Meiotic Mechanism During Gamete Maturation

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1. Introduction

Gametogenesis in both the male and female mammal represents a specialized and highly regulated series of cell cycle events, involving both mitosis and meiosis as well as subsequent differentiation. Recent advances in our understanding of the genetic control of the eukaryotic cell cycle have underscored the evolutionarily-conserved nature of these regulatory processes. However, most of the data have been obtained from yeast model systems and mammalian cell lines. Furthermore, most of the observations focus on regulation of mitotic cell cycles.

Meiosis is a specialized kind of cell cycle that reduces the chromosome number by half, resulting in the production of haploid daughter cells. In mammals, the germ cells have to undergo meiosis to produce haploid gametes, sperm and egg, and a new progeny is then developed by the fusion of these gametes at fertilization. This reduction in chromosome number is accomplished by two sequential rounds of nuclear and cell divisions after a single round of DNA replication.

In the last decade, there has been an imposing improvement in understanding the mechanisms underlying the complex process of meiosis during male and female germ cell differentiation. However, it is largely unknown how extrinsic cues from the endocrine system and surrounding somatic cells interact with intrinsic mechanisms of germ cells especially to activate signal transduction processes regulating transcription during mammalian meiosis (Eddy and O'Brien, 1998). Until recently, few meiotic protein participants had been identified and characterized in the gamete itself as well as neighboring helper cells, Sertoli cells in male and granulosa cells in female, but several recent scientific efforts have changed this situation. In addition, cytoskeletal changes with meiosis have been partially understood only in female gametes (Verlhac *et al.*, 1994; Kim *et al.*, 1996). Thus, it is the purpose of this manuscript to present the general meiotic mechanism during gamete maturation in mammals with the recent exciting findings in a given field.

2. General Aspects of Meiosis in Mammal

The overall process of meiosis consists of two sequential divisions, called meiosis I and II. DNA replication occurs before meiosis I, so that each chromosome consists of two sister chromatids (bivalent) and shows homologous pairing at the beginning of meiosis I (prophase I). This homologous chromosome pairing is not only a central event for meiotic chromosome segregation, but also allows recombination for the exchange between the paternal and maternal chromosomes.

The mechanism of homologous chromosome pairing is to be basically mediated by base pairing between complementary DNA strands and by several meiosis-specific proteins. During this process, a three-layered structure, called synaptomenal complex, is fully formed along the entire length of each pair of homologous chromosomes, which consists of four chromatids (tetrad). The chromosomes of each tetrad are attached to one or more sites of crossing over (chiasmata) to exchange of DNA between homologs, resulting in additional genetic diversity in the gametes. In female mammal, meiosis may actually arrest at this stage (prophase I) for extended times up to five decades.

During the next three phases, the nuclear membrane disappears and the bivalents move from one to another but they soon line up across the spindle equator (metaphase I). At stages of anaphase and telophase, the homologous chromosomes then separate into two groups and cytoplasm as well, resulting in producing two daughter cells which have one set of the bivalents. In these daughter cells, immediately after mitosis I, meiosis II occurs without any more DNA replication. Meiosis II resembles a normal mitosis except the separation of sister chromatids which chromosome of the daughter cell derives from a single chromatid.

3. Meiotic Mechanism in Male Gametes

As a renewable epithelium, the seminiferous epithelium of the testis supports replacement of somatic and germ cell constituents, the latter in plentiful supply to ensure male germ cell availability at any point in a reproductive cycle. Therefore, male germ cells initiate meiosis during prepubertal periods but sustain a continuous process of prophase entry throughout the reproductive life span. Transition of type B spermatogonia into the prophase of meiosis is represented by the conversion of those cells into primary spermatocytes which divide to form secondary spermatocytes. The latter, after a very short lifespan of $\sim$6 h in the human, divide to form round spermatids. These two divisions result in the conversion of the diploid to the haploid chromosomal compliment.

3-1. Degeneration of male germ cells during meiosis

During these processes, the germ cell loss occurred up to 39% in man (Johnson *et al.*, 1992), and 20% in bull (Berndtson and Desjardins, 1974). It presumed that this cell loss represents aberrant cells, especially chromosome abnormality, from processing further through spermatogenesis (Roosen-Runge, 1973). However, it is not known what causes or controls the loss of meiotic spermatocytes.

3-2. Regulation of meiosis in male germ cells

The cellular mechanisms of meiosis in male may be characterized and regulated by hormonal influences (gonadotropins, growth factors, steroid hormones) systemically, and Sertoli cells locally (review by Handel and Eppig, 1998). Recent our study (Kim *et al.*, 1996) demonstrated to establish the in vitro short-term culture system of developing male germ cells from pachytene spermatocytes to round spermatids. We found that FSH, insulin, transferrin and vitamin A were not involved directly in pachytene survival and differentiation. However, when pachytene cells were co-cultured with somatic cells (embryonic fibroblasts, testicular somatic cells), these cell

were attached the feeder layer and the percentage of viable cells was highly increased up to 65%, especially in testicular cells. Thus these results suggest indirectly that the specific environment under interactions of germ cell and Sertoli cells may need to meiosis of male germ cell. Unfortunately, our understanding the biochemical and molecular mechanisms to regulate meiosis is very limited.

Some evidences have been recently reported that the molecules involved and highlighted key steps in male meiosis. Heat-shock protein 70-2 (HSP-70-2), a unique testicular member of the HSP-70 group of proteins, was essential for the completion of meiosis and was highly expressed in pachytene spermatocytes (Dix *et al.*, 1996, 1997). In HSP-70-2 gene targeted animals, the germ cells did not complete meiosis and there was a marked increase in spermatocyte apoptosis. Other data suggest that HSP-70-2 is a important component of the synaptonemal complex, which is a crucial element in the pairing of homologous chromosomes during the prophase of meiosis (Allen *et al.*, 1996). Furthermore, recent studies are providing data that there are other meiosis specific proteins expressed in male germ cells (Table 1) as well as testicular somatic cells (Naz

Table 1. Meiotic specific proteins and transcripts during mammalian spermatogenesis

Protein	Mol. size	Expression stage	Species	Action	References
SP17	17 kD	Spermatocyte to spermatozoa	Human Rabbit Mouse	Sperm-egg interaction	Kong- (1995), Richardson- (1994)
FA-1	23 kD	2nd spermatocyte to spermatozoa	Mouse	Sperm-egg interaction	Naz- (1997)
Fertilin-β	85 kD	Pachytene spermatocyte to elongating spermatid	Guinea pig Human	Sperm-egg interaction	Burkin- (1997) Carroll- (1995)
H1 H2a H2b	207aa ? 126aa	Pachytene spermatocyte	Mouse Rat Human	Formation of chromatid	Drabent- (1996) Unni- (1995)
CREMt	?	Pachytene spermatocyte to spermatid	Mouse	Regulation of transcription	Blendy- (1996) Nantlel- (1996)
Zfp-35	24 Kb	Pachytene spermatocyte	Mouse	Regulation of transcription	Cunliffe- (1990)
HSP-70-2	70 kD	Pachytene spermatocyte	Mouse Rat	Regulation of transcription	Matsumoto- (1990)
Meg1	?	Pachytene spermatocyte	Mouse	Sex-specific differentiation	Don- (1994)
TEA95	95 kD	Pachytene spermatocyte	Mouse	Ectoplasmic specialization	Kojimizu- (1993)
Prbp	7785 bp	Pachytene spermatocyte to spermatid	Mouse	Switching DNA package	Lee- (1996)

and Vanek, 1998). These findings suggest that meiotic proteins act specifically as their critical roles in meiosis of male germ cells.

4. Meiotic Mechanism in Female Gametes

Most female mammals undergo meiotic prophase entry in the fetal ovary with all oocytes having entered the dictyate stage of prophase by the time of birth (review by Handel and Eppig, 1998). Dictyate-arrested oocytes reside within the primordial follicle pool, and as follicles initiate growth during puberty of adult reproductive cycles critical changes in metaphase competencies take place. Oocyte growth seems to be stimulated by the substances from granulosa cells and subsequently the maintenance of the dictyate-arrested oocyte is dependent on a complex balance between intracellular factors, such as cAMP, purine, and calmodulin (Eppig, 1993; Chi, 1996), and intracellular communication with cumulus cells (Eppig *et al.*, 1996). Nevertheless, as oocyte and follicle development proceeds, full competency to reinitiate and complete meiotic metaphase is acquired, apparently in steps that support the successive events of germinal vesicle breakdown, first polar body formation, arrest of meiosis at metaphase II, and completion of meiosis II upon fertilization. Therefore, oogenesis is a punctuated program of cell cycle advancement in mammals.

4-1. Coordination of cytoplasmic and nuclear maturation

Oocyte meiotic progression is characterized by a series of starts and stops imposed at specific stages of follicle development. Furthermore, few of the available oocytes ever reach a fertilizable meiotic state owing to the tremendous loss of germ cells. Such unusual demands, placed upon both the timing and number of oocytes reentering meiosis, may due to the regulatory interplay of intrinsic and extrinsic modulators from oocytes and other somatic cells in various species.

In the oocyte during pre-ovulatory development, the cytoplasmic and nuclear maturation can be viewed as separate entities. Cytoplasmic maturation and the acquisition of stores of RNA and protein dominates oocyte development between the premordial and pre-ovulatory stages of development. Initiation of nuclear maturation is marked by the germinal vesicle breakdown (GVBD) and is triggered by the midcycle LH peak which is associated with a decrease in the intracellular concentrations of cAMP. This and several subsequent steps of meiosis are controlled by the metaphase promoting factor (MPF). MPF is known to be able to phosphorylate many of the proteins involved in nuclear membrane formation, chromatin condensation, and microtubule reorganization.

4-2. Activation of metaphase promoting factor (MPF) during meiosis

While the constituents of MPF, p34cdc2 kinase and B-type cyclin, are also present in mitotically dividing cells, in meiotically dividing oocytes the regulation of MPF activity differs. In non-rodent mammals, activation of MPF (Figure 1 from review paper by Albertini and Carabatsos, 1998) requires expression and posttranslational modification of the constitutive cdc2 catalytic and cyclin regulatory subunits, and also mediation by other positive (cdc25) or negative (wee1) factors (Mitra and Schultz, 1996). In addition, an oocyte-specific protein kinase, c-mos plays an important role in up-regulating the activity of MPF at various stages of oocyte maturation up to metaphase II (Hirao and Eppig, 1997). Several lines of evidence suggest that the proper function

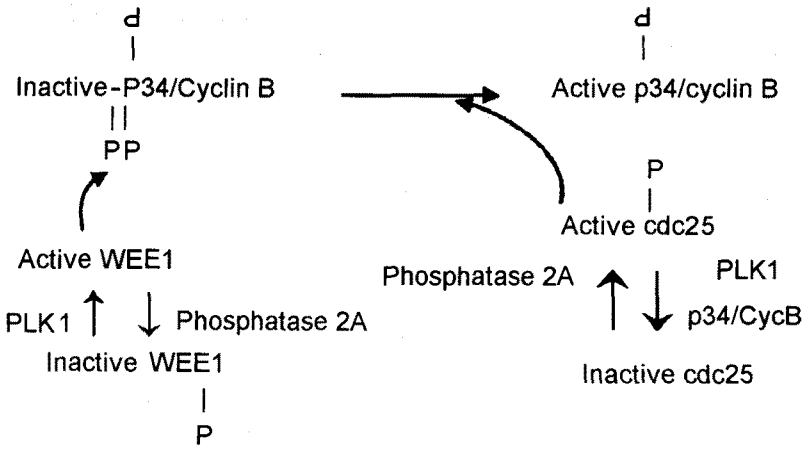

Figure 1. Positive and negative regulations of MPF activity. p34 dephosphorylation by cdc25 stimulates MPF activity (right), whereas p34 phosphorylation by wee1 inhibits (left). PLK1: polo kinase (adopted from Albertini and Carabotsos, 1998).

of the c-mos-MPF system is associated with important features of the last stages of oocyte maturation such as the resumption of meiotic maturation, inhibition of DNA replication between meiosis I and II, and the maintenance of the oocyte at metaphase II arrest until it is fertilized (Eppig *et al.*, 1996; Abertini and Carabatos, 1998). Eventually the destruction of c-mos and active MPF following fertilization allows the initiation of mitotic cell division in the pre-embryo.

Calcium and MPF

There is considerable evidence that intracellular calcium transients increases MPF activity, precedes GVBD and override cAMP maintained meiotic arrest (Homa, 1995). Thus calcium oscillation may signal the reassumption of meiosis through activation of the multiple functional calcium/calmodulin-dependent protein kinase (Winston and Maro, 1995). However, after fertilization, the transiently increased intracellular free calcium inhibit MPF activity through the protein kinase C pathway to trigger cyclin degradation, and the oocytes exits from metaphase II and enters into interphase (Colonna *et al.*, 1997).

Species-specific MPF activation

Interestingly, rodent oocytes appear to undergo a MPF activation that does not require protein synthesis and can be triggered simply by releasing oocytes from the follicle (de Vantery *et al.*, 1997). Therefore, even among mammals different mechanism exist to control metaphase entry in oocytes. Since cytoplasmic partitioning of cdc2 and cyclins has been implicated as a mechanism to limit MPF activation in the mouse, a typical model has been proposed as shown in Figure 2 (Albertini and Carabatos, 1998).

In this model it is assumed that all necessary factors for MPF activation are resident within the cytoplasm of full grown mouse oocytes and that candidate meiotic-arresting substances, such as cAMP, are delivered from somatic cells to the oocyte via gap junctions (Mitra and Schultz,

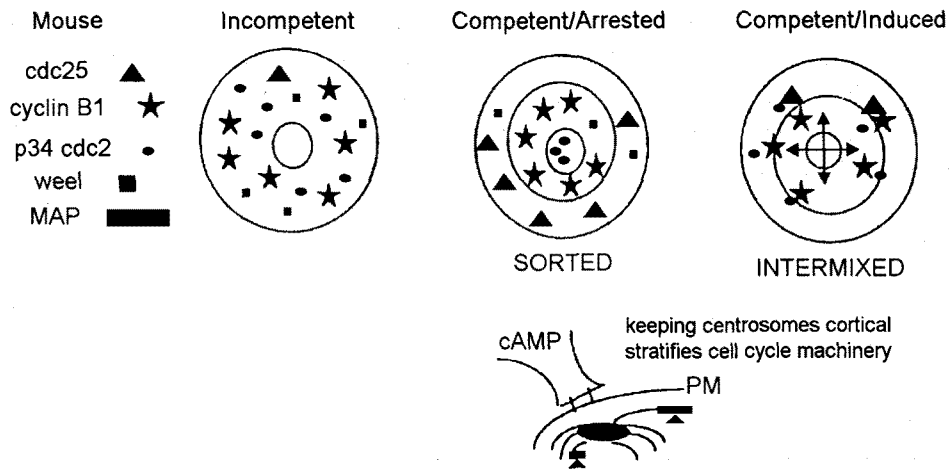

Figure 2. Model of MPF activation process with meiotic components, cdc25, cyclin, p34cdc2, cAMP and weel during maturation of mouse oocytes. Unsorted components exist in incompetent but nuclear, perinuclear, and cortical sorting shows in competent oocytes. Resumption of meiosis starts with intermixing of components after release of cortical components. From neihboring cells, cAMP stratifies centrosome microtubules bound to cdc25 (adopted from Albertini and Carabotsos, 1998).

1996). Due to cAMP stabilized interphase microtubules, these factors capable of binding to microtubules either directly of indirectly through microtubule-associated proteins would be retained in a cortical cytoskeletal scaffold. That microtubule stability influences MPF activation and inactivation has been shown in several studies (Ookada *et al.*, 1995). Stable adhesive cell contacts at the oocyte surface would be required to maintain high levels of cAMP and ensure retention of cell cycle factors. Upon stimulation of the follicle by LH, propagation of microtubule-labilizaing factor such as localized calcium would eliminate the cytoskeletal scaffold and foster intermixing of cell cycle molecules that leads to MPF activation.

Differing requirements for protein synthesis to achieve MPF activation between rodent and non-rodent mammals, speculate that oocytes in nonrodents may depend on de novo synthesis of MPF components upon reception of meiosis-inducing signals (Levesque and Sirad, 1996). Therefore, additional many studies are necessary to clarify the mechanism involved in the species-specific regulation of meiosis during oocyte maturation.

5. Conclusion

In mammal, meiosis is a specialized kind of cell cycle to produce haploid gametes, sperm and egg. This reduction in chromosome number is accomplished by two sequential rounds of nuclear and cell divisions after a single round of DNA replication. In the last decade, there has been an imposing improvement in understanding the mechanisms underlying the complex process of meiosis. However, it is largely unknown how extrinsic and intrinsic mechanisms of germ cells especially to activate signal transduction processes regulating meiosis. Until recently, few meiotic

specific protein participants had been identified and characterized in the gamete itself as well as neighboring helper cells, Sertoli cells in male and granulosa cells in female, but several recent scientific efforts have changed this situation. In the near future, the complete mechanisms of meiosis processes will be clarified and this may improve the efficiency of gamete culture systems as well as of clinical procedures.

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