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DEVELOPMENTAL HISTORY OF THE MAMMALIAN OOCYTE: INSIGHT FROM MOUSE MUTATIONS

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1. ABSTRACT

Growth and differentiation of the mammalian oocyte is regulated with the coordinate development of the granulosa cells. The complex signaling pathways that regulate the growth and development of mammalian oocytes are beginning to be elucidated through the use of gene targeting. These technologies have provided new insight into the roles of specific genes during the development of the germ cells and gonads, as well as post-pubertal development of oocytes. In many cases, these studies have resulted in a new understanding of the function of certain genes, in others they have provided new genes and pathways to be studied in mammalian reproductive biology. Ultimately, these studies will shed light on human genetic disease and infertility.

2. INTRODUCTION

It has become clear that the developmental history of the oocyte is regulated by reciprocal interactions with the surrounding somatic cells, especially the granulosa cells of the follicle. Communication between the granulosa cells and the oocyte occurs both through paracrine signaling and directly through gap junctions that form between the oocyte and the surrounding granulosa cells. The oocyte secretes signals that induce granulosa cell proliferation, regulate steroidogenesis, and maintain the architecture of the developing follicle. Similarly, Signals from granulosa cells regulate meiotic arrest, promote oocyte growth and facilitate the reinitiation of meiosis and oocyte maturation. Understanding the roles of specific genes will provide insight into these regulatory networks and ultimately into the genetic bases of infertility in humans.

Currently, the genetic basis for the regulation of these processes, and the interplay between different signaling pathways, is not well understood. However, the past decade has seen a dramatic increase in the use of genetic approaches in mice to examine the function of individual genes during folliculogenesis and oocyte maturation. Use of these technologies allows for the *in vivo* dissection of the complex interactions required for the successful development and meiotic maturation of the mammalian oocyte. Data gathered from null mutations has already had a profound impact on our understanding of the complex signaling pathways that coordinate the estrous cycle and regulate fertility. The use of molecular genetic techniques to ask questions about gene function has provided new insight into the roles of signaling molecules that regulate oocyte development. In this review, we will discuss insight gained from genetically engineered mice with a focus on phenotypes that directly impact the development of germ cells, or the functional maturation of oocytes.

3. DEVELOPMENT OF OOCYTES FROM PRIMORDIAL GERM CELLS

The origins of mammalian oocytes lie in primordial germ cells that migrate to the gonad early in fetal development (reviewed in 1). In mice, primordial germ cells (PGCs) arise from the posterior primitive streak around E6.5 to E7.5, and associate with the endodermal cells at the base of the allantoic diverticulum (2-4). Between E9.5 - E10.5, the PGCs migrate through the hindgut mesentery into the genital ridge, where they aggregate with somatic pregranulosa cells (5,6). At this stage, the PGCs (now referred to as oogonia) undergo mitotic division, expanding the germline, until E12.5 when they enter meiosis. Oogonia subsequently arrest in prophase of the first meiotic division, where they remain until ovulation. Granulosa cells of the follicle are derived from epithelial cells invaginating into the urogenital ridge. The pregranulosa cells associate with the oocytes as they enter the gonad. Oocytes enter meiosis between E13.5 and E16.5, and by birth, have progressed synchronously through the stages of meiotic prophase. Oocytes become arrested in diplotene and have an enlarged diploid nucleus, referred to as the germinal vesicle (GV). The oocytes remain arrested at this stage until the mouse reaches sexual maturity (7). In this section, we will review genes that are associated with the prefollicular events of germ cell migration, attrition, and meiosis.

3.1. Migration of Primordial Germ Cells

The migratory path of the PGCs is dependent on interactions between adhesion molecules on the cell surface with extracellular matrix glycoproteins produced by the surrounding somatic cells. Characterizing the substrate requirements for migration has been limited *in vivo*. However, in culture, PGCs are able to adhere to fibronectin, laminin, and collagen IV (8). PGCs isolated before, during, and after, migration display differences in their adhesiveness to these three substrates. For example, migration into the urogenital ridge is dependent on adherence to a long tract of laminin. This suggests that there is a corresponding change in the composition of the adhesion molecules expressed on the surface of the germ cells during migration.

Candidate molecules for mediating PGC migration include the integrins, a family of heterodimeric receptors (alpha and beta subunits). The composition of the receptor determines which extracellular matrix proteins, including fibronectin and laminin, that integrins are able to bind. Four alpha integrin subunits (alpha 3, alpha 5, alpha 6, alpha V) and two beta subunits have been detected in migrating PGCs (1). The importance of integrins to this process has been demonstrated through a chimeric analysis with beta 1 integrin deficient cells. This study demonstrated that beta1 integrin is required for normal motility of PGCs. The calcium-dependent adhesion molecules, N-, P-, and E-cadherin, are also expressed in a dynamic pattern during PGC migration. P-cadherin is expressed throughout migration, while E-cadherin is expressed in the germ cells after exiting the hindgut. N-cadherin is expressed only after the PGCs have completed migration. It has been shown that interference with the function of E-cadherin prevents the normal coalescence of the germ cells in the indifferent gonad (9).

These observations underscore the importance of the interactions between the PGCs and cell adhesion molecules expressed on the surrounding somatic cells during migration to the gonad. Changes in the requirement for specific cell adhesion molecules during, and after, migration raises the interesting issue of how the PGCs alter the expression of cell surface receptors in response to changes in the composition of the extracellular matrix along the path and the molecular triggers that regulate this process.

3.2. Attrition of the Oogonia

Germ cells undergo significant expansion in number both before and after their arrival in the developing ovary. At E8.5, approximately 145 PGCs are present, as visualized by alkaline phosphatase expression. The size of the germ cell pool increases to over 1000 by E10.5 and 26,000 by E13.5 (10). However at birth, the female mouse has lost two-thirds of the potential germ cells (11). The attrition in germ cells is due to apoptosis throughout embryogenesis, with the greatest loss at the onset of meiosis (E13- E16) (12,13). Since the size of the pool of primordial follicles is fixed at the time of birth, regulation of germ cell death directly impacts fertility and the reproductive lifespan of the animal.

One of the primary regulatory pathways for germ cell survival functions through KIT, a receptor tyrosine kinase. KIT is expressed on the surface of PGCs beginning at E7.5, the time of migration into the genital ridge. Its ligand, Steel/SCF/kit ligand (KL), is expressed in the somatic cells along the migratory path from the allantois to the genital ridge, starting at E9.0 (14,15). Mutations in either the receptor or ligand result in gonadal dysgenesis and sterility of both male and females (16). The addition of KL to PGCs in culture promotes survival, indicating its role in inhibiting apoptosis (17-21). The restricted expression of the ligand along the migratory path suggests an additional role for KL is to guide the migration of Kit-expressing cells. It is likely that the loss of germ cells in the mutant mice is due to a combination of cell death and a failure to properly migrate.

Other cytokines that influence the proliferation and survival of PGCs have been identified in culture. Factors such as leukemia inhibitory factor (LIF), basic fibroblast growth factor, tumor necrosis factor-alpha (TNF-alpha), insulin-like growth factor I (IGF-I), and interleukin-1 beta (IL1 beta) have been shown to promote PGC and oogonia proliferation in culture (19-26). Of these, only LIF has been demonstrated to have a similar role *in vivo*. Alternatively, cytokines such as transforming growth factor beta1 and beta2 (TGF beta1 and 2) and Fas ligand are able to induce death of the oogonia (27,28). The presence of apoptotic and anti-apoptotic factors in the milieu of the gonads has lead to a model in which germ cell attrition is regulated by subtle changes in the balance of cytokines (29).

The target of factors that promote or prevent apoptosis is the Bcl-2 gene family in the mitochondria of the PGC and oogonia (13). The Bcl-2 family of mitochondrial genes can be divided into anti-apoptotic (e.g. Bcl-2 and Bcl-XL) and pro-apoptotic genes (e.g. Bax and Bok) (30,31). Activation of *bcl-2* transcription by KL, LIF, and IGF-I occurs through the phosphatidylinositol 3'-kinase-mediated phosphorylation of c-Akt, a serine-threonine kinase (32-35). Other cytokines, such as IL1 beta, are able to induce the Bcl-2 pathway through the generation of sphingomyelinase 1-phosphate by activating the cytoplasmic enzyme, ceramidase. In the absence of IL1beta stimulation, pro-apoptotic factors such as acid sphingomyelinase-generated ceramides accumulate in the cell (25).

Expression of Bcl-2 or Bcl-XL in the mitochondria leads to stabilization of the organelle, and the suppression of cytochrome C. In contrast, Bax or Bok leads to a destabilization of the mitochondria and the release of cytochrome C (36,37). This ultimately leads to the activation of the caspase signaling pathway and apoptosis (36,38,39). Consistent with the predicted role of these genes in regulating germ cell number, mice homozygous for a null *bcl-2* mutation possess a significantly reduced number of oocyte-containing primordial follicles (40). In addition, expression of exogenous Bcl-2, or the inactivation of *bax*, confers resistance to apoptosis in mouse oocytes (40). Conversely, the inactivation of *Bax* in mice leads to an abrogation of apoptosis in the germ cells (42,43).

3.3. Other Genes Required for the Survival of Primordial Germ Cells

The *ataxia-telangiectasia* mutant gene (*ATM*) is associated with the autosomal recessive ataxia-telangiectasia disorder that is characterized by a broad array of maladies including gonadal atrophy (44,45). ATM belongs to a family of genes that are involved in the control of cell cycle progression, processing DNA damage, and maintenance of genomic stability (46). In *ATM*-deficient mice, oocytes in the ovaries begin to undergo apoptosis around E16.5, and the ovaries are devoid of oocytes at the time of birth (47). Telomere clustering is aberrant, and the mutant oocytes are disrupted in prophase of meiosis I (47,48). Elevated levels of p53, p21, and Bax, in *ATM*-deficient oogonia suggest that infertility may be due to an activation of the apoptotic pathway (49). Consistent with this hypothesis, compound $ATM^{-/-}/p53^{-/-}$ and $ATM^{-/-}/p21^{-/-}$ mutants partially rescue the prophase I defect.

Other genes that have been found to be required for the survival of oocytes in primordial follicles include a putative transcription factor, Zfx, and an RNA-binding protein DAZL1. With either gene, the role of the gene during oocyte development remains to be determined. *DAZL1* is the autosomal homologue of the Y-chromosomal *DAZ* gene, which is deleted in azoospermic males (50,51). Dazl1 is broadly expressed in the follicles of prepubertal ovaries and becomes restricted to the periphery of the oocyte cytoplasm in adults (52). A targeted null mutation of *Dazl1* results in a complete loss of male and female germ cells (52). Interestingly, the ovaries from $Dazl1^{-/-}$ mice contained steroidogenically active cells in the absence of any follicular structure (53).

Zfx is a gene found on the X chromosome that has been implicated in Turner syndrome and sex differentiation of the indifferent gonads (54,55). The gene encodes a protein containing 13 zinc fingers and a highly acidic domain that is able to activate transcription (56). Mice homozygous for a null allele of the *Zfx* gene are less fertile than their wild type littermates and have a shortened reproductive lifespan (57). An analysis of the indifferent gonads of E11.5 *Zfx*-null embryos revealed a reduction in the total number of primordial germ cells, implying that this gene is required for the proliferation of primordial germ cells prior to sex determination.

4. INITIATION OF EARLY FOLLICLE DEVELOPMENT AND OOCYTE GROWTH

Once a primordial follicle initiates growth and development, the oocytes are committed to ovulate or the follicles become atretic. Early follicle development is associated with a growth in the size of the oocyte and proliferation of the surrounding granulosa cells. Reciprocol interactions between the oocyte and the granulosa cells play an important role in regulating initiation and development. The rate at which primordial follicles enter the growing pool directly impacts the reproductive lifespan of the female. Hypoinitiation could result in a failure to ovulate, while hyperinitiation could lead to premature reproductive senescence. In this section, we will discuss genes that are required for proper initiation and development of primordial follicles.

4.1. Kit-Kit Ligand Signaling

As discussed earlier, an interaction between Kit and its ligand is critical for the early survival of primordial germ cells. However, there are at least two natural mutations in Kit ligand, $Mgf^{Sl\text{-}t}$ and $Mgf^{Sl\text{-}pan}$, in which follicles are present in reduced numbers (58-59). An analysis of the ovaries from these mutant mice has revealed additional roles for the Kit signaling pathway in follicle development and oocyte maturation.

The patterns of Kit and KL transcription are consistent with their role throughout follicle development. Kit expression ceases in the germ cells at the time at which they enter meiosis. A second wave of Kit transcription begins in oocytes of primordial follicles and expression is maintained in oocytes throughout follicle development (60). In antral follicles, Kit is also expressed in the thecal cell layer that surrounds the granulosa (61). KL transcripts are detectable in the granulosa cells of primary follicles, and increase with the growth of the follicle (62). With the differentiation of multiple granulosa cell types during antral follicle development, KL expression is restricted to the mural granulosa. Two splice variants of KL have been detected; both encode biologically active proteins; KL-1, a soluble form, and KL-2 a membrane bound form (62,63). The significance of the two forms of KL has not been determined. Regulation of KL-1 and KL-2 expression in the granulosa cells is positively regulated by testosterone

produced by the thecal cells. Expression can be enhanced by follicle stimulating hormone (FSH) produced by the pituitary, and inhibited by signals from the oocyte (63). Recent observations indicate that the oocyte-specific factor growth and differentiation factor 9 (GDF-9) can inhibit KL-1 and KL-2 transcription. In mice that are deficient for *gdf-9*, KL-1 and KL-2 are aberrantly expressed in the cumulus cells (64).

As mentioned above, the ovaries of $Mgf^{Sl\text{-}t}$ and $Mgf^{Sl\text{-}pan}$ mice contained follicles that arrest at a very early stage of development postnatally, prior to antrum formation (58,59,65). These follicles have exited the primordial follicle pool, but are blocked at the point at which follicles would normally undergo rapid growth. This predicted a role for Kit in the initiation of follicle growth. Experiments done that inactivated Kit in wild type females by injection of the anti-kit antibody, ACK_2 provided further evidence of a role for Kit in initiation (66). If mice were treated between birth and day 2, the subsequent growth of follicles was completely blocked due to a reduction in granulosa cell proliferation. Further, the addition of KL in culture stimulated primordial follicle development (67). Since Kit is not expressed in the granulosa cells, proliferation of these cells is likely induced by a paracrine factor(s) produced by the oocyte in response to Kit-KL signaling. The mechanism by which this occurs has not been established.

There is evidence that signaling through Kit plays a role in regulating oocyte maturation and the resumption of meiosis. In rat follicles, the addition of KL in culture resulted in a block in the progression of meiosis into metaphase I (MI), while the addition of an antisense oligonucleotide specific to Kit had the opposite effect (68,69). This predicts that KL expressed in granulosa cells around the oocyte in preantral follicles is required to maintain the oocyte in meiotic arrest. The loss of KL expression as cumulus cells differentiate in antral follicles would remove the block. It remains to be determined whether KL has the same effect in mice or humans.

The expression of Kit in the theca cells and KL in mural granulosa cells along the basal lamina predicts a role for these genes in steroidogenesis and thecal cell differentiation. There are a number of observations that support this notion. The production of the testosterone precursor androstenedione by theca cells and follicles in culture is induced by the addition of KL (70-72). KL is also able to stimulate the differentiation of stromal-interstitial cells around preantral follicles in culture. Further, it was demonstrated that KL induced proliferation of stromal and theca cells was dose-dependent (70). In small antral follicles there is higher KL expression in the mural granulosa cells than in large antral follicles (63). Since, Kit - KL signaling blocks aromatase activity in granulosa cells (71), the decrease in signaling in larger follicles would lead to increased estrogen production and expression of LHR. These data indicate the complexity of the signaling interactions that regulate the maturation process, signaling between theca and granulosa and granulosa and oocyte.

4.2. Growth and Differentiation Factor 9

Oocytes express a soluble factor, GDF-9, which acts to promote granulosa cell proliferation. GDF-9 is a member of the TGF beta superfamily of secreted signaling proteins (73). GDF-9 is expressed in oocytes beginning at the early growing follicle stage (74). In mice that are deficient for GDF-9, the follicles are arrest at the type 3b stage; follicles have a growing oocyte surrounded by a single layer of cuboidal granulosa cells. The oocytes of GDF-9-deficient mice continue to grow in size and eventually degenerate. This is followed by luteinization of the granulosa cells (75,76). Using recombinant GDF-9 in a granulosa cell culture system, it was found that this factor induces the expression of cyclooxygenase 2 (cox-2), steroidogenic acute regulator protein (StAR), and increases granulosa cell progesterone synthesis in the absence of FSH. Further, GDF-9 inhibited the expression of urokinase plasminogen activator (uPA) and LHR mRNA. The expression of HAS2 and inhibition of uPA are key events in cumulus expansion and implicated GDF-9 in this process. This was confirmed by the observation that GDF-9 could induce cumulus expansion in oocytectomized follicles (74). Thus, GDF-9 has two roles to play, inducing both the early proliferation of granulosa cells and the later differentiation of the cumulus cells. This is one of many signaling pathways that have pleiotropic functions in folliculogenesis, it will be important to understand what changes in gene expression underlie these different roles.

4.3. Growth and Differentiation Factor 9 b/Bone Morphogenetic Factor 15

Recently, a gene that is expressed exclusively in oocytes with a high degree of homology to *gdf-9* was identified, and is called either *gdf-9b* or *BMP-15*. Expression of this gene is first noted in primary follicles (one layer of cuboidal granulosa cells) and expression levels increase to a peak in antral follicles (73,77,78). *In vitro* studies in rat follicles indicate that GDF-9B/BMP-15 plays an important role in stimulating cell proliferation in the surrounding granulosa cells (78). In addition, this factor was able to selectively inhibit FSH-induced progesterone production, but not FSH-induced estradiol production, due in part to the regulation of FSH receptor transcription (79). The most compelling *in vivo* evidence for a function for GDF-9B comes from the study of a natural mutation occurring in sheep, FecXI, which maps to *gdf-9b/BMP-15* on the X-chromosome (80). Homozygous null females are infertile, due to an arrest in folliculogenesis at the primary stage. In contrast, heterozygous females are displaying an increased fertility. This suggests that fertility is regulated by GDF-9B/BMP-15 in a dosage-sensitive manner.

5. ESTABLISHING DIRECT COMMUNICATION BETWEEN THE OOCYTE AND CUMULUS GRANULOSA CELLS

5.1. Zona Pellucida

The zona pellucida is an extracellular matrix secreted by the oocyte that forms a boundary between the egg and the surrounding somatic cells. Prior to ovulation, the zona is required for the oocyte to achieve competency (81,82). After ovulation, this matrix mediates both initial

sperm-egg recognition (83) and triggers the acrosome reaction. Once the oocyte has been fertilized, cortical granule N-acetylglucosaminidase from the egg "hardens" the zona (84,85). The hardened zona is impervious to further sperm penetration, effectively preventing polyspermy. The fertilized embryo remains encased in the zona during its passage through the oviduct. Presumably, the zona prevents premature adherence of the embryo to the oviduct wall (86,87).

The mouse zona pellucida is composed of three sulfated glycoproteins, ZP1, ZP2, and ZP3 (88,89), which are 180-200, 120-140, and 83 kDa, respectively. The zona first appears as patches of coalesced extracellular matrix in primary follicles and is completely formed in fully-grown oocytes of early antral follicles (90,91). The zona does not create a complete barrier between the oocyte and the surrounding granulosa cells. Cellular processes from the cumulus granulosa cell layer penetrate the zona to form gap junctions with the oocyte (92). Transcription of the three Zp genes is regulated by the basic helix-loop-helix (bHLH) transcription factor, FIG alpha (93). A bHLH enhancer element (E box) has been identified in the promoter region of Zp1, Zp2, and Zp3, which will bind FIG alpha and direct transcription. Further, mice deficient for *fig alpha* fail to express the Zp genes (94).

The contribution of the individual Zp genes to the formation of the matrix has been examined through targeted null mutations. In the ovaries of mice deficient for *ZP1* a porous zona forms that allows the migration of cumulus cells into the perivitelline space. Early mutant embryos experience precocious hatching, which results in a reduction in survival (95). The oocytes from $Zp2^{-/-}$ mice form a thin zona pellucida, which breaks down in follicles prior to ovulation. Fewer early antral follicles are present in the ovaries of $Zp2^{-/-}$ females, and fertilized eggs fail to progress beyond the two-cell stage (82). $Zp3^{-/-}$ females are also infertile, and the primary follicles fail to form any zona pellucida even though both ZP1 and ZP2 are expressed (81). Similar to the $Zp2^{-/-}$ mice, fertilized eggs in the $Zp3^{-/-}$ mice fail to develop to the blastula stage. It has been shown that oocytes stripped of their zona can be fertilized and will progress to the blastocyst stage. This therefore indicates that the zona pellucida plays an additional role in regulating the competence of oocytes during folliculogenesis.

5.2. Gap Junctions

Intercellular communication between the oocyte and the surrounding granulosa cells is critical for proper follicle development and meiotic competency. It has been proposed that gap junction channels connecting the oocyte with the granulosa act as primary conduits of communication. Gap junctions are composed of two opposing hexameric hemi-channels consisting of six transmembrane proteins called connexins. To date, 17 members of this family have been characterized and organized into subfamilies, based on sequence homology (96). Ovarian gap junctions mediate (1) the uptake of nutrients from granulosa cells (97); (2) transmission of paracrine endocrine signaling (92,98); (3) the co ordination of steriodogenesis in the granulosa cells (99); (4) maintenance of oocytes in meiotic arrest (100-102); and (5) LH induced maturation of the oocyte (100).

Immunohistochemical studies in mouse ovaries revealed the differential expression of alpha connexins (alpha 1, -4, - 5, and -6) in the granulosa cells and beta connexins (beta 1, -2, -4, and -6) in the oocyte and thecal layer (103). Further, the expression of individual members varied with the developmental stage of the follicle (i.e. primordial, antral, preovulatory, and atretic). Targeted null mutations have begun to elucidate the role of individual connexin genes at discreet points in follicle development. Connexin 43 (alpha1) is expressed in the granulosa cells of primary follicles. The follicles in the ovaries of *connexin 43* -deficient mice are arrested at the primary stage (104). In contrast, mice deficient for *connexin 37* (alpha4), which is initially expressed in granulosa cells of early antral follicles, are ovulation incompetent (105,106), lack antral follicles, and develop numerous inappropriate CL. In addition, oocytes isolated from *connexin 37*- deficient follicles are unable to reinitiate meiosis. These results suggest that differential expression of members of the connexin family is important for the proper progression through folliculogenesis.

6. REGULATORS OF OOCYTE MATURATION

Oocyte maturation is initiated following the surge of LH from the pituitary. Oocytes competent to mature have already completed growth, formed their zona pellucida, and are surrounded by cumulus granulosa cells within an antrum. The first step usually associated with the initiation of oocyte meiotic maturation is breakdown of the GV (GVBD). The events of GVBD include, the disruption of the nuclear envelope of the GV (107), changes in the structural organization of the chromatin (108), and condensation of chromatin into discernable chromosomal bivalents comprised of synapsed homologous chromosomes. Following GVBD, the first meiotic spindle forms and meiotic anaphase follows and segregates individual homologous chromosomes at opposite poles of the spindle. The spindle orientation is accentric at the oocyte cortex, resulting in conserved, limited cytokinesis with the outside spindle pole protruding through the oocyte surface into the perivitelline space. Thus, at the conclusion of the first meiotic division, one set of segregated homologous chromosomes remains within the oocyte and the other set is abstricted in the first polar body. After establishment of the second meiotic spindle, the cell has reached the secondary oocyte stage, is genetically haploid, and has completed meiotic maturation. Ovulation follows, and the mature oocyte is fertilizable. In mammals, the secondary oocyte or mature egg, is arrested at the second meiotic metaphase, or MII, remaining at that stage until the oocyte is penetrated by sperm during fertilization.

Most emphasis in recent research directed at the regulation of maturation has focused on the mechanisms by which the oocyte progresses from the arrested GV stage of meiotic prophase at meiotic G2 to the M-phase to complete the first meiotic division. Intensive studies in *Xenopus* has lead to a model of control of cell cycle progression by the

MAP kinase cascade and cyclin-B-Cdc2 kinase (maturation promoting factor; MPF)(reviewed in 109). Regulation of the onset of maturation and the subsequent block in MII is dependent on the interplay between MAP kinase and MPF, and their activation through phosphorylation. Studies in other vertebrates, including mice, have revealed some variations in the regulation of critical components of the cell cycle control machinery (109). Here we present genetic evidence reinforcing the importance of cyclin-B, mos, and components of the prostaglandin signaling pathway.

6.1. Cyclin B1 and B2

In mammals, meiotic maturation is regulated by the activation of the cell cycle kinase, MPF, which drives cells into the division or M-phase of the cell cycle. MPF is composed of a catalytic subunit p34cdc2 and a regulatory subunit, cyclin B (110-113). The activity of MPF has been shown to increase early in oocyte maturation up to the first meiotic metaphase, to decrease at anaphase of the first meiotic division, and then to rise again at the transition to second meiotic metaphase (114,115). The modulation of cyclin protein synthesis and degradation is crucial to the control of MPF activity. The mitotic cyclins are synthesized throughout the cell cycle and destroyed during a short period, just before the metaphase-anaphase transition (116,117). In mouse oocytes, synthesis of cyclin B increases progressively during meiotic maturation, it is at its peak levels by the end of the first meiotic M phase, and is degraded at the time of polar body extrusion (118,119). There are somewhat differing reports regarding the relative amounts of p34cdc2 and cyclin B in mammalian oocytes competent to undergo maturation, however, there is general agreement that at the end of oocyte growth relatively high concentrations of p34cdc2 protein and cyclin B protein are present before initiation of GVBD (120-123).

There are two *cyclin B* genes in mammals; *B1* and *B2*, null mutants of both genes have been constructed in mice (124). Interestingly, *cyclin* $B2^{-/-}$ mice are viable and produce normal offspring, although with reduced litter size, but *cyclin* $B1^{-/-}$ mutants die embryonically. The subfertility of the *cyclin B2* mutants has not been studied, but could indicate a role for this gene in oocyte maturation. More recently, Ledan *et al.* (125) injected oocytes with cyclin B1 antisense RNA and found two distinct phenotypes. Most of the injected oocytes were blocked in MI, but others extruded the first polar body prematurely and were unable to enter MII. Injection of antisense cyclin B2 had no effect on maturation. It might be interesting in light of this data to study compound mutations, such as cyclin $B2^{-/-}/B1^{+/-}$, for possible changes in fertility due to decreased levels of cyclin B. These observations indicate that the regulation of the timing of events during meiotic maturation is tightly tied to the synthesis of cyclin B.

6.2. Mos

The c-mos proto-oncogene product (Mos) is a serine/threonine kinase essential for oocyte maturation. Mos is a component of cytostatic factor (CSF), that was originally described as a regulator of arrest at metaphase II. In *Xenopus*, Mos activates and/or stabilizes the MPF complex through a pathway that involves the MAP kinase cascade. This results in the resumption of meiosis, extrusion of the first polar body, suppression of DNA synthesis, and arrest in metaphase II. To determine the function of Mos in mice, *c-mos*-deficient mice were generated by gene targeting. MAP kinase failed to be activated in these mice. The female mice were subfertile; producing only a few litters consisting of 1 or 2 pups. Oocytes from $mos^{-/-}$ mice progress normally through folliculogenesis and meitotic maturation, but activated parthenogenetically due to a failure to arrest at metaphase II (134,135). During the transition between the two meiotic metaphases, the microtubules and chromosomes evolve towards an interphase-like state in $mos^{-/-}$ oocytes, while in wild type oocytes they remained in an M-phase configuration (136). Thus Mos appears to be required for chromatin organization and arrest at metaphase II in the mouse though it is necessary throughout maturation in *Xenopus.*

Mos is a MAPKKK and thus is predicted to activate MAP kinase through an intermediate kinase. However, microinjected constitutively active forms of the MAPKKs, MEK1 and Raf, failed to activate MAP kinase in $mos^{-/-}$ oocytes (137). In contrast, MAP kinase could be activated by the phosphatase inhibitor okadaic acid. This suggests that MAP kinase is normally inactivated by phosphatases and that one function of Mos is to inhibit protein phosphatases. Activated MEK1 was able to induce the phosphorylation of a MAP kinase mutant that was resistant to dephosphorylation. This has led to dual paths for Mos during the activation of MAP kinase, inducing phosphorylation and inhibiting phosphatases.

6.3. Prostaglandin signaling pathway

It is known that some functions of gonadotropins are mediated by prostaglandins, these compounds modulate the actions of hormones and can have profound effects on cellular functions. Prostaglandins are synthesized from arachidonic acid; the rate limiting step in prostaglandin synthesis is mediated by the enzyme cyclooxygenase (cox). There are two isoforms of this enzyme (cox-1 and -2), encoded by separate genes (138,139). Cox-1 is expressed in cells constitutively, including cells of ovarian follicles, however, expression of cox-2 is induced in follicles by gonadotropins (140,141). *Cox-1* and *cox-2* deficient mice have been generated (142-144). *Cox-1* null mice are fertile, whereas $cox\text{-}2^{-/-}$ mutant female mice have multiple reproductive defects (145). The ovarian phenotype of these mice includes a defective ovulatory response to gonadotropins and a lack of proper oocyte maturation. The latter conclusion was based on the observation that the few eggs released by these mutants were not fertilizable and did not extrude a first polar body (145). Interestingly, mice deficient for the prostaglandin E receptor, EP2, exhibit reduced fertility that also was attributed to a fertilization failure. It was noted that the $EP2^{-/-}$ follicles did not undergo cumulus expansion, and this was concluded to directly lead to the fertilization defect (146). Interestingly, GDF-9 has been shown to induce signaling through the EP2 receptor, and to directly regulate the expression of *cox-2* (147). These studies further highlight the importance of reciprocal signaling in the eventual release of a fertilizable oocyte.

7. PERSPECTIVE

Fertility in mammals depends upon a tightly coordinated sequence of events that lead to the growth, maturation, and ovulation of the oocyte. These events are regulated by a complex autocrine/paracrine signaling network that includes the brain, ovary, oocyte, and granulosa and theca cells. *In vitro* approaches have been successfully used to examine the role of individual genes during oocyte development. However, the interpretation of these experiments are compromised by the removal of the oocyte or follicle from the entire signaling mileau. Genetic manipulations of the mouse, including targeted null mutations and transgenics, have provided complementary approaches to examine the same gene within the *in vivo* signaling context. This review has concentrated on the advances in our understanding of the development of the oocyte based on genetic mutations in mice.

Embedded in the developmental history of each oocyte are several checkpoints integral to the reproductive success of the animal. Most notably are the cell cycle checkpoints that follow the switch from mitosis to meiosis, that maintain the oocyte in prophase I (prior to maturation), and anaphase II (prior to activation). Other checkpoints include, the initiation of primordial germ cell migration, and the initiation of primordial follicle growth. Though much is known about the genes that regulate the onset of oocyte maturation and activation, little is known about the molecular triggers for earlier checkpoints. Both the loss, or permanent block, of these checkpoints are likely to result in subfertile females through a failure to produce fertilizable eggs or a premature depletion of the primordial follicle pool. The past decade has seen a dramatic increase in the identification of genes involved in regulating follicle and oocyte development. These genes can now be used to design approaches to identify upstream regulators that are proximate to these important developmental checkpoints.

8. REFERENCE

1. Anderson, R., R. Fassler, E. Georges-Labouesse, R.O. Hynes, B.L. Bader, J.A Kreidberg, K. Schaible, J. Heasman, & C. Wylie: Mouse primordial germ cells lacking beta1 integrins enter the germline but fail to migrate normally to the gonads. *Development* 126, 1655-1664 (1999)

2. Copp, A.J., H.M. Roberts, & P.E. Polani: Chimaerism of primordial germ cells in the early postimplantation mouse embryo following microsurgical grafting of posterior primitive streak cells *in vitro*. *J Embryol Exp Morphol* 95, 95-115 (1986)

3. Ginsburg, M., M.H. Snow & A. McLaren: Primordial germ cells in the mouse embryo during gastrulation. *Development* 110, 521-528 (1990)

4. Anderson, R., T.K. Copeland, H. Scholer, J. Heasman, & C. Wylie: The onset of germ cell migration in the mouse embryo. *Mech Dev* 91, 61-68 (2000)

5. Clark, J.M. & E.M. Eddy: Fine structural observations on the origin and associations of primordial germ cells of the mouse. *Dev Biol* 47, 136-155 (1975)

6. Gomparts, M., M. Garcia-Castro, C. Wylie. & J. Heasman: Interactions between primordial germ cells play a role in their migration in mouse embryos. *Development* 120, 135-141 (1994)

7. Chun, S.Y. & A. J. Hsueh: Paracrine mechanisms of ovarian follicle apoptosis. *J Reprod Immunol* 39, 63-75 (1998)

8. Garcia-Castro, M.I., R. Anderson, J. Heasman, & C. Wylie: Interactions between germ cells and extracellular matrix glycoproteins during migration and gonad assembly in the mouse embryo. *J Cell Biol* 138, 471-480 (1997)

9. Bendel-Stenzel, M.R., M. Gomperts, R. Anderson, J. Heasman & C. Wylie: The role of cadherins during primordial germ cell migration and early gonad formation in the mouse. *Mech Dev* 91, 143-152 (2000)

10. Tam, P. & M. Snow: Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. *J Embryol Exp Morphol* 64, 133-147 (1981)

11. Bakken, A.H. & M. McClanahan: Patterns of RNA synthesis in early meiotic prophase oocytes from fetal mouse ovaries. *Chromosoma* 67, 21-40 (1978)

12. Reynaud, K. & M.A. Driancourt: Oocyte Attrition. *Mol Cell Endocrinol* 163, 101-108 (2000)

13. Morita, Y., T.F. Manganaro, X.J. Tao, S. Martimbeau, P.K. Donahoe & J.L. Tilly: Requirement for phosphatidylinositol-3'-kinase in cytokine-mediated germ cell survival during fetal oogenesis in the mouse. *Endocrinology* 140, 941-949 (1999a)

14. Matsui, Y., K.M. Zsebo & B.L. Hogan: Embryonic expression of a haematopoietic growth factor encoded by the *Sl* locus and the ligand for *c-kit*. *Nature* 347, 667-669 (1990)

15. Keshet, E., S.D. Lyman, D.E. Williams, D.M. Anderson, N.A. Jenkins, N.G. Copeland & L.F. Parada: Embryonic RNA expression patterns of the c-kit receptor and its cognate ligand suggest multiple functional roles in mouse development. *EMBO J* 10, 2425-2435 (1991)

16. Mintz, B.& E.S. Russell: Gene-induced embryological modification of primordial germ cells in the mouse. *J Exp Zool* 134, 207-230 (1957)

17. De Felici, M. & S. Dolci: Leukemia inhibitory factor sustains the survival of mouse primordial germ cells cultured on TM4 feeder layers. *Dev Biol* 147, 281-284 (1991)

18. Godin, I., R. Deed, J. Cooke, K. Zsebo, M. Dexter & C.C. Wylie: Effects of the steel gene product on mouse primordial germ cells in culture. *Nature* 352, 807-809 (1991)

19. Matsui, Y., D. Toksoz, S. Nishikawa, S. Nishikawa, D. Williams, K. Zsebo & B.L. Hogan: Effect of Steel factor and leukaemia inhibitory factor on murine primordial germ cells in culture. *Nature* 353, 750-752 (1991)

20. Dolci, S., M. Pesce & M. De Felici: Combined action of stem cell factor, leukemia inhibitory factor, and cAMP on *in vitro* proliferation of mouse primordial germ cells. *Mol Reprod Dev* 1993 35, 134-139 (1993)

21. Pesce, M., M.G. Farrace, M. Piacentini, S. Dolci & M. De Felici: Stem cell factor and leukemia inhibitory factor promote primordial germ cell survival by suppressing programmed cell death (apoptosis). *Development* 118, 1089-1094 (1993)

22. Matsui, Y., K.Zsebo & B.L. Hogan: Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. *Cell* 70, 841-847 (1992)

23. Kawase, E., H. Yamamoto, K. Hashimoto & N. Nakatsuji: Tumor necrosis factor-alpha (TNF-alpha) stimulates proliferation of mouse primordial germ cells in culture. *Dev Biol* 161, 91-95 (1994)

24. Van Dissel-Emiliani, F.M., M. De Boer-Brouwer & D.G. De Rooij: Effect of fibroblast growth factor-2 on Sertoli cells and gonocytes in coculture during the perinatal period. *Endocrinology* 137, 647-654 (1996)

25. Nikolova-Karakashian, M., E.T. Morgan, C. Alexander, D.C. Liotta & A.H. Merrill Jr: Bimodal regulation of ceramidase by interleukin-1beta. Implications for the regulation of cytochrome p450 2C11. *J Biol Chem* 272, 18718-8724 (1997)

26. Resnick, J.L., M. Ortiz, J.R. Keller & P.J. Donovan: Role of fibroblast growth factors and their receptors in mouse primordial germ cell growth. *Biol Reprod* 59, 1224-1229 (1998)

27. Guo, M.W., E. Mori, J.P. Xu & T. Mori: Identification of Fas antigen associated with apoptotic cell death in murine ovary. *Biochem Biophys Res Commun* 203, 1438-1446 (1994)

28, Olaso, R., C. Pairault, B. Boulogne, P. Durand & R. Habert: Transforming growth factor beta1 and beta2 reduce the number of gonocytes by increasing apoptosis. *Endocrinology* 139, 733-740 (1998)

29. Anderson, R., J. Heasman & C. Wylie: Early events in the mammalian germ line. *Int Rev Cytol* 203, 215-230 (2001)

30. Adams, J.M. & S. Cory: The Bcl-2 protein family: Arbiters of cell survival. *Science* 281, 1322-1326 (1998)

31. Korsmeyer, S.J.: BCL-2 family and the regulation of programmed cell death. *Cancer Res* 59, 1693s-1700s (1998)

32. Kennedy, S.G., A.J. Wagner, S.D. Conzen, J. Jordan, A. Bellacosa, P.N. Tsichlis & N. Hay: The PI 3-kinase/Akt signaling pathway delivers an anti-apoptotic signal. *Genes Dev* 11, 701-713 (1997)

33. Kulik, G., A. Klippel & M.J. Weber: Antiapoptotic signalling by the insulin-like growth factor I receptor, phosphatidylinositol 3-kinase, and Akt. *Mol Cell Biol* 117, 1595-1606 (1997)

34. Cardone, M.H., N. Roy, H.R. Stennicke, G.S. Salvesen, T.F. Franke, E. Stanbridge, S. Frisch & J.C. Reed: Regulation of cell death protease caspase-9 by phosphorylation. *Science* 282, 1318-1321 (1998)

35. Jurgensmeier, J.M., Z. Xie, Q. Deveraux, L. Ellerby, D. Bredesen & J.C. Reed: Bax directly induces release of cytochrome c from isolated mitochondria. *Proc Natl Acad Sci* U S A 95, 4997-5002 (1998)

36. Morita, Y., T.F. Manganaro, X.J. Tao, S. Martimbeau, P.K. Donahoe & J.L. Tilly: Requirement for phosphatidylinositol-3'-kinase in cytokine-mediated germ cell survival during fetal oogenesis in the mouse. *Endocrinology* 140, 941-949 (1999a)

37. Kluck, R.M., M.D. Esposti, G. Perkins, C. Renken, T. Kuwana, E. Bossy-Wetzel, M. Goldberg, T. Allen, M.J. Barber, D.R. Green & D.D. Newmeyer: The pro-apoptotic proteins, Bid and Bax, cause a limited permeabilization of the mitochondrial outer membrane that is enhanced by cytosol. *J Cell Biol* 147, 809-822 (1999)

38. Kluck, R.M., E. Bossy-Wetzel, D.R. Green & D.D. Newmeyer: The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 275, 1132-1136 (1997)

39. Yang, J., X. Liu, K. Bhalla, C.N. Kim, A.M. Ibrado, J. Cai, T.I. Peng, D.P. Jones & X. Wang: Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* 275, 1129-1132 (1997)

40. Ratts, V.S., J.A. Flaws, R. Kolp, C.M. Sorenson & J.L. Tilly: Ablation of *bcl-2* gene expression decreases the numbers of oocytes and primordial follicles established in the post-natal female mouse gonad. *Endocrinology* 136, 3665-3668 (1995)

41. Morita, Y., G.I. Perez, D.V. Maravei, K.I. Tilly & J.L. Tilly: Targeted expression of *Bcl-2* in mouse oocytes inhibits ovarian follicle atresia and prevents spontaneous and chemotherapy-induced oocyte apoptosis *in vitro*. *Mol Endocrinol* 13, 841-50 (1999b).

42. Knudson, C.M., K.S. Tung, W.G. Tourtellotte, G.A. Brown & S.J. Korsmeyer: *Bax*-deficient mice with lymphoid hyperplasia and male germ cell death. *Science* 270, 96-99 (1995)

43. Perez, G.I., C.M. Knudson, L. Leykin, S.J. Korsmeyer & J.L. Tilly: Apoptosis-associated signaling pathways are required for chemotherapy-mediated female germ cell destruction. *Nat Med* 3, 1228-1232 (1997)

44. Boder, E: Ataxia-telangiectasia: some historic, clinical and pathologic observations. *Birth Defects* 11, 255-270 (1975)

45. Barlow, C., S. Hirotsune, R. Paylor, M. Liyanage, M. Eckhaus, F. Collins, Y. Shiloh, J.N. Crawley, T. Ried, D.Tagle & A. Wynshaw-Boris: Atm-deficient mice: a paradigm of ataxia telangiectasia. *Cell* 85, 159-171 (1996)

46. Hoekstra, M.F.: Responses to DNA damage and regulation of cell cycle checkpoints by the ATM protein kinase family. *Curr Opin Genet Dev* 7, 170-175 (1997)

47. Barlow, C., M. Liyanage, P.B. Moens, M. Tarsounas, K. Nagashima, K. Brown, S. Rottinghaus, S.P. Jackson, D. Tagle, T. Ried & A. Wynshaw-Boris: *Atm* deficiency results in severe meiotic disruption as early as leptonema of prophase I. *Development* 125, 4007-4017 (1998)

48. Pandita, T.K., C.H. Westphal, M. Anger, S.G. Sawant, C.R. Geard, R.K. Pandita & H. Scherthan: *Atm* inactivation results in aberrant telomere clustering during meiotic prophase. *Mol Cell Biol* 19, 5096-5105 (1999)

49. Barlow, C., M. Liyanage, P.B. Moens, C.X. Deng, T. Ried & A. Wynshaw-Boris: Partial rescue of the prophase I defects of *Atm*-deficient mice by p53 and p21 null alleles. *Nat Genet* 17, 462-466(1997)

50. Ma, K., J.D. Inglis, A. Sharkey, W.A. Bickmore, R.E. Hill, R,J. Prosser, R.M. Speed, E.J. Thomson, M. Jobling, K. Taylor, J. Wolfe, H.J. Cooke, T.B. Hargreave & A.C. Chandley: A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell* 75, 1287-1295 (1993)

51. Reijo, R., T.Y. Lee, P. Salo, R. Alagappan, L.G. Brown, M. Rosenberg, S. Rozen, T. Jaffe, D. Straus, O. Hovatta, A. de la Chapelle, S. Silber & D.C. Page: Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nat Genet* 10, 383-393 (1995)

52. Ruggiu, M., R. Speed, M. Taggart, S.J. McKay, F. Kilanowski, P. Saunders, J. Dorin & H.J. Cooke: The mouse *Dazla* gene encodes a cytoplasmic protein essential for gametogenesis. *Nature* 389, 73-77 (1997)

53. McNeilly, J.R., P.T. Saunders, M. Taggart, M. Cranfield, H.J. Cooke & A.S. McNeilly: Loss of oocytes in *Dazl* knockout mice results in maintained ovarian steroidogenic function but altered gonadotropin secretion in adult animals. *Endocrinology* 141, 4284-4294 (2000)

54. Ashworth, A., S. Rastan, R. Lovell-Badge & G. Kay: X-chromosome inactivation may explain the difference in viability of XO humans and mice. *Nature* 351, 406-408 (1991)

55. Hawkins, J.R., P. Koopman & P. Berta:Testis-determining factor and Y-linked sex reversal. *Curr Opin Genet Dev* 1, 30-33 (1991)

56. Mardon, G., S.W. Luoh, E.M. Simpson, G. Gill, L.G. Brown & D.C. Page: Mouse Zfx protein is similar to Zfy-2: each contains an acidic activating domain and 13 zinc fingers. *Mol Cell Biol* 10, 681-688 (1990)

57. Luoh, S.W., P.A. Bain, R.D. Polakiewicz, M.L. Goodheart, H. Gardner, R. Jaenisch & D.C. Page: *Zfx* mutation results in small animal size and reduced germ cell number in male and female mice. *Development* 124, 2275-2284 (1997)

58. Kuroda, H., N. Terada, H. Nakayama, K. Matsumoto & Y. Kitamura: Infertility due to growth arrest of ovarian follicles in *Sl/Slt* mice. *Dev Biol* 126, 71-9 (1988)

59. Huang, E.J., K.H. Nocka, J. Buck & P. Besmer: Differential expression and processing of two cell associated forms of the kit-ligand: KL-1 and KL-2. *Mol Biol Cell* 3, 349-362 (1992)

60. Manova, K., K. Nocka, P. Besmer & R.F. Bachvarova: Gonadal expression of c-kit encoded at the *W* locus of the mouse. *Development* 110, 1057-1069 (1990)

61. Horie, K., K. Takakura, S. Taii, K. Narimoto, Y. Noda, S. Nishikawa, H. Nakayama, J. Fujita & T. Mori: The expression of c-kit protein during oogenesis and early embryonic development. *Biol Reprod* 45, 547-552 (1991)

62. Manova, K., E.J. Huang, M. Angeles, V. De Leon, S. Sanchez, S.M. Pronovost, P. Besmer & R.F. Bachvarova: The expression pattern of the c-kit ligand in gonads of mice supports a role for the c-kit receptor in oocyte growth and in proliferation of spermatogonia. *Dev Biol* 57, 85-99 (1993)

63. Joyce, I.M., F.L. Pendola, K. Wigglesworth & J.J. Eppig: Oocyte regulation of kit ligand expression in mouse ovarian follicles. *Dev Biol* 214, 342-53 (1999)

64. Elvin, J.A., C. Yan, P. Wang, K. Nishimori & M.M. Matzuk: Molecular characterization of the follicle defects in the *growth differentiation factor 9*-deficient ovary. *Mol Endocrinol* 13, 1018-1034 (1999)

65. Terada, N., T. Yamane, K. Matsumoto, H. Asai & Y. Kitamura: Estrogen-induced increase in eosinophil number and peroxidase activity in uterus of genetically mast cell-deficient W/*Wv* mice. *Biol Reprod* 33, 899-901 (1995)

66. Yoshida, H., N. Takakura, H. Kataoka, T. Kunisada, H. Okamura & S.I. Nishikawa: Stepwise requirement of c-kit tyrosine kinase in mouse ovarian follicle development. *Dev Biol* 184, 122-137 (1997)

67. Parrott, J.A. & M.K. Skinner: Kit-ligand/stem cell factor induces primordial follicle development and initiates folliculogenesis. *Endocrinology* 140, 4262-4271 (1999)

68. Ismail, R.S., Y. Okawara, J.N. Fryer & B.C. Vanderhyden: Hormonal regulation of the ligand for c-kit in the rat ovary and its effects on spontaneous oocyte meiotic maturation. *Mol Reprod Dev* 43, 458-469 (1996)

69. Ismail, R.S., M. Dube & B.C. Vanderhyden: Hormonally regulated expression and alternative splicing of kit ligand may regulate kit-induced inhibition of meiosis in rate oocytes. *Dev Biol* 184, 333-342 (1997)

70. Parrott, J.A. & M.K. Skinner: Direct actions of kit-ligand on theca cell growth and differentiation during follicle development. *Endocrinology* 138, 3819-3827 (1997)

71. Parrott, J.A. & M.K. Skinner: Thecal cell-granulosa cell interactions involve a positive feedback loop among keratinocyte growth factor, hepatocyte growth factor, and Kit ligand during ovarian follicular development. *Endocrinology* 139, 2240-2245 (1998)

72. Reynaud K., R. Crotvrindt, J. Smitz & M.A. Driancourt: Effects of kit ligand and anti-kit antibody on growth of cultured mouse preantral follicles. *Mol Reprod Devel* 55, 1-12 (2000)

73. Dube, J.L., P. Wang, J. Elvin, K.M. Lyons, A.J. Celeste & M.M. Matzuk: The *bone morphogenetic protein 15* gene is X-linked and expressed in oocytes. *Mol Endocrinol* 12, 1809-1817 (1998)

74. Elvin, J. A., A.T. Clark, P. Wang, N.M. Wolfman & M.M. Matzuk: Paracrine actions of growth factor-9 in the mammalian ovary. *Mol Endocrinol* 13, 1035-1048 (1999)

75. Dong, J. D., F. Albertini, K. Nishimori, T.R. Kumar, N. Lu & M.M. Matzuk: Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 383, 531-535 (1996)

76. Carabatsos, M. J., J. Elvin, M. M. Matzuk & D.F. Albertini: Characterization of oocyte and follicle development *in growth differentiation factor-9*-deficient mice. *Dev Biol* 204, 373-384 (1998)

77. Laitinen, M., K. Vuojolainen, R. Jaatinen, I. Ketola, J. Aaltonen, E. Lehtonen, M.Heikinheimo & O. Ritvos: A novel *growth differentiation factor-9* (GDF-9) related factor is co-expressed with GDF-9 in mouse oocytes during folliculogenesis. *Mech Dev* 78, 135-140 (1998)

78. Otsuka, F., Z. Yao, T. Lee, S. Yamamoto, G.F. Erickson & S. Shimasaki: Bone morphogenetic protein-15. Identification of target cells and biological functions. *J Biol Chem* 275, 39523-39528 (2000)

79. Otsuka, F., S. Yamamoto, G.F. Erickson & S. Shimasaki: Bone morphogenetic protein-15 inhibits follicle stimulating hormone (fsh) action by suppressing fsh receptor expression. *J Biol Chem* 276, 11387-11392 (2001)

80. Galloway, S.M., K.P. McNatty, L.M. Cambridge, M.P. Laitinen, J.L. Juengel, T.S. Jokiranta, R.J. McLaren, K. Luiro, K.G. Dodds, G.W. Montgomery, A.E. Beattie, G.H. Davis & O. Ritvos: Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nat Genet* 25, 279-283 (2000)

81. Rankin, T., M. Familari, E. Lee, A. Ginsberg, N. Dwyer, J. Blanchette-Mackie, J., Drago, H. Westphal & J. Dean: Mice homozygous for an insertional mutation in the *Zp3* gene lack a zona pellucida and are infertile. *Development* 122, 2903 2910 (1996)

82. Rankin, T.L., M. O'Brien, E. Lee, K. Wigglesworth, J. Eppig & J. Dean: Defective zonae pellucidae in *Zp2*-null mice disrupt folliculogenesis, fertility and development. *Development* 128, 1119-1126 (2001)

83. Bleil, J.D., J.M. Greve & P.M. Wassarman: Identification of a secondary sperm receptor in the mouse egg zona pellucida: role in maintenance of binding of acrosome-reacted sperm to eggs. *Dev Biol* 128, 376-385 (1988)

84. Bleil, J.D., C.F. Beall & P.M. Wassarman: Mammalian sperm-egg interaction: fertilization of mouse eggs triggers modification of the major zona pellucida glycoprotein, ZP2. *Dev Biol* 86, 189-197 (1981)

85. Miller, D.J., X. Gong, G. Decker & B.D. Shur: Egg cortical granule N-acetyl- glucosaminidase is required for the mouse zona block to polyspermy. *J Cell Biol* 123, 1431-1440 (1993)

86. Bronson, R.A. & A. McLaren: Transfer to the mouse oviduct of eggs with and without the zona pellucida. *J Reprod Fertil* 22, 129-137 (1970)

87. Modlinski, J.A.: The role of the zona pellucida in the development of mouse eggs *in vivo*. *J Embryol Exp Morphol* 23, 539-547 (1970)

88. Bleil, J.D. & P.M.Wassarman: Structure and function of the zona pellucida: identification and characterization of the proteins of the mouse oocyte's zona pellucida. *Dev Biol* 76, 185-202 (1980

89. Shimizu, S., M. Tsuji & J. Dean: *In vitro* biosynthesis of three sulfated glycoproteins of murine zonae pellucidae by oocytes grown in follicle culture. *J Biol Chem* 258, 5858-5863 (1983)

90. Odor, D.L. & R.J. Blandau: Ultrastructural studies on fetal and early postnatal mouse ovaries. I.Histogenesis and organogenesis. *Am J Anat* 124, 163-186 (1969)

91. Dietl, J.: Ultrastructural aspects of the developing mammalian zona pellucida. In:The Mammalian Egg Coat. Ed: Dietl J., Springer-Verlag, Berlin 49-60 (1989)

92. Eppig, J.J.: Intercommunication between mammalian oocytes and companion somatic cells. *Bioessays* 13, 569-574 (1991)

93. Liang, L., S.M. Soyal & J. Dean: *FIG alpha*, a germ cell specific transcription factor involved in the coordinate

expression of the zona pellucida genes. *Development* 124, 4939-4947 (1997)

94. Soyal, S.M., A. Amleh & J. Dean: *FIG alpha*, a germ cell-specific transcription factor required for ovarian follicle formation. *Development* 127, 4645-4654 (2000)

95. Rankin, T., P. Talbot, E. Lee & J. Dean: Abnormal zonae pellucidae in mice lacking *ZP1* result in early embryonic loss. *Development* 126, 3847-3855 (1999)

96. Kumar, N.M.: Molecular biology of the interactions between connexins. *Novartis Found Symp* 219, 6-16 (1999)

97. Biggers, J.D., D.G. Whittingham & R.P. Donahue: The pattern of energy metabolism in the mouse oocyte and zygote. *Proc Natl Acad Sci U S A* 58, 560-567 (1967)

98. Eppig, J.J., F. Chesnel, Y. Hirao, M.J. O'Brien, F.L. Pendola, S. Watanabe & K. Wigglesworth: Oocyte control of granulosa cell development: how and why. *Hum Reprod* 12, 127-132 (1997)

99. Grazul-Bilska, A.T., L.P. Reynolds & D.A. Redmer: Gap junctions in the ovaries. *Biol Reprod* 57, 947-957 (1997)

100. Dekel, N.: Spatial relationship of follicular cells in the control of meiosis. *Prog Clin Biol Res* 267, 87-101 (1988)

101. Downs, S.M., S.A. Daniel, E.A. Bornslaeger, P.C. Hoppe & J.J. Eppig: Maintenance of meiotic arrest in mouse oocytes by purines: modulation of cAMP levels and cAMP phosphodiesterase activity. *Gamete Res* 23, 323-334 (1989)

102. Eppig, J.J.: The participation of cyclic adenosine monophosphate (cAMP) in the regulation of meiotic maturation of oocytes in the laboratory mouse. *J Reprod Fertil Suppl* 38, 3-8 (1989)

103. Wright, C.S., D.L. Becker, J.S. Lin, A.E. Warner & K. Hardy: Stage-specific and differential expression of gap junctions in the mouse ovary: connexin-specific roles in follicular regulation. *Reproduction* 121, 77-88 (2001)

104. Juneja, S.C., K.J. Barr, G.C. Enders & G.M. Kidder: Defects in the germ line and gonads of mice lacking *connexin 43*. *Biol Reprod* 60, 1263-1270 (1999)

105. Simon, A.M., D.A. Goodenough, E. Li & D.L. Paul: Female infertility in mice lacking *connexin 37*. *Nature* 385, 525 529 (1997)

106. Carabatsos, M.J., C. Sellitto, D.A. Goodenough & D.F. Albertini: Oocyte-granulosa cell heterologous gap junctions are required for the coordination of nuclear and cytoplasmic meiotic competence. *Dev Biol* 226, 167-179 (2000)

107. Motlik, J. & M., Kubelka: Cell-cycle aspects of growth and maturation of mammalian oocytes. *Mol Reprod Dev* 27, 366-375 (1990)

108. Albertini, D.F.: Cytoplasmic reorganization during the resumption of meiosis in cultured preovulatory rat oocytes. *Dev Biol* 120, 121-131 (1987)

109. Abrieu, A., M. Doree & D. Fisher: The interplay between cyclin-B-Cdc2 kinase (MPF) and MAP kinase during maturation of oocytes. *J Cell Sci* 114, 257-267 (2001)

110. Gautier, J., J. Minshull, M. Lohka, M. Glotzer, T. Hunt & J. L. Maller: Cyclin is a component of maturation promoting factor from *Xenopus*. *Cell* 60, 487-494 (1990)

111. Draetta, G., F. Luca, J. Westendorf, L. Brizuela, J. Ruderman & D. Beach: Cdc2 protein kinase is complexed with both cyclin A and cyclin B: Evidence for proteolytic inactivation of MPF. *Cell* 56, 829-838 (1989)

112. Labbe, J. C., A.Picard, G. Peaucelier, J.C. Cavadore, P. Nurse & M. Doree: Purification of MPF from starfish: Identification as the H1 histone kinase p34cdc2 and a possible mechanism for its periodic activation. *Cell* 57, 253-263 (1989)

113. Mejier, L., D. Arion, R. Golsteyn, J. Pines, L. Brizuela, T. Hunt & D. Beach: Cyclin is a component of the sea urchin egg M-phase specific histone H1 kinase. *EMBO J* 8, 2275-2282 (1989)

114. Hashimoto, N. & T.Kishimoto: Regulation of meiotic metaphase by a cytoplasmic maturation-promoting factor during mouse oocyte maturation. *Dev Biol* 126, 242-252 (1988)

115. Choi, T., F. Aoki, M. Mori, M. Yamashita, Y. Nagahama & K. Kohmoto: Activation of p34cdc2 protein kinase activity in meiotic and mitotic cell cycles in mouse oocytes and embryos. *Development* 113, 789-795 (1991)

116. Murray, A. W. & M. W. Kirschner: Cyclin synthesis drives the early embryonic cell cycle. *Nature* 339, 275-280 (1989)

117. Evans, T., E.T. Rosenthal, J. Youngblom, D. Distel & T. Hunt: Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* 33, 3893-96 (1983)

118. Hampl, A. & J. J. Eppig: Analysis of the mechanism(s) of metaphase 1 arrest in maturing mouse oocytes. *Development* 121, 925-933 (1995a)

119. Winston, N: Stability of cyclin B protein during miotic maturation and the first meiotic cell cycle division in mouse oocyte. *Biol Cell* 89, 211-219 (1997)

120. Chesnel, F. & J.J. Eppig: Synthesis and accumulation of p34cdc2 and cyclin B in mouse oocytes during acquisition of competence to resume meiosis. *Mol Reprod Dev* 40, 503-508 (1995)

121. Mitra, J. & R.M. Schultz: Regulation of the acquisition of meiotic competence in the mouse: changes in the subcellular localization of cdc2, cyclin B1, cdc25C and wee1, and in the concentration of these proteins and their transcripts. *J Cell Sci* 109, 2407-2415 (1996)

122. de Vantery Arrighi, C., A. Campana, & S. Schorderet-Slatkine: A role for the MEK-MAPK pathway in okadaic acid induced meiotic resumption of incompetent growing mouse oocytes. *Biol Reprod* 63, 658-665 (2000)

123. Tay, J., R. Hodgman & J. D. Richter: The control of cyclin B1 mRNA translation during mouse oocyte maturation. *Dev Biol* 221, 1-9 (2000)

124. Brandeis, M., I. Rosewell, M. Carrington, T. Crompton, M.A. Jacobs, J. Kirk, J. Gannon &T. Hunt: Cyclin B2-null mice develop normally and are fertile whereas *cyclin B1*-null mice die *in utero*. *Proc. Natl. Acad. Sci USA* 95, 4344-4349 (1998)

125. Ledan, E., Z. Polanski, M-E. Terret & B. Maro: Meiotic maturation of the mouse oocyte requires an equilibrium between cyclin B synthesis and degradation. *Dev Biol* 232, 400-413 (2001)

126. Nebreda, A. R. & T. Hunt: The *c-mos* proto-oncogene protein kinase turns on and maintains the activity of MAP kinase, but not MPF, in cell-free extract of *Xenopus* oocytes and eggs. *EMBO J* 12, 1979-1986 (1993)

127. Posada, J., N., Yew, N.G. Ahn, G.F. Vande Woude & J. Cooper: Mos stimulates MAP kinase in Xenopus oocytes and activates a MAP kinase kinase *in vitro*. *Mol Cell Biol* 13, 2546-2553 (1993)

128. Shibuya, E. K. & J. V.Ruderman: Mos induces the *in vitro* activation of mitogen-activated protein kinases in lysates of frog oocytes and mammalian somatic cells. *Mol Biol Cell* 4, 781-790 (1993)

129. Sagata, N., I. Daar, M. Oskarsson, S.D. Showalter & G.F.Vande Woude: The product of the mos proto-oncogene as a candidate initiator for oocyte maturation. *Science* 245, 643-646 (1989a)

130. Carnero, A., B. Jimenz & J. C. Lacal: Progesterone but not ras requires MPF for *in vivo* activation of MAPK and S6KII: MAPK is an essential connexion point of both signaling pathways. *J Cell Biochem* 55, 465-476 (1994)

131. Kosako, H., Y. Gotoh & E.Nishida: Mitogen-activated protein kinase kinase is required for the mos-induced metaphase arrest. *J Biol Chem* 269, 28354-28358 (1994a)

132. Haccard, O., B. Sarcevic, A. Lewellyn, R. Hartley, L. Roy, T. Izumi, E. Erikson & J. L. Maller: Induction of metaphase arrest in cleaving *Xenopus* embryos by MAP kinase. *Science* 262, 1262-1265 (1993)

133. Haccard, O., A. Lewellyn, R. S. Hartley, E. Erikson & J. L.Maller: Induction of Xenopus Oocyte meiotic maturation by MAP kinase. *Dev Biol* 168, 677-682. (1995)

134. Hashimoto, N., N. Watanabe, Y. Furuta, H. Tamemoto, N. Sagata, M. Yokoyama, K. Okazaki, M. Nagayoshi, N. Takeda, Y. Ikawa & S. Aizawa: Pathenogenetic activation of oocytes in *c-mos*-deficient mice. *Nature* 370, 68-71 (1994)

135. Colledge, W.H., M.B.L. Carlton. G.B. Udy & M.J. Evans: Disruption of *c-mos* causes parthenogenetic development of unfertilized mouse eggs. *Nature* 370, 65-68 (1994)

136. Verlhac, M-H., J. Z. Kubiak, M. Weber, G. Geraud, W.H. Colledge, M.J. Evans & B. Maro: Mos is required for MAP kinase activation and is involved in microtubule organization during meiotic maturation in the mouse. *Development* 122, 815-822 (1996)

137. Verlhac, M.H., C. Lefebvre, J.Z. Kubiak, M. Umbhauer, P. Rassinier, W. Colledge & B. Maro: Mos activates MAP kinase in mouse oocytes through two opposite pathways. *EMBO J* 19, 6065 6074 (2000)

138. Morita, I., M. Schindler, M. K. Regier, J.H. Otto, T. Hori, D. L. Dewitt & W. L. Smith: Different intracellular locations for prostaglandin endoperoxide H synthase-1 and-2. *J Biol Chem* 270, 10902-10908 (1995)

139. Smith, W. L. & D. L. DeWitt: Prostaglandin endoperoxide H synthase-1 and -2. *Adv Immunol* 62, 167-214 (1996)

140. Sirios, J., D.L. Simmons & J. S. Richards: Hormonal regulation of messenger ribonucleic acid encoding a novel isoform of prostaglandin endoperoxide H synthase in rat preovulatory follicles. *J Biol Chem* 267, 11586-11592 (1992)

141. Sirois, J: Induction of prostaglindin endoperoxide H synthase-2 by human chorionic gonadotropin in bovine preovulatory follicles *in vivo*. *Endocrinology* 135, 841-848 (1995)

142. Dinchuk, J. E., B. D. Car, R. J. Focht, J. J. Johnston, B. D. Jaffee, M. B. Covington, N R. Contel, V. M. Eng, R. J Collins & P.M. Czerniak *et al*.: Renal abnormalities and altered inflammatory response in mice lacking *cyclooxygenase II*. *Nature* 378, 406-409 (1995)

143. Langenbach, R., S.G. Morham, H.F. Tiano, C.D. Loftin, B.I. Ghanayem, P.C. Chulada, J.F. Mahler, C.A. Lee, E. H. Goulding & Kluckman, K. D. *et al.: Prostaglandin synthase 1* gene disruption in mice reduces arachidonic acid induced inflammation and indomethacin-induced gastric ulceration. *Cell* 83, 483-492 (1995)

144. Morham, S. G., R. Langenbach, C.D. Loftin, H.F. Tiano, N. Vouloumanos, J. C. Jennette, J.F. Mahler, K.D. Kluckman, A. Ledford, C.A. Lee & O. Smithies: *Prostaglandin sythase 2* gene disruption causes severe renal pathology in the mouse. *Cell* 83, 473-482 (1995)

145. Lim, H., B.C. Paria, S.K. Das, J.E. Dinchuk, R. Langenbach, J.M. Trzaskos & S. K. Dey: Multiple female

reproductive failures in *cyclooxygenase 2*-deficient mice. *Cell* 91, 197-208 (1997)

146. Hizaki, H., E. Sefi, Y. Sugimoto, M. Hirose, T. Saji, F. Ushikubi, T. Matsuoka, Y. Noda, T. Tanaka, N. Yoshida, S. Narumiya & A. Ichikawa: Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP2. *Proc Natl Acad Sci USA* 96, 10501-10506 (1999)

147. Elvin, J. A., C. Yan & M. M. Matzuk: Growth differentiation factor-9 stimulates progesterone synthesis in granulosa cells via a prostaglandin E2/EP2 receptor pathway. *Proc Natl Acad Sci USA* 97, 10288-10293 (2000)

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