stimulation of oocyte AC with forskolin raises cAMP levels in isolated rodent oocytes and delays the onset of germinal vesicle breakdown (GVBD) (Olsiewski & Beers 1983, Schultz *et al.* 1983*a*, Urner *et al.* 1983, Bornslaeger & Schultz 1985). (3) Microinjection of the non-hydrolyzable GTP analog, GTPγS, which activates G-proteins including G_s, transiently and dose-dependently maintains meiotic arrest in isolated mouse oocytes (Downs *et al.* 1992). (4) cAMP levels increase in isolated oocytes maintained in meiotic arrest with the phosphodiesterase inhibitors, IBMX or hypoxanthine (Bornslaeger & Schultz 1985, Webb *et al.* 2002*b*). (5) Cholera toxin, which irreversibly activates G_s (De Haan & Hirst 2004), has been shown to delay oocyte maturation in isolated oocytes (Dekel & Beers 1978, Schultz *et al.* 1983*b*, Urner *et al.* 1983, Vivarelli *et al.* 1983, Downs *et al.* 1992, Grøndahl *et al.* 2000*a*). The inability of cholera toxin to completely prevent maturation may be a result of G_s degradation within the oocyte following its activation (Levis & Bourne 1992, Fong & Milligan 1999, Moravcova *et al.* 2004).

Direct evidence for an essential role of G_s in the maintenance of meiotic arrest has been obtained recently by microinjecting either a function-blocking antibody or a dominant negative form of the α subunit of G_s into follicle-enclosed oocytes (Mehlmann *et al.* 2002, Kalinowski *et al.* 2004). This pathway is supported further by the finding that oocytes from mice lacking the AC3 AC isoform, which is present in the oocyte, spontaneously undergo GVBD within ovarian follicles (Horner *et al.* 2003). Because G_s activity requires stimulation by a G-protein-coupled receptor, it has been postulated that such a receptor exists in the mouse oocyte membrane. This receptor could exhibit constitutive activity, and/or could be stimulated by a ligand produced by the surrounding follicle cells. Inhibiting G_s in isolated oocytes maintained in meiotic arrest with hypoxanthine stimulates meiotic resumption (Mehlmann *et al.* 2002, Kalinowski *et al.*

2004), supporting the hypothesis that the receptor in the oocyte has some constitutive activity.

Recently, the G_s-coupled receptor, GPR3, has been identified as an essential regulator of meiotic arrest in the mouse oocyte (Mehlmann *et al.* 2004). *Gpr3* RNA is localized in oocytes, with ~14 times lower expression in the follicle cells. Oocytes from mice lacking the *Gpr3* gene undergo spontaneous oocyte maturation within fully grown, intact follicles, independent of an increase in LH. Competence to undergo meiosis develops when an oocyte reaches its full size and when the follicle begins to form an antral space (Sorensen & Wassarman 1976, Mehlmann *et al.* 2004). Correspondingly, ~40% of the oocytes within smaller, early antral follicles from *Gpr3* $^{-/-}$ mice also undergo spontaneous oocyte maturation. The ability of oocytes from *Gpr3* $^{-/-}$ mice to maintain meiotic arrest can be rescued by microinjecting *Gpr3* RNA into incompetent *Gpr3* $^{-/-}$ oocytes within preantral follicles, followed by a 4-day culture period during which an antrum forms, indicating that the presence of *Gpr3* is needed specifically in the oocyte rather than in the follicle cells (Mehlmann *et al.* 2004).

GPR3 is an orphan receptor that exhibits a high degree of constitutive activity when overexpressed in numerous tissue culture cell lines, resulting in a high level of cAMP production (Eggerickx *et al.* 1995, Uhlenbrock *et al.* 2002). This indicates that it is coupled to G_s. It is currently not known whether constitutive activity of GPR3 in the oocyte is sufficient to produce the amount of cAMP required to maintain meiotic arrest, or whether the follicle cells surrounding the oocyte produce a ligand that increases the activity of GPR3. Structurally, GPR3 is closely related to the lysophosphatidic acid receptors, sphingosine-1-phosphate (edg) receptors, cannabinoid receptors, and melanocortin receptors (Uhlenbrock *et al.* 2002, Ignatov *et al.* 2003, Kostenis 2004*a*, 2004*b*). With the exception of the melanocortin receptors, these receptor families are activated by lipids. It is therefore possible that a lipid present in the regions of membrane contact between cumulus cells and oocyte stimulates GPR3. Another possibility for how the follicle cells might keep the oocyte arrested in prophase I until the LH surge is that they may inhibit oocyte phosphodiesterase(s) (Conti *et al.* 2002). Both of these possibilities need to be explored further to determine how the follicle cells interact with the oocyte to keep cAMP levels high prior to the LH surge.

How does LH trigger meiotic resumption?

The mechanism(s) by which LH, acting on the granulosa cells, triggers the oocyte to resume meiosis is still unknown. As mentioned previously, LH acts on the outermost, mural granulosa cells of the follicle; cumulus cells and oocytes lack LH receptors (Peng *et al.* 1991, Eppig *et al.* 1997). The LH signal must therefore be transmitted from the mural granulosa cells to the oocyte. The action of LH could either remove an inhibitory, or maturation-arresting, substance or it could provide a positive, maturation-promoting substance to the oocyte (see Conti *et al.* 2002, Eppig *et al.* 2004). This review focuses on some of the current ideas in this field, taking into account recent data elucidating the maintenance of meiotic arrest.

Recent work has shed some light on how the LH signal transmits from the exterior to the interior of the follicle. Mural granulosa cells express RNA encoding epidermal growth factor (EGF)-like proteins within 1–3 h after LH receptor stimulation (Park *et al.* 2004, Ashkenazi *et al.* 2005), and these proteins, in particular amphiregulin and epiregulin, cause follicle-enclosed as well as cumulus-enclosed oocytes to mature as effectively as LH, though with a faster time-course. They do not cause maturation of isolated oocytes. Pharmacological inhibition of the EGF receptor in cultured follicles completely inhibits LH-induced oocyte maturation, further supporting a link between these EGF-like proteins and LH (Park *et al.* 2004). These results are in agreement with previous studies showing that EGF promotes meiotic maturation of cumulus-enclosed oocytes (Das *et al.* 1991, De La Fuente *et al.* 1999, Coticchio *et al.* 2004). The signaling pathway between cumulus cells and oocytes remains unknown however.

LH acting on follicle cells surrounding frog and fish oocytes has long been known to stimulate the production of steroid hormones that trigger oocyte maturation (Masui & Clarke 1979, Nagahama *et al.* 1995, Maller 1998, Thomas *et al.* 2002, Hammes 2004, Tsafriri *et al.* 2005). However, steroids have little if any stimulatory effect on mammalian oocyte maturation (Dekel & Beers 1978, Schultz *et al.* 1983*b*, Andersen & Byskov 2002, Gill *et al.* 2004), and in some cases have a slight inhibitory effect (Schultz *et al.* 1983*b*, Kaji *et al.* 1987). Moreover, complete inhibition of steroidogenesis in cultured follicles does not prevent oocyte maturation in response to LH (Lieberman *et al.* 1976).

The sterol, follicular fluid–meiosis-activating sterol (4,4-dimethyl-5α-cholesta-8,14,24-trien-3β-ol; FF-MAS), is a candidate oocyte maturation-inducing substance. FF-MAS, which was first isolated from human follicular fluid (Byskov *et al.* 1995), is an intermediate in the cholesterol biosynthetic pathway (Schroepfer 1982). In the mouse, FF-MAS levels increase following injection of human chorionic gonadotropin (hCG), which stimulates the LH receptor (Baltsen 2001). Both purified and synthetic FF-MAS stimulate the resumption of meiosis in isolated oocytes of a variety of mammalian species including mouse, rat, and human (Byskov *et al.* 1995, Grøndahl *et al.* 1998, 2000*b*, Hegele-Hartung *et al.* 1999, 2001). However, it is not clear whether FF-MAS becomes detectable in mouse ovaries earlier than 3 h after hCG injection (Baltsen 2001). Because GVBD is observed within 1.5 to 3 h after hCG injection (Schultz *et al.* 1983*a*), FF-MAS levels should increase earlier if it is an oocyte maturation inducer. In addition, FF-MAS-induced GVBD in isolated oocytes

maintained in hypoxanthine takes 6–20 h (Hegele-Hartung *et al.* 1999, Downs *et al.* 2001). FF-MAS is therefore not a likely candidate for the initiation of oocyte maturation. However, there is evidence that it improves the ability of oocytes to complete meiosis to metaphase II, as well as the ability of fertilized oocytes to develop to the two-cell and blastocyst stages (Hegele-Hartung *et al.* 1999, Cukurcam *et al.* 2003, Griffin *et al.* 2004, Marin Bivens *et al.* 2004*a*, 2004*b*). For further discussion, see Byskov *et al.* (2002) and Tsafriri *et al.* (2002, 2005).

A meiosis-inducing factor could affect targets downstream of cAMP, perhaps by interacting with cell cycle-regulatory proteins. However, because cAMP levels fall early in oocyte maturation (Schultz *et al.* 1983*a*, Törnell *et al.* 1990, Conti *et al.* 2002), it seems more likely that the targets for such a meiosis-inducing substance are upstream of cAMP. There are several possible targets on which a meiosis-inducing factor could act within the oocyte (see Fig. 3).

(1) GPR3. Constitutive activity of G-protein-coupled receptors can be reduced by inverse agonists (Milligan 2003). Such an inverse agonist turning off GPR3 would lower cAMP in the oocyte. Alternatively, LH stimulation could affect the activity of a ligand that in the unstimulated follicle would activate GPR3, either by inactivating the ligand or by decreasing its synthesis, to ultimately lower the activity of GPR3. GPR3 could also be inactivated by other mechanisms, such as phosphorylation by G-protein receptor kinases (GRKs), which would result in its downregulation (Penn *et al.* 2000, Lefkowitz 2004). Indeed, analysis of the GPR3 protein sequence shows several serine and threonine residues in the carboxyl terminus, as well as in the intracellular loops, that could be targets of GRKs or other kinases, which could cause receptor desensitization (Saeki *et al.* 1993, Eggerickx *et al.* 1995).

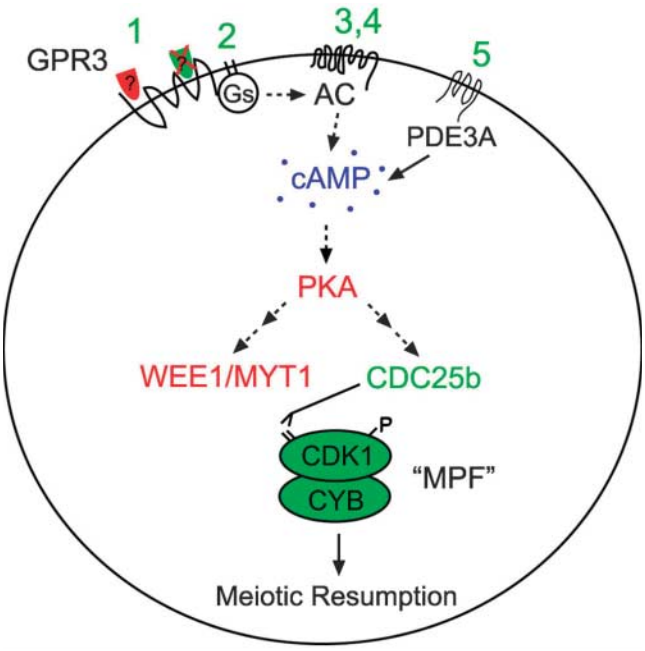

Figure 3 Potential targets through which LH could act to stimulate meiotic resumption. Each target is indicated with a green number. See corresponding numbers in the text for details.

(2) G_s. G-proteins can be inactivated by GTPase-activating proteins, or 'GAPs', also known as regulators of G-protein signaling (RGS) proteins (Kehrl & Sinnarajah 2002, Cabrera-Vera *et al.* 2003). These proteins accelerate the exchange of GTP for GDP on the G-protein α subunit, thereby turning off the G-protein. Although nothing is known about RGS proteins in oocytes, it is interesting to note that RGS2 can inhibit G_s-mediated cAMP production (Sinnarajah *et al.* 2001, Kehrl & Sinnarajah 2002, Roy *et al.* 2003). Thus, an RGS protein could potentially inhibit cAMP production in the oocyte following LH stimulation.

(3) G_i. A well-characterized pathway for inactivating ACs is by stimulating the G_i G-protein subunit, which lowers cAMP (Simonds 1999, Hanoune & Defer 2001). Indeed, the three AC isoforms that have been found to be expressed in mouse and rat oocytes, AC1, AC3, and AC9 (Horner *et al.* 2003), are all inactivated by G_i (Hanoune & Defer 2001), and a G_i pathway is known to be responsible for triggering oocyte maturation in echinoderm oocytes (Shilling *et al.* 1989, Chiba *et al.* 1992, Tadenuma *et al.* 1992, Jaffe *et al.* 1993, Kalinowski *et al.* 2003). In mammals, a role for G_i has not been examined with regard to LH signaling to cause oocyte maturation. It is possible that activation of G_i within mammalian oocytes, in response to LH, stimulates meiotic maturation. This hypothesis could be tested by examining the effects of specifically inhibiting G_i within follicle-enclosed oocytes, using pertussis toxin or antibodies, on LH-induced maturation.

(4) Calcium. All three of the AC isoforms found in rodent oocytes (Horner *et al.* 2003) can be inactivated by Ca^{2+} (Defer *et al.* 2000, Hanoune & Defer 2001, Wang & Storm 2003). In mouse oocytes, forskolin-stimulated cAMP production is prevented by raising intracellular Ca^{2+} (Horner *et al.* 2003). This effect is reversed by an inhibitor of Ca^{2+}/calmodulin-dependent kinase II, suggesting that mouse oocyte AC is inhibited by Ca^{2+}. It is possible that Ca^{2+} rises in the oocyte following LH stimulation such that it could inactivate ACs. Under some experimental conditions, Ca^{2+} release can be induced in cumulus cells, resulting in a subsequent increase in Ca^{2+} in oocytes as long as functional gap junctions exist between the oocyte and the cumulus cells (Mattioli *et al.* 1998, Webb *et al.* 2002*a*). Measurements of Ca^{2+} within follicle-enclosed oocytes following LH stimulation should

be able to clarify whether Ca^{2+} has a role in triggering oocyte maturation, perhaps at the level of turning off AC.

(5) cAMP phosphodiesterase (PDE). An attractive hypothesis is that LH stimulation leads to the activation of oocyte PDE, which hydrolyzes cAMP (Conti *et al.* 2002). The PDE3A isoform is a prevalent PDE in the mouse oocyte (Tsafriri *et al.* 1996, Shitsukawa *et al.* 2001). PDE3A activity in cumulus-enclosed mouse oocytes has been shown to increase following LH receptor stimulation (Richard *et al.* 2001); however, PDE3A activity has not been examined in isolated oocytes following stimulation of the LH receptor. Microinjection of active PDE into isolated mouse oocytes arrested with the PDE inhibitor IBMX causes GVBD (Bornslaeger *et al.* 1986). A critical role for PDE3A in mouse oocyte maturation has recently been demonstrated by generating PDE3A-deficient mice by homologous recombination (Masciarelli *et al.* 2004). The oocytes of female *Pde3A* $^{-/-}$ mice are unable to undergo meiotic resumption, remaining arrested at prophase I despite normal follicular growth and ovulation. Likewise, oocytes from *Pde3A* $^{-/-}$ mice do not spontaneously mature when released from the ovary into culture medium. The ability of these oocytes to resume meiosis is restored, however, by inhibiting PKA or by microinjecting RNA encoding the phosphatase CDC25 (Masciarelli *et al.* 2004). Although these studies highlight the importance of PDE for meiotic resumption, experiments in which PDE activity is measured specifically in oocytes within intact follicles before and after LH treatment would provide stronger evidence that PDE is a major target of regulation by LH.

Concluding remarks

The mechanisms of mammalian meiotic arrest and resumption have been technically challenging to study because the oocyte is embedded in multiple layers of cells and is therefore difficult to manipulate and observe. The follicle must remain intact in order to preserve its normal function. In addition, there is limited material available for biochemical studies of mammalian oocytes. However, new methods for studying oocyte maturation have recently provided useful information about the mechanisms of meiotic arrest. Recent methods have utilized genetically altered mice, as well as directly inhibiting oocyte-specific proteins by microinjecting follicle-enclosed oocytes. With these methods, the pathway leading to high cAMP levels in meiotically arrested oocytes has been clarified, and the receptor GPR3 has been implicated as a major regulator of cAMP production by the oocyte. The ability to manipulate follicle-enclosed oocytes should also pave the way for elucidating the mechanisms whereby LH stimulates mammalian oocyte maturation.

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