—Review—

Acquisition of Meiotic Competence during Oocyte Growth in Mammals

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Mammalian ovaries contain a large number of small oocytes [1], and a small number of these small oocytes grow to their final size. Oocytes reaching full size resume meiosis in response to gonadotropic stimuli in the ovary. Meiotic resumption in oocytes destined for ovulation is manifested by the condensation of chromosomes, disassembly of the nuclear membrane (germinal vesicle breakdown: GVBD), assembly of the first metaphase spindle, the first meiotic division, and progression to the second metaphase, where a second period of cell cycle arrest is imposed [2].

These sequential processes can be induced *in vitro* by culturing fully-grown follicular oocytes under the appropriate conditions [3, 4]. In an extension of this work, *in vitro* culture systems have been used to determine the meiotic potential of growing oocytes in a variety of mammalian species. These studies have revealed that the capacity to resume meiosis is first acquired in oocytes at an intermediate stage of growth; intermediate-sized oocytes do not progress beyond the first metaphase. The ability to progress to the second metaphase is only acquired in oocytes approaching their full size. Hence it is thought that oocytes acquire meiotic competence in a stepwise manner during the growth phase; first, the capacity for GVBD and for progression to the first metaphase and, second, the ability to reach the second metaphase.

During the acquisition of meiotic competence in growing oocytes, it has been thought that the oocytes store the necessary information and materials to carry their own nucleus through meiosis and beyond. Several studies have revealed that the acquisition of meiotic competence is correlated either with changes in nuclear morphology [5–8] or with some biochemical characteristics of growing oocytes [9], but the molecular nature of the acquisition process remains unclear.

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The progression of the meiotic cell cycle is controlled by the so-called maturation promoting factor (MPF) [10]. In the late 1980s, it was determined that MPF consists of a 34 kD catalytic subunit ($p34^{cdc2}$) and a cyclin B regulatory subunit [11]. Since then it has been speculated that the gradual acquisition of meiotic competence of the oocyte is based on the acquisition of the competence necessary to produce active MPF at the appropriate time [12]. In this review, a succinct account of the relevant aspects of the cell cycle in mammalian female germ cells is provided along with a discussion of changes in the cell cycle molecules during the acquisition of meiotic competence based on recent findings on aspects of the cell cycle.

Cell Cycle in Female Germ Cells

During fetal life, mammalian oogonia proliferate mitotically and become oocytes that begin meiotic division (Fig. 1). In the mitotic cell cycle, which is the means by which somatic cells proliferate, the oogonium replicates its DNA, segregates its chromosomes into two identical sets, and divides into two genetically identical progeny oogonia. In contrast, in the meiotic cell cycle of the oocyte, two successive rounds of chromosome segregation follow a round of DNA replication to produce a gamete with half as many chromosomes as its parent.

The mitotic cell cycle is divided into four phases. DNA in the nucleus is replicated during the S phase (S: synthesis) and segregates during the M phase (M: mitosis) of the cell cycle. Between the end of the M phase and the beginning of DNA synthesis, there is usually an interval known as the G_1 phase (G: gap). There is a second interval, known as the G_2 phase, between the end of DNA synthesis and the beginning of the next M phase, so that the mitotic cell cycle progresses through G_1, S, G_2, and M phases.

Mammalian oocytes enter the meiotic cell cycle and are then arrested in prophase of the first meiosis in the

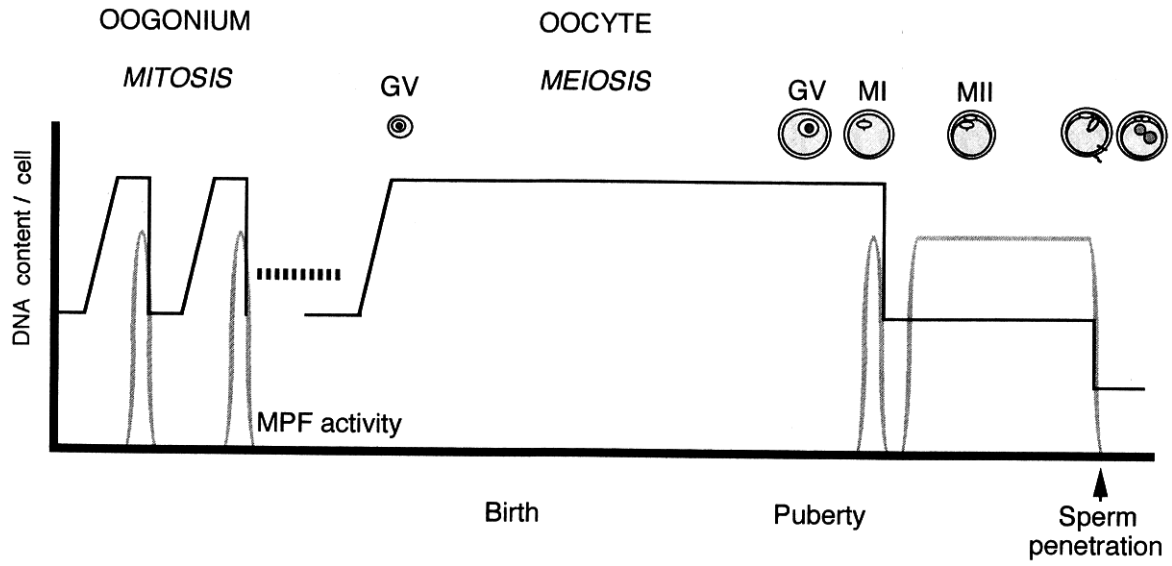

Fig. 1. Fluctuations in DNA content and MPF activity during the cell cycle of female germ cells.

fetal ovaries. This phase can be considered equivalent to the G_2 phase of the somatic cell cycle, in the sense that DNA replication is completed and the next cell cycle events will be chromosome condensation, disassembly of the nuclear membrane (germinal vesicle) breakdown (GVBD) and assembly of the first metaphase spindle. By the time of birth, the oocytes in most mammals have had meiosis arrested in the first prophase, but mammalian oocytes never resume meiosis until the females reach puberty, when gonadotropins from the pituitary gland start to induce meiotic resumption of the oocytes. Because the period between birth and puberty differs among mammalian species, for example 6–7 months in the pig, and 10–15 years in man, oocytes are meiotically arrested in the ovaries for months or years, depending on the species.

Evidence from a variety of cell cycle studies shows that both the mitotic and meiotic cycles are driven by a protein kinase, generally known as MPF. MPF activity was first discovered by Masui and Markert [10] in the cytoplasm of metaphase-arrested frog (*Rana pipience*) oocytes in 1971. In their experiment, transferred cytoplasm of the metaphase-arrested frog oocytes induced the recipient oocytes to enter meiosis. MPF activity fluctuates in both mitotic and meiotic cell cycles. The activity rises rapidly to a peak in the metaphase and then falls around the time of chromosome segregation [13]. MPF is now known to consist of a 34 kD catalytic subunit ($p34^{cdc2}$) and a cyclin B regulatory subunit (Fig. 2). Cyclins were originally identified in marine invertebrates as proteins displaying a striking periodicity in synthesis and degradation during the early embryonic cell cycle [14, 15]. B-type cyclins are components of MPF [16–19], and they act through association with the highly conserved protein kinase $p34^{cdc2}$, the product of the cell division cycle gene cdc2 in the fission yeast *Schizosaccaromyces pombe* [20].

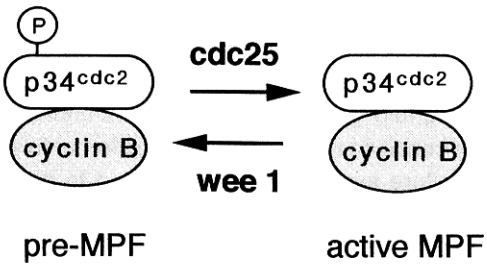

Fig. 2. A model of MPF activation.

In *Xenopus* and some marine invertebrates, it has been reported that the fully grown oocytes contain both $p34^{cdc2}$ and cyclin B, and the increased synthesis of cyclin B occurs in the cytoplasm during oocyte maturation [19, 21]. Cyclins associate $p34^{cdc2}$ molecules, the specific tyrosine residue of $p34^{cdc2}$ is in turn dephosphorylated, and the kinase becomes activated. The increased activity is maintained during the metaphase and declines sharply after fertilization or artificial activation of the oocytes. The disappearance of the kinase activity is thought to be associated with cyclin degrada-

tion via the ubiquitine pathway [22].

Cell cycle studies have recently been conducted with mammalian oocytes [23]. It has been revealed in several mammalian species that MPF activity, measured as histone H1 kinase activity, fluctuates in a manner similar to that observed in the *Xenopus* oocyte during maturation and fertilization (Fig. 1) [24–28]. Several reports have also described the molecular changes in the MPF components, $p34^{cdc2}$ and cyclin B, during oocyte maturation and fertilization [24, 29], but the changes in the growing oocytes are now under examination.

G_2-arrested Oocytes Grow and Acquire Meiotic Competence in the Ovary

Mammalian oocytes are arrested in the G_2 phase of the cell cycle in the ovary for a long time, as described above. During this period, the oocytes undergo a remarkable enlargement of their volume to about 100-fold. It is well known that a large number of primordial follicles are contained in the mammalian ovary. Several hundred thousand follicles are contained in the ovaries of the pig and cow, as well as in women [1, 30–32]. A small population of oocytes in the primordial follicles begin to grow and reach their final size. Oocytes 15–20 μm in diameter in the mouse [33, 34], and 30 μm in the pig [30, 35] and cow [36] grow to full size (without zona pellucida), 70–75 μm [34] and 120 μm [6, 8, 36], respectively. The remaining large number of oocytes are quiescent and never enter the growth phase. Alternatively, they degenerate before or during their growth phase in the ovary.

During the growth phase, oocytes acquire the competence to resume meiosis. For example, the smallest pig oocytes (30 μm in diameter) in primordial follicles have no competence to resume meiosis. Although the oocytes from 0.2 to 0.4 mm preantral follicles are already about three times their non-growing diameter, they are nevertheless still totally incapable of resuming meiosis (Fig. 3, Lee *et al.*, unpublished data). A small number of oocytes about 100 μm in diameter from 0.5–1.5 mm early antral follicles undergo GVBD and reach diakinesis or the first metaphase, although no significant number of oocytes reach the second metaphase. Over 90% of oocytes from middle- and large antral follicles undergo GVBD. Less than half of the oocytes from the middle antral follicles reach the second metaphase, but most of the oocytes which have grown to full size from the large antral follicles reach the second metaphase. This stepwise acquisition of meiotic competence of growing oocytes has been identified in the mouse [37, 38], rabbit [39], pig [6, 8] and cow [40, 41] by using various culture conditions. Mouse oocytes acquire competence to resume the first meiotic division at a diameter of 65 μm [37,38]. Bovine and porcine oocytes achieve complete nuclear maturation to the second metaphase, when at a diameter of 110 μm [41], and 110–115 μm [6, 8], respectively.

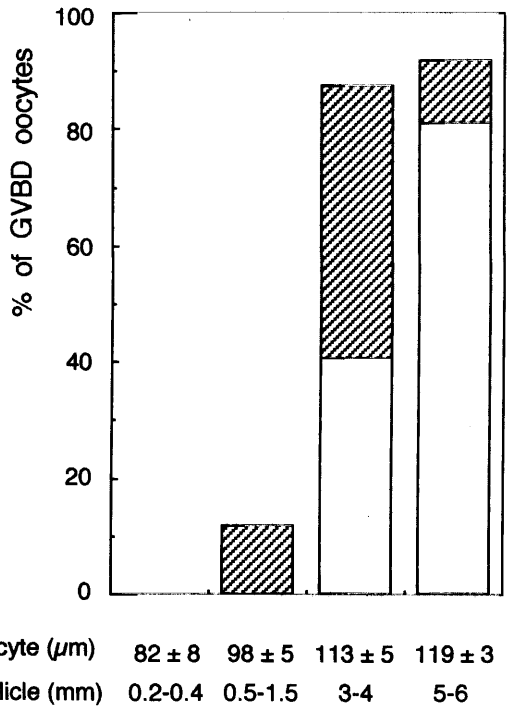

Fig. 3. Acquisition of meiotic competence in growing porcine oocytes. Various sized oocytes enclosed in cumulus cells were collected from preantral and antral follicles, cultured in medium 199 supplemented with FCS and hMG for 48 h, and examined. Each column shows the % of GVBD oocytes, and the included open column represents the % of oocytes reaching the second metaphase.

It is noteworthy that changes in nuclear morphology correlate with the acquisition of meiotic competence in the growing oocytes. It has been reported that the morphology of the chromosomes changes from a diffuse to a perinucleolar condensed state in the mouse [7] and the pig [6, 8]. Fibrillo-granular and vacuolated nucleoli are compacted in the oocytes in the pig [5] and in cattle [42, 43]. It has been thought that these changes reflect a significant decrease in rRNA synthesis [5, 44], but it has not been known which molecules are involved in the changes.

Even after the acquisition of meiotic competence in

growing oocytes, the actual progression of meiosis is restrained by the inhibitory influence of the follicular environment. Heterologous gap junctions between oocytes and granulosa cells are present throughout the oocyte growth phase [45], and granulosa cells efficiently transfer nutrients and metabolic precursors to the oocyte [46–50]. Beside this nutritional role, it has been suggested that an inhibitor(s) of oocyte meiosis is produced by granulosa cells, because the resumption of meiosis is restricted when intact cumulus oocyte complexes are cultured in contact with granulosa cells [51–53]. Several studies have attempted to identify this inhibitor, but the results have been inconclusive [54–56], although hypoxanthine appears to be a potential candidate [57–60].

Molecular Basis of the Acquisition of Meiotic Competence of Growing Oocyte

Before reaching the specific growing stage, small G_2-arrested oocytes have no competence to resume meiosis, as described above. An explanation of this inability of the oocytes associated with cytoplasmic events has been provided by cell-fusion experiments. Balakier [61] first demonstrated that the chromosome condensation of meiotically incompetent growing mouse oocytes from juvenile mice occurs after fusion with maturing fully-grown oocytes. In the pig, formation of the first meiotic spindle in small oocytes has been observed after fusion with homologous oocytes arrested at the second metaphase [62]. Moreover, two recent reports have demonstrated that in the mouse the chromosomes in the nucleus of small oocytes in the primordial follicles can be condensed and arranged in the first meiotic spindle when the oocytes are fused with either fully-grown and metaphase arrested oocytes [63], or fully-grown G_2-oocytes from which the germinal vesicles have previously been removed, and subsequently the fused oocytes are matured *in vitro* [64]. All of these results demonstrate that the inability of the small oocytes to resume meiosis is not due to their nucleus but to their cytoplasm. Probably, during the gradual acquisition of meiotic competence in the oocytes, they accumulate in their cytoplasm the necessaries which will allow for the production of active MPF at the appropriate time [12, 65].

It has recently been reported that accumulation of MPF component $p34^{cdc2}$ in the mouse [66, 67] and pig [8] increases during the period of oocyte growth, when these oocytes become competent to resume meiosis. Actually incompetent growing porcine oocytes in preantral follicles have a much lower concentration of $p34^{cdc2}$ than meiotically competent oocytes (Fig. 4). The synthesis and accumulation of $p34^{cdc2}$ increase as the oocytes increase in size until the middle-antral follicle stage (oocyte diameter: 115 μm).

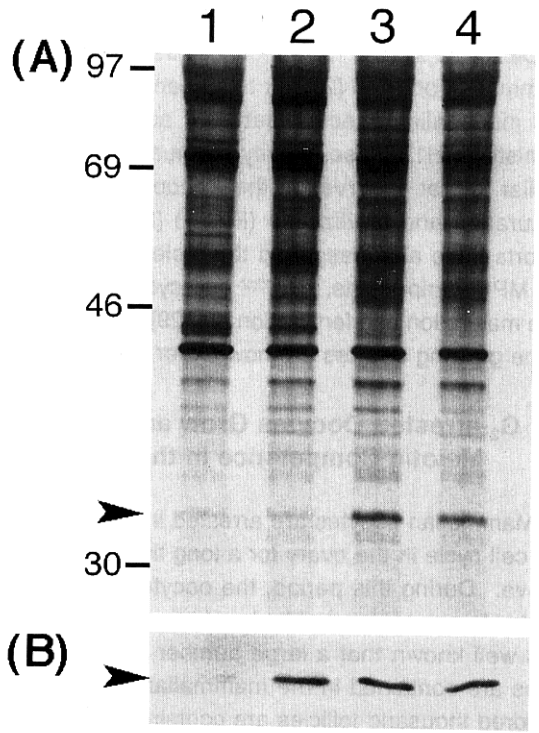

Fig. 4. Synthesis and accumulation of $p34^{cdc2}$ in pig oocytes at various stages of growth. Lanes 1, 2, 3 and 4 contain oocytes of about 80, 105, 115 and 120 μm in diameter, respectively. (A) Lysates of [^{35}S]-methionine-labeled oocytes at various growth stages were run on 13% polyacrylamide gel, and autoradiographed. (B) Immunoblotting of $p34^{cdc2}$ in pig oocytes. The numbers of oocytes were calculated to ensure that the total oocyte mass in each lane was similar to all other lanes. $p34^{cdc2}$ protein was detected by mouse anti- PSTAIR antibody [80].

In the pig, cyclin B1 is never observed in G_2-arrested oocytes in either the growing or the fully-grown stage (Lee *et al.*, unpublished data). On the other hand, it has been reported in the mouse that meiotically incompetent oocytes in the growth phase already contain cyclin B1, and this level does not undergo any significant change during the acquisition of meiotic competence [66, 67]. Fully-grown mouse oocytes can resume meiosis without the synthesis of new protein [68], but porcine oocytes need protein synthesis for GVBD [69]. Pig cyclin B1 appears just before nuclear membrane breakdown,

increases sharply, and then remains at a high level during the metaphase (Lee *et al.*, unpublished data). The difference in the necessity for protein synthesis may reflect the accumulation of cyclin B1 protein during the growth phase, but the differences between species in cyclin B1 levels in meiotically incompetent oocytes is not completely understood [70].

MPF is activated through the phosphorylation/dephosphorylation cascade. Although the precise nature of the cascade from gonadotropic stimulation to MPF activation is still not understood in mammalian oocytes. Okadaic acid, an inhibitor of protein phosphatases 1 and 2A, is known to bypass the phosphorylation cascade required for the activation of MPF [71,72]. In the mouse, small oocytes (50 μm in diameter) are entirely unresponsive to okadaic acid, which is thought to be due to inadequate levels of MPF component $p34^{cdc2}$. On the other hand, microinjection of okadaic acid induces GVBD in growing oocytes (55 to 60 μm) which are unable to achieve either hormone-stimulated or spontaneous meiotic resumption *in vitro* [72]. It has also been reported that okadaic acid accelerates the transition into the M-phase of the meiotic cell cycle in fully grown porcine and bovine oocytes [73], and small porcine oocytes from preantral follicles (80–90 μm in diameter) were incapable of resuming meiosis after treatment with okadaic acid because of inadequate levels of MPF-kinase subunits [74]. Quite recently Mitra and Schultz [75] reported that the concentration of tyrosine phosphatase cdc25C [76, 77] increases, but the concentration of wee1 kinase [78, 79], which phosphorylates the tyrosine-residue of $p34^{cdc2}$ and inactivates MPF, decreases during the acquisition of meiotic competence in growing mouse oocytes (Fig. 2).

Based on this evidence, I would postulate that inadequate levels of $p34^{cdc2}$ block meiotic progression in early growing mammalian oocytes. During the acquisition of meiotic competence growing oocytes gradually accumulate the MPF component which cannot be mobilized because of lesions in the phosphorylation cascade required to activate the molecule, and finally the oocytes reaching full size equip all of the components to progress from the G_2- to the M-phase. Considering the relatively stable levels of $p34^{cdc2}$ in somatic cells during the mitotic cell cycle, it will be interesting to establish whether the suppressor mechanism in oocytes during the early growth phase, namely the inadequate synthesis of the $p34^{cdc2}$ protein, is regulated at the transcriptional or translational level.

Conclusion

Mammalian oocytes begin meiotic division in fetal ovaries and are arrested in the G_2 phase of the cell cycle for a long time. These small G_2-arrested oocytes start to grow and acquire the competence to resume meiosis toward the end of the growth phase. Recent studies have revealed that the growing oocytes gradually accumulate the MPF component $p34^{cdc2}$. Although in this review I do not refer to Mos and MAP kinase, the results suggest that they play an important role in the meiotic resumption of mammalian oocytes. A study of the oocyte cell cycle is like attempting to put together a jigsaw puzzle without knowing the number of pieces, but the accumulation of $p34^{cdc2}$ may be crucial for the acquisition of meiotic competence of oocytes, when we recall that the MPF is a universal cell cycle engine. Because MPF is activated through a cascade(s) consisting of different protein kinases and phosphatases, future work should be conducted to understand their nature in order to better understand the acquisition of meiotic competence in mammalian oocytes.

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