

## Meiotic spindle, spindle checkpoint and embryonic aneuploidy

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### TABLE OF CONTENTS

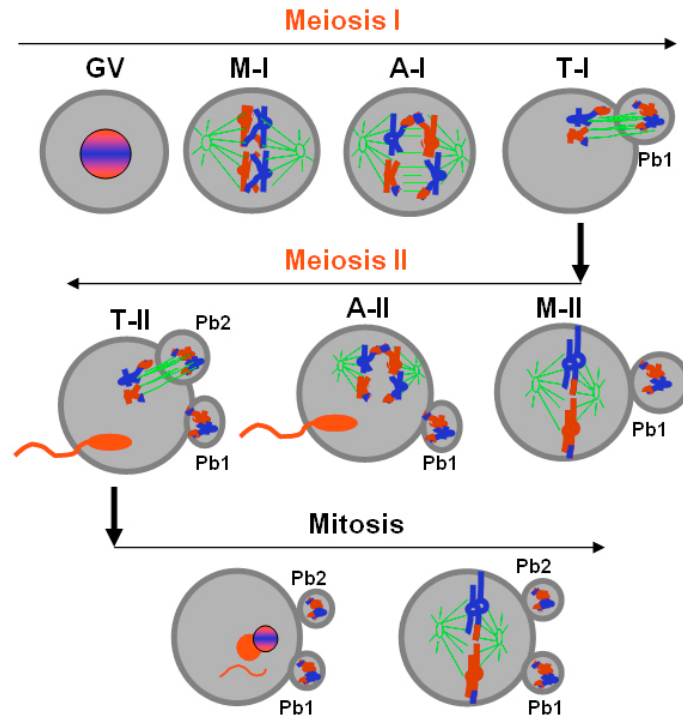
1. Abstract
2. Introduction
3. Spindle formation during meiosis
4. Kinetochore and kinetochore proteins
5. Spindle checkpoint signaling pathway
6. Checkpoint proteins in mammalian meiosis
  - 6.1. Mad1
  - 6.2. Mad2
  - 6.3. Bub1
  - 6.4. CENP-E
  - 6.5. MAP kinase
  - 6.6. Cytoplasmic dynein
7. Embryonic aneuploidy
  - 7.1. Advanced maternal age and embryonic aneuploidy
  - 7.2. Abnormal spindle morphology and embryonic aneuploidy
  - 7.3. Defective checkpoint and embryonic aneuploidy
  - 7.4. Diagnosis of embryonic aneuploidy
8. Perspective
9. Acknowledgment
10. References

## 1. ABSTRACT

In mitosis, a spindle checkpoint plays important roles at the metaphase-anaphase transition to ensure the formation of a bipolar spindle, the completion of connecting chromosomes to microtubules and the alignment of all chromosomes at the spindle equator before initiation of anaphase. Components of the spindle checkpoint were first identified through genetic screens in budding yeast and some checkpoint proteins later were found in a wide range of cells from yeast to human. However, the presence and function of the spindle checkpoint in mammalian meiosis, especially female meiosis, have long been disputed but evidence is now accumulating to support the existence. Recent studies indicate that a spindle checkpoint system participates in the regulation of mammalian female meiosis and prevention of embryonic aneuploidy. Here we review recent progress on checkpoint studies in both mitosis and meiosis, toward an understanding of the checkpoint signal transduction pathway and its role in preventing chromosome abnormalities during meiosis. Furthermore, the causes of embryonic aneuploidies and diagnosis are discussed.

## 2. INTRODUCTION

The accurate segregation of homologous chromosomes (meiosis I) or sister chromatids (mitosis and meiosis II) in the metaphase-to-anaphase transition is essential for maintaining the integrity of the genome in both mitosis and meiosis. Failure of equal segregation will cause aneuploidy, which is thought to be the origin of some genetic disorders and cancers in somatic cells (1-4) and aneuploid embryos (5-8). Aneuploid embryos usually arrest development at an early stage (9), but some develop further, implant into the uterus, and undergo spontaneous abortion (7, 10-12), while others develop to term and carry genetic disorders (13, 14). Aneuploidy occurs when chromosomes or chromatids separate unequally during cell division. To prevent aneuploidy, both mitotic and meiotic cells have developed a high-fidelity surveillance system to monitor the coordinated and precise operation of the segregation machinery (15, 16). This system is referred to as spindle checkpoint in mitotic cells (15, 17), which delays the onset of anaphase (sister chromatid or chromosome segregation) until all chromatids or chromosomes are correctly oriented in a bipolar position at the metaphase



**Figure 1.** Meiosis and meiotic spindle formation in mammalian oocytes. Meiosis I starts at a very early stage of oocyte growth, which consists of an extended prophase. The oocyte has a large nucleus, called the germinal vesicle (GV) in the last stage of prophase. Some GV stage oocytes become fully-grown before the prepubertal phase. The oocyte is blocked at the GV stage until stimulation by a gonadotropin hormone, luteinizing hormone. Resumption of meiosis I is recognized by breakdown of the GV followed by pro-metaphase I and then metaphase I (M-I), in which a spindle forms and homologous chromosomes align at the spindle's equator. After all chromosomes align at the spindle equator, the oocyte enters anaphase I (A-I) and chromosomes start to separate, followed by telophase I (T-I) in which a polar body (Pb1) containing half of chromosomes is extruded from the oocyte. Completion of meiosis I is indicated by the extrusion of the first polar body. Then, the oocyte re-organizes a second meiotic spindle and chromosomes re-align at the equator of the spindle, and the oocyte is arrested at metaphase II (M-II). Meiosis II is initiated in the oocyte by fertilization or parthenogenic activation, which causes segregation of chromatids through anaphase II (A-II) and telophase II (T-II) stages, and extrusion of the second polar body (Pb2). After fertilization, a female pronucleus and a male pronucleus form in the oocyte, followed by formation of a mitotic spindle, allowing the oocyte (embryo) to enter mitosis.

plate of the bipolar spindle (15-17). The first breakthrough in uncovering the mechanisms of the spindle checkpoint began with the successful screen of several genes from mitotic arrest deficient (Mad) and budding uninhibited by benzimidazole (Bub) mutants of budding yeast: Mad1-3 (18), Bub1-3 (19) and Mps1 (20). Homologues have been identified for the majority of these checkpoint genes in some organisms including humans (15). Checkpoint proteins have also been identified by using immunofluorescent microscopy and immunoblotting for some eukaryotes and found to function similarly in the mitotic checkpoint system (21-26). Biochemical, genetic and cell biological analyses show that most checkpoint proteins are linked to the same checkpoint pathway that controls anaphase onset (27-32) although a second pathway may also be present (33-35). The checkpoint system has been widely studied in mitotic cells, from yeast to human, but its mechanism(s) is still poorly understood and the exact signal transduction pathways are unknown. Information compiled for mitosis suggests that a common mechanism may be present from budding yeast to mammals and between

mitosis and meiosis (17, 18, 21, 22, 27, 28, 30, 32, 36, 37). Recently, it has been found that some checkpoint proteins observed in mitosis also exist in meiosis in mammals (36-42). However, compared with the studies in mitosis, information for meiosis is lagging behind and it is not yet clear if meiosis follows the exact same checkpoint pathway as that known for mitosis. In this paper, we review recent progress on checkpoint studies in both mitosis and meiosis toward an understanding of the signal pathway of the spindle checkpoint mechanism and its role in preventing chromosome abnormalities during cell division. We also have included an attempt to understand the relationship between spindle checkpoint and embryonic aneuploidies in mammals, including humans.

### 3. SPINDLE FORMATION DURING MEIOSIS

Figure 1 shows the formation of meiotic spindles during meiosis I and II, and the first mitotic spindle after fertilization in mammalian oocytes. The spindle, mainly composed of microtubules and centrosomes, is one of the

most essential cellular structures that are responsible for the accurate separation of homologous chromosomes (meiosis I) or two sets of chromatids (meiosis II) during germ cell division. According to the "search-and-capture" model of spindle formation (43-45), two major events take place during spindle assembly: First, the two centrosomes, which nucleate microtubules, translocate to opposite sides of the nucleus, establishing spindle bipolarity before nuclear envelope breakdown. Second, after nuclear envelope breakdown and chromatin condensation, kinetochores randomly capture and stabilize the dynamic microtubules that originate from centrosomes and form kinetochore fibers. Then chromosomes become organized at the equatorial plane of the spindle and the sister kinetochores become connected to the opposite poles by kinetochore fibers. This random and continuous process ends when all chromosomes are aligned at the metaphase plate (46, 47). However, in mammalian oocytes, the bipolarity of the spindle is not predefined during early maturation.

Oogonia enter meiosis at the beginning of the second trimester of pregnancy and after the last round of DNA synthesis. The oocyte undergoes two meioses (meiosis I and II) before entering embryonic mitosis. Meiosis I has an extended prophase I, and the mammalian oocyte is arrested in diakinesis at birth. With follicle growth, the oocyte develops to full size (fully-grown), and a large nucleus, termed the germinal vesicle (GV), is present in the oocyte. The oocyte is arrested at the GV stage until stimulation by gonadotropins-FSH to induce meiotic competence and LH to trigger the resumption of meiosis and ovulation. Resumption of meiosis is indicated by GV breakdown (GVBD). After GVBD, tubulin begins to polymerize into microtubules around the condensed chromatin and chromosomes form in the oocytes before entering metaphase I (M-I). This stage is termed prometaphase I (ProM-I) stage and it is thought that the connection between microtubules and chromosomes are established at this stage. As microtubules become organized into a bipolar spindle and all chromosomes align at the spindle equator, the oocyte enters the M-I stage. After M-I, oocytes soon enter a short anaphase I (A-I) during which chromosomes start to separate, followed by telophase I (T-I). During the first meiosis, half of the chromosomes are separated from the oocyte into the first polar body. The second meiotic spindle becomes organized soon after the first polar body is extruded, and the oocyte becomes arrested at the M-II stage until fertilization. Morphologically, no significant differences are observed between M-I and M-II spindles, although the M-I spindle appears to be longer than the M-II spindle in some mammals (48) and more sensitive to temperature fluctuations (49). Similar patterns of spindle organization have been observed during oocyte maturation in *Drosophila*, *Xenopus* and in various mammalian species (43, 44, 50). It is still unclear which specific component(s) that initiates polymerization of microtubules, but gamma-tubulin, nuclear mitotic apparatus (NuMA) protein, dynein/dynactin, and mitogen-activated protein (MAP) kinase are thought to play important role(s) in microtubule polymerization and spindle formation (51). At the onset of

microtubule polymerization, kinetochores start to capture the plus ends of microtubules (43, 44). In mouse oocytes, it has been reported that the interactions between kinetochores and microtubules are not established until ~8h after GVBD (44), just before final alignment of chromosomes in the spindle that is accomplished by checking cytoplasmic linker protein-170 (CLP-170), a 170 KDa transient kinetochore protein (52).

Meiosis II is initiated when the arrested M-II oocytes are fertilized by sperm or activated by chemical or physical stimuli (parthenogenetic activation) (53). Meiosis II in mammalian oocytes is short as compared with meiosis I. Once fertilized by a spermatozoon or artificially activated, the oocyte soon enters the A-II stage, followed by the T-II stage, and finally extrudes the second polar body that contains half of the chromatids. It has been shown that intracellular calcium rises during sperm penetration and parthenogenetic activation is the most important factor to initiate intracellular signal transduction during meiosis II (53). From this point, it appears that the signal transduction pathways are different between meiosis I and meiosis II. However, mechanisms (spindle checkpoint pathway) that control chromosome (meiosis I) and chromatid (meiosis II) segregation may be similar (as discussed later).

After fertilization or activation, half of the chromatids remaining in the oocyte form a female pronucleus. The chromatin from the spermatozoon forms a male pronucleus. Both male and female pronuclei move toward the center of the oocyte and membranes from both pronuclei break down. Chromatin from male and female pronuclei mix followed by mitotic spindle formation. The embryo then divides by mechanisms known for mitotic cell division.

## 4. KINETOCHORES AND KINETOCHORE PROTEINS

The kinetochores are macromolecular structures (identified as trilaminar in electron micrographs) that are composed mainly of proteins (54) that are responsible for establishing and maintaining the connection between chromosomes and microtubules of the spindles. In mitotic cells, for accurate chromosome segregation, sister kinetochores must attach to microtubules extending from opposite spindle poles prior to the onset of anaphase. This state is called sister kinetochore bi-orientation or chromosome bi-orientation. The mechanism ensuring chromosome bi-orientation is central and crucial for chromosome segregation. To guide homologous chromosomes to opposite poles during meiosis I, each pair of sister kinetochores must function as a single unit, establishing an attachment to the same pole. Recent studies have identified a group of *S. cerevisiae* proteins, termed "monopolins," that are required for monopolar attachment in meiosis I.

So far, two types of kinetochore (or centromere) proteins (CENPs) have been identified (Table 1). One type represents constitutive proteins, such as CENP-A~D. These

**Table 1.** Kinetochore proteins and their locations and functions

Proteins	Mitosis	Meiosis <sup>1</sup>	Major functions
<b>Constitutive Proteins</b>			
CENP-A	+	+	Structural protein
CENP-B	+	+	Structural protein
CENP-C	+	ND	Structural protein
CENP-D	+	+	Structural protein
<b>Checkpoint proteins</b>			
Mad1	+	+	Bind to Mad2 and recruit it to kinetochore
Mad2	+	+	Bind to Cdc20 & inhibit APC/C
Mad3 (BubR1)	+	ND	Bind to Bub3 & inhibit APC/C
Bub1	+	+	Required for kinetochore to localize BubR1, CENP-E, CENP-F, Mad1, Bub3 & Mad2. Bind to BubR1
Bub2	+	ND	Has a different checkpoint pathway from other checkpoint proteins
Bub3	+	ND	Bind to BubR1 and Bub1, perhaps bind to APC
<b>Motor proteins</b>			
CENP-E	+	+	Required for kinetochore to associate Mad1, Mad2 & other checkpoint proteins. Required for chromosome movement.
Dynein	+	+	Transport checkpoint proteins, deactivate checkpoint and participate in chromosome movement
Mps1	+	ND	Required for recruiting CENP-E at kinetochore
Zw10	+	ND	Required for dynein to localize at kinetochore
Rod	+	ND	Together with Zw10 to localize dynein at kinetochore
<b>Others</b>			
MAP kinase	+	+	Required for kinetochore to associate CENP-E. Required for spindle formation
Aurora B	+	ND	Required for Bub1, Mps1, BubR1, CENP-E, Mad1 & Mad2 to associate with kinetochore

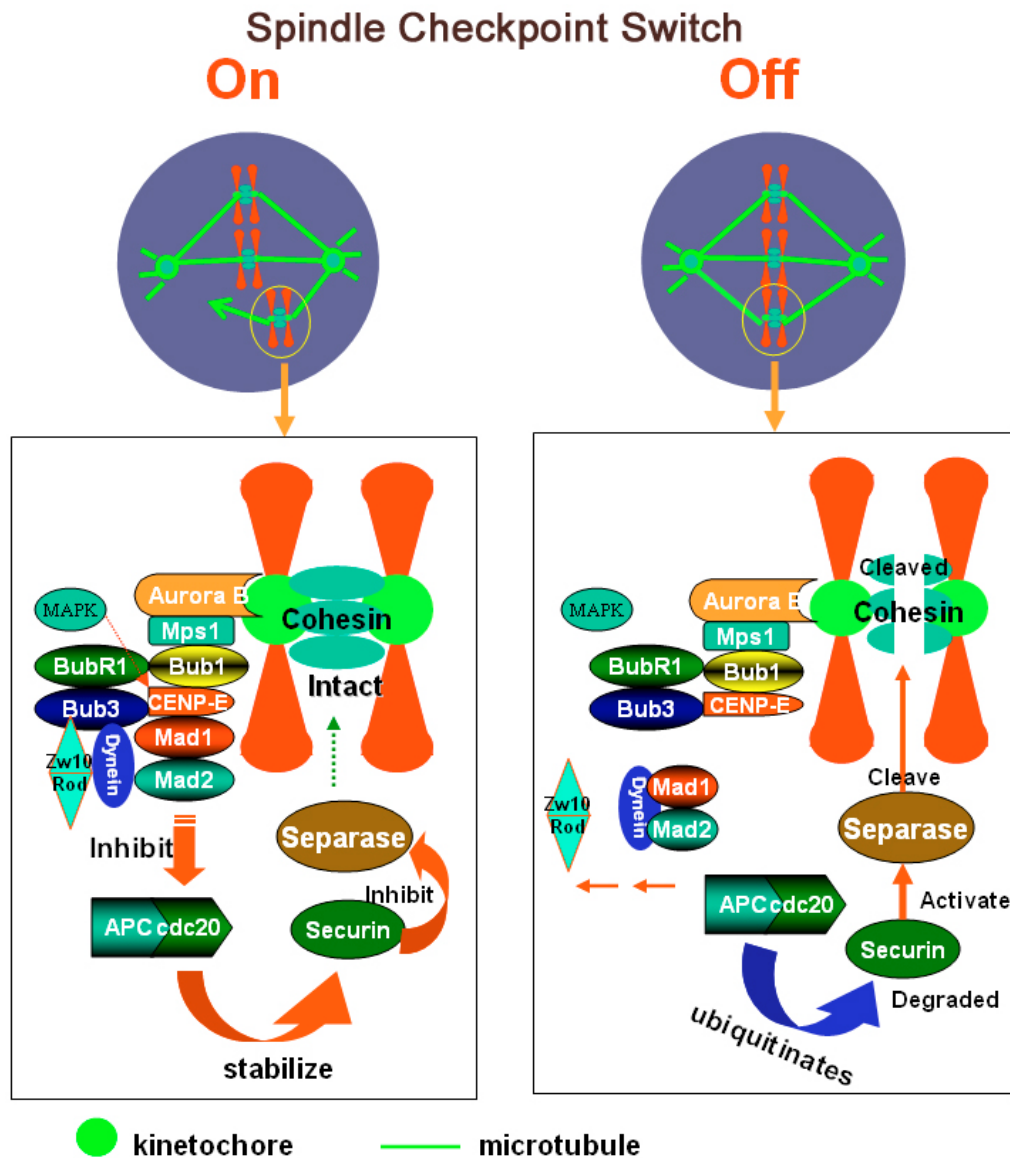
<sup>1</sup> mammalian meiosis; ND: not determined; +: reported to exist.

CENPs are present at the centromeres/kinetochores throughout the cell cycle and can be detected by anticentromere antibodies (ACA), autoimmune sera from patients with calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia (CREST) (54-59). Cell cycle changes do not alter their location (59). The second type represents transient proteins or checkpoint proteins. Checkpoint proteins can be detected in specific cell cycle stages by specific antibodies (59) and will be discussed later.

CREST has been used to identify constitutive proteins at the kinetochore of various cells including mammalian oocytes. Studies in mouse (60) and porcine (61) oocytes showed that at least three CENPs, CENP-A (17 kDa), CENP-B (80kDa) and CENP-D (50kDa) are present in oocytes from GV to M-II stages. However, CENP-A, CENP-B and CENP-C have been detected by CREST in human mitotic cells (57) and CENP-A~D have been detected in HeLa cells (62). We do not yet know whether differences observed among these studies are due to different species, different cells, different material or other factors. Quantification of CENPs indicates that both CENP-B and CENP-D in pig oocytes are significantly increased after GVBD and reach their highest level in oocytes at M-II (61). These results suggest that CENPs are synthesized not only in fully-grown oocytes, but also in oocytes undergoing meiotic maturation. The increases in quantity also suggest that CENPs are associated with chromosome segregation during meiosis.

The functions of the constitutive proteins are unclear. They may participate in DNA binding, microtubule capture and transient protein binding (59). The most detailed data for these proteins' function has come from antibody microinjection experiments. It has been reported that CENP-B null mice are viable (61) and can undergo normal meiosis and mitosis (58, 63). However, microinjection of anti-kinetochore/centromere antibodies in mouse oocytes interfered with chromosome congression at ProM-I stage and caused unaligned chromosomes in the spindle when they entered metaphase, resulting in abnormal meiosis (60). Furthermore, injection of anti CENP-B antibodies into human and mouse somatic cells also resulted in disruption of centromere assembly during interphase and inhibited kinetochore function in mitosis (64, 65). Dysfunctional kinetochores or depletion of some CENPs may cause premature anaphase, then induce unequal distribution of sister chromatids during cell division, yield aneuploidy, and consequently result in tumor or severe congenital syndromes (46, 47, 66). The effects may be indirect, as transient proteins may be unable to bind to kinetochores if constitutive proteins are destroyed. Although the structure and function of kinetochores and associated CENPs have been widely studied in mitotic cells, little is known about their role for meiosis, especially female meiosis (43, 50, 67).

As kinetochore functions are largely dependent on the transient proteins, the localization and function of



**Figure 2.** Signal transduction during checkpoint switch (on and off) for normal chromosome (chromatid) segregation during mitosis and meiosis. Checkpoint switch is turned on when a spindle has not formed and/or chromosomes/chromatids have not aligned at the spindle equator by inactive (inhibited) APC/Cdc20, and sequential binding of all other checkpoint proteins (Aurora B, Mps1, Bub1, CENP-E, Bub2, BubR1, Bub3, Mad1 and Mad2) at kinetochores, leading to the stabilization of securin, which in turn inhibits separase. Cohesin between chromosomes/chromatids is intact. MAP kinase may participate in CENP-E binding to kinetochores, and Zw10/Rod may help dynein to transport Mad1 and Mad2 to bind to kinetochores. When spindle formation is completed and all chromosomes/chromatids align at the spindle equator, checkpoint proteins (Mad1 and Mad2) are released from the kinetochore, APC/C cdc20 inhibition is released, and securin is ubiquitinated. The degradation of securin activates separase that in turn cleaves cohesin, resulting in chromosome or chromatid segregation. Zw10/Rod aid dynein to transport Mad1 and Mad2 to spindle poles during checkpoint silencing (turn off).

the transient proteins are described in the following sections.

## 5. THE CHECKPOINT SIGNALING PATHWAY

Kinetochore localization of the checkpoint proteins occurs in a defined order (25, 32, 68). The initial

binding of checkpoint protein is very important. Recent studies in *Xenopus* egg extracts (30, 32, 69) and mitotic cells (22, 25, 29, 30, 68) are presented as a schematic diagram on the spindle checkpoint pathway in both mitosis and meiosis. As shown in Figure 2, it is thought that Aurora B (that binds to the inner centromere protein, INCENP) is the first transient protein that binds to the kinetochore (32).

## Checkpoint pathway in mammalian meiosis

It is thought that Aurora B plays important roles in metaphase chromosome alignment (70, 71) and microtubule-kinetochore interactions (72). Recent evidence suggests that Aurora B is required for kinetochore localization of Bub1, BubR1, CENP-E and Mad2 (73) and Aurora kinase inhibitor reduces kinetochore binding of these proteins (73). In *Xenopus* egg extracts, it was also found that kinetochores could not bind Mps1, Bub1, CENP-E, Bub3, Mad1 and Mad2 after Aurora B depletion (32) indicating that Aurora B also participates in the spindle checkpoint functions in meiosis.

The second protein that binds to kinetochores is thought to be Mps1 and/or Bub1. Bub1 localizes to kinetochores as soon as chromosome condensation becomes visible (68). It is required for kinetochore localization of BubR1, CENP-E and Mad2 (74). Depletion of Bub1 prevented CENP-E, Bub3, Mad1 and Mad2 from localizing to the kinetochores (30). Mps1 is also required for recruitment of CENP-E at kinetochores (75), which in turn is necessary for kinetochore association of Mad1 and Mad2 (69). In Mps1-depleted *Xenopus* egg extracts, kinetochore staining of CENP-E, Mad1 and Mad2 completely disappeared (32, 69), thus Mps1 is also thought to act at the top of the mitotic and meiotic checkpoint cascade after Aurora B action (69). However, it was recently found that in *Xenopus* egg extracts Mps1 and Bub1 bound to kinetochores at almost the same time (32), as Mps1 depletion prevented Bub1 binding to kinetochores and Bub1 inhibition removed Mps1 from kinetochores (32). These results indicate that Bub1 and Mps1 bind to kinetochores at the same time and are dependent on each other, but they bind to kinetochores earlier than other checkpoint proteins, such as CENP-E, BubR1, Bub3, Mad1 and Mad2.

CENP-E is a kinase-related microtubule motor protein (62, 75) and located in the outer kinetochore plate and fibrous corona (76). The studies on the subcellular localization of CENP-E in different vertebrate somatic cells indicate that it accumulates in the cytoplasm during interphase and first appears at the kinetochore in early prometaphase to late anaphase (62, 75, 76). CENP-E mainly acts as a motor protein to participate in chromosome movement (62, 77-79) but recently it was found that it participates in spindle checkpoint by acting as a binding partner of BubR1 (80, 81) or forming a link between microtubules and kinetochores (82, 83). It binds to kinetochores after Aurora B (32, 73) but later (30) or together with Mps1 and Bub1, as Mps1, Bub1 and CENP-E depend on each other for binding (32). CENP-E forms a complex with BubR1 after BubR1 binding, which is important for Mad1 and Mad2 binding to kinetochores (29, 84). Reduced CENP-E also reduces BubR1 binding to kinetochores (83, 84) and activation of BubR1 is dependent on CENP-E (84, 85), suggesting that CENP-E is an activator of BubR1. CENP-E is also required for efficient recruitment of Mad1 and Mad2 to attached and newly unattached kinetochores (85). Taken together, there is evidence that CENP-E appears at kinetochore earlier than BubR1, Mad1 and Mad2, and acts as a checkpoint protein in addition to being a motor protein.

The next proteins that bind to kinetochores are thought to be Bub3 and BubR1. Bub3 is found in most eukaryotes (24, 86) and is one of the proteins that transmit the spindle assembly checkpoint signal. During mitosis, Bub3 appears on kinetochores during prophase, but is lost by metaphase (23, 28, 31). When kinetochores are not attached to the spindle, or lagging attachment, the amount of kinetochore-associated Bub3 antigen increases (86). Bub3 may serve as a platform for interactions between kinetochore checkpoint proteins, and its association with Mad2, Mad3 and Cdc20 (28) thus may directly participate in inhibition of APC/C (28, 31). However, it was found that the Bub3-binding site is on Bub1 and both form a Bub3-Bub1 complex (23, 25), thus the role of Bub3 is to facilitate kinetochore localization of Bub1 (25). hBub3 also appears to be required for kinetochore localization of hBubR1 (23). From these observations, it appears that Bub3 may bind to kinetochores after Bub1, but that it also has a down-stream regulation role on the binding of Bub1. BubR1 (mammalian homolog of yeast Mad3) is also a checkpoint protein participating in other protein binding to kinetochore (25). BubR1 is required for kinetochore loading of Mad1 and Mad2 (29), but it may also directly inhibit cdc20 and then inhibit APC/C together with Mad2 (28, 87, 88).

The last two checkpoint proteins binding to kinetochores are Mad1 and Mad2. The localization and functions of Mad1 and Mad2 have been widely studied in mitotic spindle checkpoint from yeast to mammalian cells. In budding yeast, a tight complex between Mad1 and Mad2 is crucial for checkpoint function (89), which was subsequently verified by analysis of tetrameric Mad1-Mad2 crystal structure (90). *Xenopus* Mad1 also recruits *Xenopus* Mad2 to unattached kinetochores (22). A study in *Xenopus* egg extracts showed that only a part of Mad2 formed a complex with Mad1, while the other part of Mad2 did not bind to Mad1; the ratio between Mad1 and Mad2 was critical for maintaining a pool of Mad1-free Mad2 that is necessary for the spindle checkpoint (91). Mad2 may become activated and dissociated from Mad1 at kinetochores and is replenished by a pool of Mad1-free Mad2 (91).

The final component in the checkpoint signal transduction pathway is the anaphase promoting complex or cyclosome (APC/C)/cdc20. Prior to spindle attachment, sister chromatids or chromosomes remain associated until the onset of anaphase. The establishment and maintenance of chromatid and chromosome cohesion depend on a protein complex called cohesin, a glue holding the sister chromatids together (92-95). All checkpoint proteins bind to unattached kinetochores that generate a diffusible wait anaphase signal which inhibits APC/C activator, termed cdc20 in yeast, fizzy in *Drosophila*, or p53cdc in mammals, thus the subsequent events associated with sister chromatid separation are prevented. When microtubules attach to kinetochores and all chromosomes align at the spindle equator, kinetochores release the checkpoint proteins Mad1 and Mad2, and then inhibition to cdc20 or p53cdc is released and APC/C is activated. After activation, APC/Ccdc20 ubiquitinates securin, and degradation of securin in turn activates an enzyme termed separase, which

**Table 2.** Checkpoint proteins on kinetochores in mammalian oocytes at various stages during meiosis I

Proteins	GV	ProM-I	M-I	Early A-I	Late A-I	M-II	Species	References
Mad1	+	++	-	-	-	-	mouse	39
Mad2	+	++	-	-	-	-	mouse, rat, human, pig	36,37,40-42
Bub1	?	++	++	++	-	++	mouse	38
CENP-E	+	++	++	++	-	++	mouse, pig	122,123
Dynein	+?	+?	-	-	-	-	mouse	Unpublished data
MAP kinase	-	++	++	+?	+?	++	almost all mammals	51, 128-131

+: some binding, ++: strong binding, -, no binding, ?: unclear.

cleaves cohesin (92, 93). Finally the sister chromatids or chromosomes separate. The homologous chromosome segregation at meiosis I only requires loss of cohesion along the chromosome arms but not the cohesion around the centromeres. Sister chromatid separation is achieved by releasing centromere cohesion. All these steps are essential for faithful transmission of chromosomes and thereby must be regulated precisely. Although other checkpoint proteins may also participate in activation/deactivation of APC/C, such as BubR1 (96), most data provide evidence that Mad2 is the direct protein that binds to cdc20 or p55cdc and participates in activation/deactivation of APC/C cdc20 (97).

The kinetochores bind and release checkpoint proteins after checking spindle formation and chromosome alignment. Some proteins, such as Mad1 and Mad2, relocate between kinetochores and cytoplasm/spindle pole. How these proteins transport/relocate between the kinetochore and spindle pole is still unclear. Cytoplasmic dynein may play a key role in the relocation of these proteins. It has been suggested that dynein also participates in the mitotic spindle checkpoint by transporting checkpoint proteins (98). For example, in Ptk1 cells, microinjection of anti-dynein antibody prevented Mad2, Bub1 and CENP-E from translocating to the spindle poles, prevented Mad2 detachment from the kinetochores, decreased kinetochore tension and caused a mitotic block at metaphase (98), indicating that dynein might de-activate spindle checkpoint after all chromosomes have bioriented at the metaphase plate. On the other hand, prometaphase kinetochores had more dynein signals than metaphase kinetochores in mitotic cells (99, 100) and dynein was recruited to metaphase kinetochores after depolymerization of microtubules (99, 101). Once the attachment starts in early stage during mitosis, most of the dynein detaches from kinetochores (101). These results suggest that dynein also participates in the transport of some checkpoint proteins toward the kinetochores.

Zeste white 10 (Zw10) and Rough deal (Rod) proteins also display remarkable dynamics in their intracellular location during cell division (102, 103) and may also serve as spindle checkpoint proteins (104, 105). Both proteins accumulate strongly at the outer kinetochore plates during prometaphase (102). At metaphase, Zw10 and Rod depart from the kinetochores and relocate onto spindle microtubules (102, 104). During anaphase, the proteins are no longer found on kinetochore microtubules and instead localize exclusively to the kinetochores of the separating chromosomes. It was suggested that Zw10 and Rod are responsible for localization of dynein to kinetochores (103) via a direct contact with dynamitin, a component of the

dynactin complex (102). Recently, it was also suggested that Human Zw10 and Rod are mitotic checkpoint proteins (104), as cells lacking these proteins at kinetochores fail to arrest in mitosis when exposed to microtubule inhibitors. However, it appears that both Zw10 and Rod affect checkpoint pathway through dynein (103), which participates in the transport of some checkpoint proteins (98), but Bub3 loading to kinetochore needs neither Zw10 nor Rod3 (106), suggesting that Bub3 detachment from the kinetochores is not dependent upon dynein. However, the transportation of Mad2 and BubR1 to the spindle poles relies on dynein's presence, suggesting that Zw10 and Rod function as indirect checkpoint proteins (4).

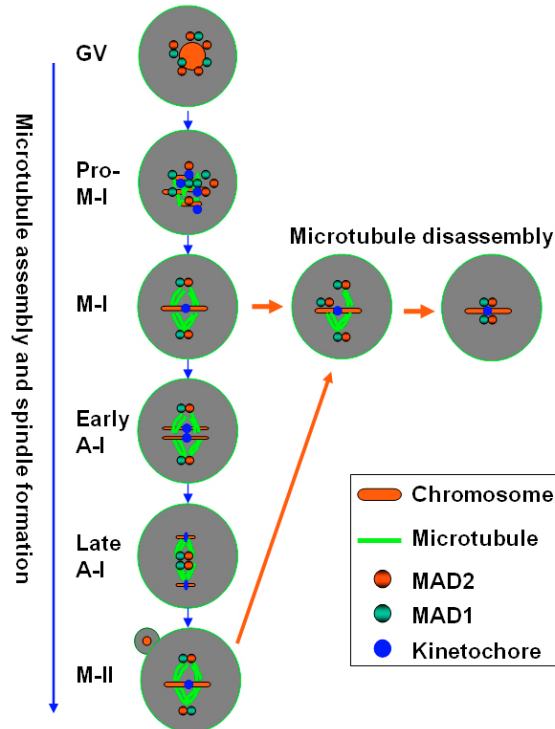
## 6. CHECKPOINT PROTEINS IN MAMMALIAN MEIOSIS

As shown in Table 2, only a few checkpoint proteins have been reported to exist in mammalian meiotic cells, and others have not been determined and/or reported. The following is a summary of the detailed localization and functions of these checkpoint proteins.

### 6.1. Mad1

Recently, it was found that Mad1 is present in mouse oocytes (39). However, its localization appears different from that observed in mitosis and *Xenopus* egg extracts. In budding yeast, Mad1 was distributed within the nucleus (107). In Hela cells, hMad1 was localized to nuclear pores during interphase, on kinetochores at prometaphase, and disappeared at metaphase (108). In *Xenopus* egg extracts, Mad1 was localized to the nuclear envelope and nucleus during interphase, dissociated from the nuclear envelope at prophase, moved to the chromosomes at prometaphase, and then disappeared from chromosomes at metaphase and anaphase (22). However, as shown in Figure 3, in mouse oocytes, Mad1 was observed around nuclei at the GV stage (prophase), on kinetochores at ProM-I stage, and moved to spindle poles at M-I, early A-I and M-II stages (39). These results suggest that differences for Mad1 localization are present between mitosis and meiosis.

After nocodazole treatment, Mad1 in mouse oocytes was relocated: some moved close to the chromosomes in an aggregated form (did not bind to kinetochore) while others bound to kinetochores (39). This relocation of Mad1 during spindle disruption is obviously different from that in mitosis (107), in which all Mad1 was at kinetochores when the spindle was disrupted. This may suggest that an excess of Mad1 is present in mouse oocytes but not in somatic cells. However, Mad1 localization was not altered in mouse oocytes after tension had been



**Figure 3.** Model for Mad1 and Mad2 localization in mouse oocytes during spindle assembly and disassembly. Immature oocyte at GV stage contains a nucleus enveloped by intact membranes, and Mad1 and Mad2 are localized around the nucleus. No microtubules are observed. When GV breaks down, the oocyte reaches prometaphase (Pro-M-I) and microtubules start to polymerize to form the spindle. Chromosomes disperse, and both Mad1 and Mad2 bind to kinetochores. When a bipolar spindle is formed and all chromosomes align at the spindle equator, the oocyte reaches metaphase I (M-I). Mad1 and Mad2 move to the spindle poles. Soon after M-I, chromosomes start to separate and the oocyte reaches early anaphase I (A-I). Mad1 and Mad2 are still at the spindle poles. However, at late A-I, Mad1 and Mad2 move to the spindle midzone. When the first polar body is extruded from the oocyte, microtubules re-organize to form a spindle and all chromatids align at the spindle equator, and the oocyte reaches metaphase II (M-II). Mad1 and Mad2 relocate to the spindle poles. When the oocytes at M-I and M-II stages are treated with nocodazole or cold temperature to disassemble the spindle, Mad1 and Mad2 move back to the chromosomes and re-bind to unattached kinetochores. When the spindle is completely disassembled, Mad1 and Mad2 bind to all unattached kinetochores.

changed (39). These results indicate that Mad1 merely senses attachment of chromosomes to microtubules, but not the tension between microtubules and chromosomes.

The exact mechanism by which Mad1 affects chromosome alignment is still unknown in mammalian oocytes. When anti-Mad1 antibody was injected into mouse oocytes, it was found that it did not affect oocyte nuclear

maturation and spindle formation, but induced chromosome misalignment (39). However, whether the oocytes with misaligned chromosomes have chromosome abnormalities, such as gain or loss of chromosomes after completion of meiosis, needs further investigation by chromosome analysis. According to studies in *Xenopus* egg extracts and mitosis, it appears that Mad1 affects Mad2 binding to kinetochores. For example, adding an anti-Mad1 antibody to *Xenopus* egg extracts de-activates the checkpoint and prevents Mad2 from binding to unattached kinetochores (22). It has been suggested that Mad2 might become activated and dissociated from Mad1 at kinetochores and is replenished by the pool of Mad1-free Mad2 (109). In mouse oocytes, we found that Mad2 could not bind to kinetochores at the ProM-I stage when anti-Mad1 antibody was injected into oocytes at GV stage (unpublished data). These results indicate that Mad1 participates in spindle checkpoint in both mitosis and meiosis by recruiting Mad2 binding to kinetochores.

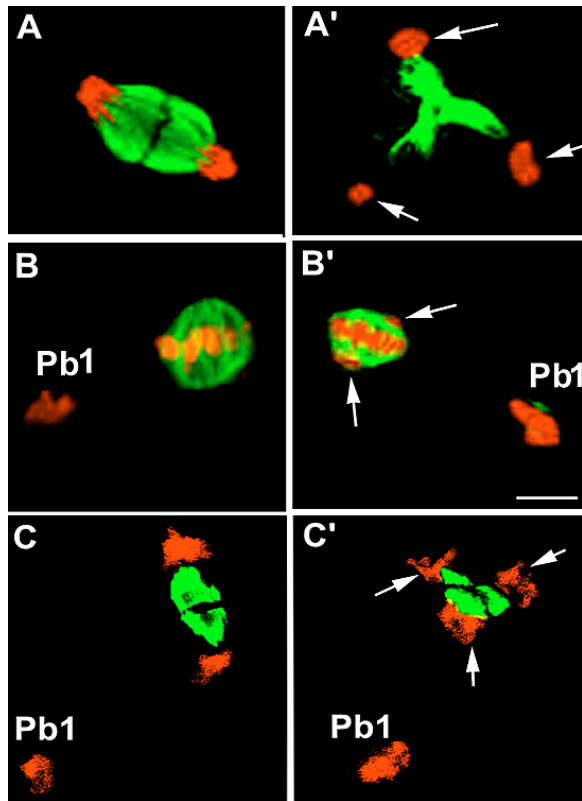
## 6.2. Mad2

Mad2 is one of the best-studied checkpoint proteins in mammalian meiosis. Species-specific differences of Mad2 localizations during meiosis were observed in mammals. ProM-I stage oocytes had similar Mad2 localization in all mammals examined including rat (40), mouse (37) and pig (Ma et al., unpublished data), i.e., Mad2 bound to all unattached kinetochores. Species differences were observed in the oocytes after reaching M-I and beyond M-I stage. Mad2 completely disappeared in rat oocytes at M-I~M-II stages (40), while Mad2 relocated to spindle poles at M-I, early A-I and M-II stages and spindle midzone at late A-I stage in mouse oocytes, as shown in Figure 3 (37, Zhang et al, unpublished data) and Mad2 still existed on some chromosomes at M-I and M-II stages in pig oocytes (Ma et al., unpublished data). What caused the different Mad2 localizations among mammals is still unknown. Mad2 binding to kinetochore was increased when oocytes were treated with nocodazole or cold (40), both causing microtubule depolymerization, thus it is possible that different Mad2 localizations reflect the connection between microtubules and kinetochores. It is well known that temperature-sensitivity of microtubules differs in different animal species (110-113), thus microtubules in rat and mouse oocytes may be more stable than in pig oocytes during temperature changes (113). In vitro manipulation may affect the connection between microtubules and kinetochores in pig oocytes but not in rat and mouse oocyte, and thus some Mad2 rebinds to kinetochores in pig oocytes during in vitro manipulation (113).

Different localization of Mad2 was also observed between mitosis and meiosis, male meiosis and female meiosis. However, one common characteristic is that Mad2 binds to unattached kinetochores and is then released from the kinetochores when microtubules attach to kinetochore. This localization of Mad2 is similar to that of Mad1, but is clearly different from other checkpoint proteins, which still bind to kinetochores of metaphase stage cells.

The function of Mad2 has been studied by Mad2 depletion, inhibition, over-expression, microtubule





**Figure 4.** Effects of anti-Mad2 antibody injection on chromosome alignment and segregation in porcine oocytes during meiosis I and meiosis II. (A-C) Control oocytes show normal chromosome alignment at M-II (B) and normal chromosome segregation at A-I (A) and A-II (C). (A'-C') Anti-Mad2 antibody injected oocytes show abnormal chromosome alignment at M-II (B') and abnormal chromosome segregation at A-I (A') and A-II (C'). Anti-Mad2 antibody was injected into the oocytes at GV stage and oocytes were then cultured until examination. Meiosis II was initiated by parthenogenetic activation of the matured oocytes with calcium ionophore. Arrows indicate abnormal chromosomes/chromatids. Pb1: first polar body. Red color represents chromosomes and green color represents microtubules. Bar = 10 micrometer.

disruption and stabilization. Anti-Mad2 antibody-injected rat (40), mouse (37, 42) and pig (Ma et al., unpublished data) oocytes enter a premature anaphase in which chromosomes start to separate before they are completely aligned at the metaphase plate. Chromosomes undergo abnormal, unequal alignment and separation as shown in Figure 4, which is among the causes of embryonic aneuploidy (42). These results are similar to those observed in somatic cell lines after anti-Mad2 antibody injection or Mad2 knockout (114-116).

On the other hand, overexpression of Mad2 in the oocytes resulted in metaphase arrest by activation of the spindle checkpoint, which is similar between mammalian oocytes (37, 42) and somatic cell lines (117). In other words, excess of Mad2 prevents silencing or deactivation

of the checkpoint. There are two other possible explanations. One is that an excess of Mad2 inhibits release from the kinetochore, and the other is that an excess of Mad2 can not be transported to other locations, thus a wrong signal may be transduced between the kinetochore and other checkpoint proteins.

It was previously found that Mad2 localized to kinetochores and depended on microtubule attachment, not tension (21, 114, 117-119). The same results were also reported in mammalian oocytes in which microtubule depolymerization with nocodazole or cooling caused Mad2 to re-attach to kinetochores at M-I and M-II stages (40). These results indicate that when the attachment between kinetochores and microtubules is interrupted, Mad2 binds to the kinetochore, thus terminating the next phase of the cell cycle (anaphase). These results suggest that before homologous chromosomes separate, Mad2 may check microtubule detachment from the kinetochores and inhibit anaphase initiation. When the nocodazole-treated oocytes were allowed to recover, Mad2 localization was totally different from that in normal oocyte spindle formation (40). During normal spindle formation, Mad2 can check chromosome misalignment and its activity disappears only when all chromosomes are aligned at the equator of the spindle. However, during spindle recovery in nocodazole-treated oocytes, we found that most oocytes still had misaligned chromosomes and Mad2 had disappeared (40), suggesting that Mad2 can check unattached kinetochores, but cannot check misaligned chromosomes. These results indicate that microtubule disassembly can cause Mad2 binding or rebinding to kinetochores at any stage and the re-bound Mad2 in these oocytes can check kinetochore attachment, but cannot check unaligned chromosomes, which may explain why aneuploidy is so common in human oocytes observed during in vitro fertilization (IVF). A high proportion of oocytes contain abnormal spindles when oocytes are retrieved from women and exposed to in vitro conditions (120). Taxol-treated oocytes show similar Mad2 dynamics as control oocytes (40), suggesting that Mad2 does not check for changes of tension at kinetochores in mammalian oocytes.

### 6.3. Bub1

The only evidence observed in mammalian oocytes for the presence of Bub1 comes from a study in mouse oocytes (38). Bub1 was observed on kinetochores of mouse oocytes from GVBD to early A-I stages and disappeared only at late A-I stage. It reappeared on kinetochores in M-II oocytes (38). Treatment of M-II stage oocytes with nocodazole did not change Bub1 at the kinetochores, indicating that Bub1 binding to kinetochores is not dependent on a connection between microtubules and kinetochores once it has bound to kinetochores. The localization of Bub1 appears different from that of Mad1 or Mad2 in the oocytes at metaphase stages (M-I and M-II). The localization is also different from that in mouse somatic cells in which Bub1 also moves away from kinetochores at the metaphase stage (23). The functions of Bub1 in the checkpoint pathway during meiosis are still unknown. Studies in DLD-1, TA-Hela cells, and *Xenopus* egg extracts suggest that Bub1 is a master regulator

required for assembly of the kinetochore signing domain and required for other checkpoint proteins such as CENP-E, BubR1, and Bub3 to bind to kinetochores, thus affecting binding of Mad1 and Mad2 to kinetochores as discussed above (30, 32, 69, 74, 75).

### 6.4. CENP-E

CENP-E is a kinesin-like protein that accumulates in both mitotic and meiotic cells. Studies of CENP-E in mitosis (62, 76, 77), male meiosis (121) and female meiosis (122, 123) revealed the same localization during the cell cycle. It is a transient kinetochore component that binds to kinetochores soon after breakdown of the nuclear envelope (GVBD in female meiosis) and remains fully bound throughout chromatid or chromosome congress to the metaphase plate. In late anaphase or telophase, it is relocated to midzonal microtubules of the spindles (121-123). The functions of CENP-E are complicated. Its main function is thought to be a motor protein to regulate chromosome movement (62, 75, 77-79). However, recent evidence has indicated that CENP-E is a spindle checkpoint protein participating in the kinetochore binding of other checkpoint proteins, such as BubR1, Bub1, Bub3, Mad1 and Mad2 (80-84), thus suggesting that it also participates in the spindle checkpoint pathway. Injection of an anti-CENP-E antibody into mouse oocytes at prophase completely prevented the oocytes from progressing to the A-I stage and all oocytes were blocked at the M-I stage (122), suggesting that CENP-E participates in chromosome movement, rather than the checkpoint pathway. Further experiments are necessary to address these differences observed between mitosis and meiosis.

### 6.5. MAP kinase

It is well known that MAP kinase plays important roles in microtubule organization and spindle formation (51). Recently, experimental evidence indicates that MAP kinase also participates in the spindle checkpoint pathway in some cell lines and *Xenopus* egg extracts (124-127). It was found that active MAP kinase appeared on kinetochores during early prometaphase and became undetectable by mid-anaphase (127). In mammalian oocytes, immunofluorescence staining indicated that subcellular localization of MAP kinase is similar to some checkpoint proteins: MAP kinase was translocated from the cytoplasm to the nucleus and was activated shortly after GVBD (128, 129). When the oocytes reached metaphase, it was localized to the spindle pole and then translocated to the spindle midzone at anaphase (130, 131). The localization of MAP kinase appears to be similar to localization of Mad1 and Mad2 in mouse oocytes. Immunodepletion of MAP kinase prevents checkpoint activation, which can be rescued by adding external MAP kinase (125). In addition, MAP kinase staining on kinetochores could also be enhanced after checkpoint activation by microtubule depolymerization (51). These results indicate that MAP kinase occupies a similar location as Mad1 and Mad2 during the cell cycle. Although the mechanism(s) by which MAP kinase participates in the spindle checkpoint is unknown, it was found that MAP kinase is necessary for phosphorylation of CENP-E. Subcellular staining of MAP kinase and CENP-E is the

same during the cell cycle and both interact *in vivo* (127). Somatic cells that arrested in metaphase through activation of the spindle checkpoint contain active MAP kinase on kinetochores (127). Taken together, these results indicate that MAP kinase is involved in the checkpoint pathway but further studies are needed to solidify these data and elucidate the underlying mechanisms of its action(s).

### 6.6. Cytoplasmic dynein

It has been shown that dynein also participates in the spindle checkpoint mechanisms (98, 101). In mammalian meiosis, we found that dynein was responsible for transport of Mad1 and Mad2 between kinetochores and the cytoplasm (spindle poles) (Zhang et al., unpublished data). This was verified by dynein's co-localization with Mad1 and Mad2. We found that dynein displayed a similar dynamic localization as Mad1 and Mad2 during meiosis I in mouse oocytes. Dynein was mainly localized around the nucleus in the oocytes at GV and ProM-I stages and translocated to the spindle poles in the oocytes at M-I and M-II stages. When the M-I or M-II stage oocytes were treated with nocodazole, dynein moved back to the chromosomes. When dynein's function was inhibited by injection of anti-dynein antibody, transport of Mad1 and Mad2 was inhibited. These results indicate that dynein might participate in the checkpoint pathway by transport of Mad1 and Mad2 between kinetochores and the spindle poles. For checkpoint activation, dynein may bind to Mad1 and Mad2 and transport them to kinetochores and then returns to the cytoplasm; for checkpoint deactivation, dynein may bind to Mad1 and Mad2 that are depleted from kinetochores followed by transport to spindle poles. Therefore, dynein plays an important role in checkpoint activation and silencing. These results are similar to those reported for mitosis. In mitotic cells, prometaphase kinetochores display more dynein signals than metaphase kinetochores (99, 100). Dynein is localized to metaphase kinetochores after depolymerization of microtubules (100). Dynein is also sensitive to the attachment of microtubules to kinetochores (101). Once the attachment starts in early mitosis, dynein detaches from the kinetochores (101). Microinjection of anti-dynein antibody prevents Mad2, Bub1, CENP-E and 3F3/2 phosphoantigen from transport to the spindle poles, prevents Mad2 detachment from the kinetochores, decreases kinetochore tension and causes mitotic arrest at metaphase (98). Therefore, dynein might de-activate the spindle checkpoint after all chromosomes have bioriented at the metaphase plate. In addition, it has been shown that inhibition (132, 133) or overexpression of dynein (100) prevents spindle assembly and chromosome movement indicating that dynein also functions in chromosome movement and spindle assembly.

## 7. EMBRYONIC ANEUPLOIDY

Accurate segregation of chromosomes requires a highly ordered spindle. Mistakes in chromosome distribution and segregation during meiosis result in aneuploid embryo formation, which causes early embryo death, spontaneous abortion, or genetic diseases. Embryonic aneuploidies are produced if abnormal chromosomes are present in sperm, oocyte, or both. Most

embryonic aneuploidies are derived from oocytes and their frequency is high in human embryos produced by IVF. The exact causes are still unclear although maternal aging is thought to play a role as one of the most important risk factors.

### 7.1. Advanced maternal age and embryonic aneuploidy

Human fertility, especially female fertility declines with age. The age related female fertility decline is due to limited follicle development in the ovaries and poor oocyte quality. When older women undergo infertility treatment, external gonadotropins can stimulate a couple of follicles to grow and the oocytes can undergo meiotic maturation. Fertilization and early development of the oocytes appear normal but clinical pregnancy and implantation rates are significantly reduced with maternal ageing (133). This decline in pregnancy and implantation rates is not related to uterine function, as oocyte donation produced very high pregnancy and implantation rates in this group of infertile women (134). Recent studies using preimplantation genetic diagnosis (PGD) indicated that reduced pregnancy and implantation rates in advanced women are related to embryonic aneuploidies (135-137). Although the reason for the increased aneuploid formation in older women is unclear, examination of chromosomes during meiosis I and meiosis II revealed that more than one third of oocytes had chromosome abnormalities and about 40% of the embryos were aneuploid (137, 138). These data suggest that most embryonic aneuploidies are the result of abnormal meiosis of oocytes.

Currently, it is unknown how aging alters oocyte quality and/or chromosome instability. Some possible factors, such as reduced protein accumulating within the oocytes, accumulation of radiation or toxic agents, production of reactive oxygen species (139), mutation of mitochondria DNA (140) and telomere shortening (141), may be responsible for the failure of oocytes to organize a normal meiotic spindle and synthesize the necessary proteins to participate in checkpoint mechanisms.

### 7.2. Abnormal spindle morphology and embryonic aneuploidy

The meiotic spindle is an important structure in the oocyte that is very sensitive to changes in the cytoplasm and environmental factors (110-113), therefore spindle morphology may reflect oocyte quality. Higher fertilization and embryo developmental rates were observed in human oocytes containing intact spindles compared to those without intact spindles as evaluated by using polarized light microscope imaging (142). Abnormal meiotic spindles are observed in human oocytes and their rates increase significantly with maternal age (120). Abnormal spindles in human oocytes are thought to be among the main factors associated with abnormal chromosome segregation and embryonic aneuploidy. This has also been confirmed by improved in vitro temperature control during IVF, in which meiotic spindle stability was correlated with higher pregnancy rates (143).

It is not known how abnormal spindles are formed in oocytes collected from older women. Among a number of

reasons, it is possible that proteins that participate in spindle organization and formation, such as MAP kinase, are reduced in the oocytes. This may result in meiotic spindle instability and morphology changes related to environmental changes. Another reason may include defective checkpoint mechanisms in the oocytes, as reported for porcine oocytes in which in vitro aging significantly reduced Mad2, MAP kinase and other protein expression (41).

### 7.3. Defective checkpoint and embryonic aneuploidy

Theoretically, inhibition or deletion of any protein in the checkpoint pathway would cause aneuploidy (4). This has recently been reported in mammalian female meiosis (39, 40, 42). When anti-Mad1 or Mad2 antibody was injected into GV stage oocytes, anti-Mad injection induced early anaphase (42), abnormal spindle organization (39-42), abnormal chromosome alignment (39, 40) and abnormal chromosome segregation (39, 40, 42). Early anaphase caused premature cell division before the metaphase plate moved to the oocyte cortex and a big first polar body and multiple polar bodies were observed at M-II stage oocytes (42). Chromosome analysis indicated that 16.3% of oocytes had hyperploidy, and most of them had a single extra 21 monovalent in Mad2 depleted mouse oocytes (42). Similar results were obtained in porcine oocytes, as shown in Figure 4. Chromosome abnormalities were observed in about 20% in meiosis I and 32% in meiosis II oocytes when Mad2 was depleted. When oocytes were subjected to in vitro ageing, Mad2 expression was significantly reduced, which in turn caused abnormal chromosome segregation and embryo fragmentation (41).

When anti-CENP-E antibody was injected into mouse GV stage oocytes, all oocytes were blocked at M-I stage during subsequent culture, indicating that anaphase was not initiated in the CENP-E-inhibited oocytes (122). This study suggested that the checkpoint was activated in CENP-E antibody-injected oocytes. However, checkpoint activation needs kinetochore binding of the checkpoint proteins Mad1, Mad2 and others, which requires the presence of CENP-E (83-85), thus it is not clear how the checkpoint was activated in CENP-E-inhibited oocytes. The answer may be found in the complicated functions of CENP-E. CENP-E is not only a checkpoint protein, but it is also a motor protein that participates in chromosome movement (62, 77-79). Thus the CENP-E-caused metaphase arrest in the antibody-injected oocytes is probably due to inhibited chromosome movement, rather than checkpoint activation. Further studies are needed in both mitosis and meiosis, as similar results have also been reported in mitosis (62).

### 7.4. Diagnosis of embryonic aneuploidy

Currently, diagnosis of embryonic aneuploidy is mainly being performed in humans. Aneuploidy is one of the major factors resulting in early embryo loss in human infertility treatment. Clinical physicians and basic scientists have developed technologies for diagnosis of embryonic aneuploidy, referred to as PGD. PGD has been used to detect genetic defects and to screen aneuploid embryos. At present, it is possible to separate aneuploid embryos from

normal embryos and to only transfer normal embryos back to patients thus significantly improving clinical pregnancy and implantation rates. The main technology used in PGD is fluorescence in-situ hybridization (FISH). FISH technology can be used for one-color (one chromosome) or multi colors (multi-chromosomes) at the same time. The same sample can be used to perform FISH for more than two sequential hybridizations, thus all chromosomes can be screened by this technology. Currently, FISH is being performed mainly in early stage embryos, but sperm or oocyte FISH is also possible.

Studies on human spermatozoa reveal that chromosome 21 structural abnormalities occur in 5-10% of cases (144). FISH for 15, 18, X and Y chromosomes had also shown that very high rates of sperm nullisome were present in recurrent pregnancy loss patients (145). By using probes for some other chromosomes, the sperm aneuploidy rate was shown to be higher when sperm parameters are abnormal as compared to men with normal sperm parameters (146). Although the contribution of sperm chromosome abnormalities to embryonic aneuploidy is less when compared to oocyte chromosome abnormalities, such a possibility can not be excluded, especially when no obvious reason is found in maternal sources. FISH in spermatozoa can be used to identify the possible cause for aneuploid embryo formation, but cannot be used for selection of normal sperm for IVF.

Aneuploidy most commonly arises during maternal meiosis. Therefore, clinical aneuploidy diagnosis is being performed in oocytes during both meiosis I and meiosis II. This approach includes analysis of the first and the second polar bodies (147). Testing of oocytes by FISH analysis of polar bodies showed that more than half of the oocytes from advanced women (age 35 years or older) undergoing IVF had abnormal chromosomes, which originated from errors in meiosis I and/or meiosis II (138). The majority of meiosis I abnormalities were represented by chromatid errors, which seems to be the major source for chromosomal abnormalities in the resulting embryos (138). Although polar body aneuploidy analysis could detect the great majority of autosomal aneuploidy, aneuploidy from paternal sources is not detected. Therefore, currently, FISH of polar bodies is usually combined with FISH of blastomeres in human IVF clinics to detect the presence of aneuploidies.

FISH analysis of blastomeres from early stage embryos (usually day 3 at 6-8 cell stages) is the most common and practical method to detect embryo aneuploidies in human IVF clinics. This technology requires both embryo biopsy and blastomere FISH. By using this technology, it has been found that trisomic offspring, spontaneous abortion and multiple gestations (reduced the number of embryos for transfer) have been significantly reduced; accordingly, implantation and pregnancy rates were significantly increased. Currently, PGD of aneuploidy has been used for patients with advanced maternal age, recurrent spontaneous abortions and repeated IVF failure (148).

## 8. PERSPECTIVES

In summary, it appears that at least three groups of proteins participate in the spindle checkpoint signal pathway. The first are "transporting proteins", such as dynein, Zwi10, Rod, and perhaps MAP kinase. These proteins are responsible for transporting checkpoint proteins between kinetochores and cytoplasm (including spindle microtubules and spindle poles). The second are "binding proteins", such as Mps1, Bub1, BubR1 and CENP-E. These proteins bind to kinetochores at very early stages of the cell cycle and remain at kinetochores even when the cells are at the metaphase stage in which the spindle has formed and chromosomes have aligned at the spindle equator. Without these "binding proteins" checkpoint proteins cannot bind to kinetochores and the checkpoint cannot be activated. These proteins bind to kinetochores at almost all stages of the cell cycle (except late A-I stage) and are ready to recruit checkpoint proteins once the connection between microtubules and chromosomes is destroyed. The third are "real" checkpoint proteins, such as Mad1 (perhaps partial) and Mad2. These proteins are the final signal transduction components to perform checkpoint functions. When spindle formation and chromosome alignment are completed, these proteins are immediately released from kinetochores, thus the checkpoint is deactivated or silenced, leading to anaphase onset. In contrast, once the connection between microtubules and chromosomes is affected, they are moved back to kinetochores and re-bind to kinetochores to re-activate the checkpoint, resulting in metaphase arrest. These three groups of proteins work together and form a complicated checkpoint system. When any component (protein) in this system is inhibited, affected or deleted, final checkpoint signal transduction and chromosome segregation will be affected, causing aneuploid formation. The information and evidence on the spindle checkpoint in mammalian meiosis are still limited, thus further studies remain necessary to address the exact mechanism(s) by which aneuploid formation is prevented.

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**Abbreviations:** APC/C: anaphase promoting complex or cyclosome, CENP: centromere proteins, ACA: anticentromere antibody, CREST: calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia, MAD: mitotic arrest deficient, Bub: budding uninhibited by benzimidazole, Zw10: Zeste White 10, Rod: Rough Deal, GV: germinal vesicle, GVBD: GV breakdown, ProM-I: Prometaphase I, M-I: Metaphase I, A-I: anaphase I, T-I: Telophase I, M-II: Metaphase II, A-II: Anaphase II, MAP: mitogen-activated protein, GFP: green fluorescence protein. FISH: fluorescence in-situ hybridization, PGD: preimplantation genetic diagnosis, IVF: in vitro fertilization

**Key Words:** Spindle, Spindle Checkpoint, Meiosis, Oocytes, Embryonic Aneuploidy, Review

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