Review

Biochemical and cytogenetic changes in postovulatory and in vitro aged mammalian oocytes: a predisposition to aneuploidy

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Abstract

Aneuploidy represents the most prevalent genetic disorder of man. Its association with spontaneous abortions, mental and physical retardation, and numerous malignant cells is well-known. Unfortunately, little is known about the causes and even less about the underlying molecular mechanisms of aneuploidy, especially in mammalian germ cells. Although several etiologies have been proposed for describing human aneuploidy, the only consistent finding remains its positive correlation with maternal age. At the outset, it is essential to point out that there exist numerous potential causes and mechanisms for the etiology of aneuploidy. Nevertheless, information about the molecular mechanisms of chromosome segregation in various species is providing a foundation for research designed to investigate the causes and mechanisms of aneuploidy. The intent of this review is to propose that the biochemical reactions and cellular organelles responsible for accurate chromosome segregation become compromised during postovulatory and in vitro oocyte aging; thus, increasing the probability of faulty chromosome segregation. Recent data have shown that the efficacies of the spindle assembly checkpoint and the chromosome cohesion proteins diminish as oocytes age postovulation and during in vitro culture. Such changes represent potential models for studying aneuploidy. Prior to describing the biochemical and cellular organelle changes found in aged oocytes and their effect on chromosome segregation, an overview of the molecular details surrounding chromosome segregation is presented.

Introduction

This review is based on the premise that postovulatory and in vitro oocyte aging are accompanied by a progressive and functional deterioration of the biochemical pathways and cellular organelles responsible for chromosome segregation. This review does not delve into the extensive literature dealing with the relationship between maternal age and aneuploidy.

The identification, chronology, and interaction of the biochemical events associated with chromosome segregation are undergoing extensive investigation - mainly in models other than mammalian oocytes. Such data provide a foundation for designing experiments to study aneuploidy in mammalian germ cells. To preserve genomic integrity, the interactions between unique biochemical pathways and cellular organelles must be choreographed precisely during mitosis and meiosis. Disarray among these events can result in aneuploidy. At the least, accurate chromosome segregation requires the temporally-coordinated interaction among: protein kinases and phosphatases, topoisomerases, DNA decantination, chiasmata resolution, chromatin condensation, microtubule kinetics, centrosomes, kinetochore-microtubule attachment and tension, kinetochores and their associated proteins, motor and passenger proteins, chromosome biorientation, spindle checkpoint proteins, anaphase promoting complex, proteasomes, securin, cohesin, and separin proteins. Furthermore, the complexity of studying aneuploidy is illustrated by the approximate 5,000 yeast genes that have been directly or indirectly associated with chromosome segregation. Many of these genes are also found in humans, and it appears that the basic mechanisms of chromosome segregation are similar between budding yeast (*Saccharomyces cervisiae*), fission yeast (*Schizosaccharomyces pombe*), and other eukaryotes (Yanagida 2005).

Numerous reports have described biochemical and cellular organelle changes in aged oocytes. However, relatively few studies were designed to concomitantly study such changes and aneuploidy under the same experimental design. Those that have done so, found a positive correlation between biochemical and cellular organelle alterations and aneuploidy (Emery *et al.* 2005, Mailhes *et al.* 1998, Plachot *et al.* 1988, Rodman 1971, Sakurada *et al.* 1996, Yamamoto & Ingalls 1972). It is suggested that experimental manipulation of the reported changes in aged oocytes can be used as models for investigating some of the numerous potential mechanisms of aneuploidy. Recent data from mammalian oocytes showed that degradation of spindle-assembly checkpoint (SAC) proteins (Homer *et al.* 2005a, Steuerwald *et al.* 2005) and chromosome cohesion proteins (Hodges *et al.* 2001, 2005, Prieto *et al.* 2004) increased the probability of premature centromere separation (PCS) and aneuploidy. The reader is referred to other reviews involving aneuploidy in male germ cells (Adler *et al.* 2002, Handel *et al.* 1999) and neoplastic cells (Bharadwaj & Yu 2004, Rajagopalan & Lengauer 2004, Yuen *et al.* 2005).

Overview of Mammalian Oocyte Aneuploidy Research

Although aneuploidy represents the greatest genetic affliction of man, little is known about its causes and even less about its underlying molecular mechanisms, especially in mammalian germ cells. Human aneuploidy is linked with embryonic loss, mental and physical anomalies in newborns, and cancer. Approximately 10-30% of human zygotes (Ford 1981, Hansmann 1983, Hassold & Hunt 2001), 50% of spontaneous abortuses (Bond & Chandley 1983, Hook 1985a), and 0.31% (204/64887) of human newborns (Hecht & Hecht 1987) have an abnormal chromosome number. Furthermore, data based on cytogenetic analyses of human oocytes and preimplantation embryos indicate that over 50% are aneuploid (Kuliev *et al.* 2003, Magli *et al.* 2001, Munne 2002). When considering preimplantation genetic diagnosis for aneuploidy, it seems relevant to note that within embryonic variation was reported among blastomeres when FISH technology was employed (Coulam *et al.* 2007).

For decades, numerous hypotheses have been proposed for the etiology of human germ cell aneuploidy. However, the only constant finding remains its positive correlation with maternal age (Bond & Chandley 1983, Chandley 1987, Hook 1985b). Even for this relationship, definitive data about the underlying mechanisms are lacking (Pellestor *et al.* 2005, Warburton 2005). Besides maternal age, other findings also appear relevant for understanding the genesis of germ cell aneuploidy. Earlier reports showed that the incidence of aneuploidy for specific chromosomes occurred more frequently during female meiosis I than either meiosis II or male meiosis (Bond & Chandley 1983, Hassold & Sherman 1993, Hook 1985b). However, this finding has recently been questioned (Rosenbusch 2004). Also pertinent are the reports showing that certain human chromosomes are more susceptible to missegregation than others (Hassold 1985, Hassold & Hunt 2001, Hassold *et al.* 1984, Lamson & Hook 1980, Nicolaidis & Petersen 1998). Such variability among chromosomes requires prudence when only specific chromosomes (instead of the entire complement) are used to estimate the incidence of aneuploidy.

Another significant factor for consideration is the sexual dimorphism that exists for both spontaneous and induced germ cell aneuploidy (Eichenlaub-Ritter *et al.* 1996, Pacchierotti *et al.* 2007). Currently, data are unavailable from a study that was specifically designed to evaluate gender differences for mammalian germ cell aneuploidy. Other distinctive features of meiosis include the influence of neighboring somatic cells on germ cell differentiation and entry into meiosis (Geijsen *et al.* 2004, Toyooka *et al.* 2003) and the intrinsic sexual dimorphism between oogenesis and spermatogenesis (Handel & Sun 2005, Hodges *et al.* 2001). Thus, the fundamental distinctions between oogenesis and spermatogenesis, the differential susceptibility among chromosomes, the differences between mitosis and meiosis, and the numerous potential mechanisms demonstrate the complexity of studying aneuploidy.

Although it is not surprising that a unique precept explaining the etiology of aneuploidy has been adopted, it is now generally accepted that both nondisjunction and PCS represent primary events that lead to human aneuploidy (Anahory *et al.* 2003, Angell 1991, Angell *et al.* 1994, Pellestor *et al.* 2006, Wolstenholme & Angell 2000). Moreover, it appears that aneuploidy more often results from PCS of sister chromatids than from nondisjunction of whole chromosomes (Plachot 2003, Rosenbusch 2004). Additionally, variation has been shown to exist among specific chromosomes for the probabilities of both PCS and nondisjunction (Eichenlaub-Ritter 2003, Pellestor *et al.* 2003, Sun *et al.* 2000).

Early germ cell studies concentrated on the ability of various chemicals (mainly those that damaged microtubules) to induce aneuploidy (Adler 1990, 1993, Allen *et al.* 1986, Eichenlaub-Ritter 1996, Mailhes 1995, Mailhes *et al.* 1986, Miller & Adler 1992). Other investigations involved assay development and validation (Pacchierotti 1988, Mailhes & Marchetti, 1994, Eastmond *et al.* 1995, Parry *et al.* 1995) and gender differences (Eichenlaub-Ritter 1996, Eichenlaub-Ritter *et al.* 1996, Pacchierotti *et al.* 2007, Wyrobek *et al.* 1996). Based on the results from some of these studies, a broad-working hypothesis emerged. Several investigators proposed that endogenous-and exogenous-induced perturbations during the temporal sequence of oocyte maturation (OM) predispose oocytes to aneuploidy (Eichenlaub-Ritter 1993, Hansmann & Pabst, 1992, Mailhes & Marchetti 1994a). This proposal suggested that an induced temporal disarray (usually detected as a transient delay during metaphase I) among the cellular organelles and biochemical reactions controlling OM increased the probability of aneuploidy.

Although considerable data have shown that a chemically-induced delay during OM is often associated with chromosome missegregation, exceptions can be found. Phorbol 12,13-dibutyrate (Eichenlaub-Ritter 1993), colchicine (Mailhes & Yuan 1987), vinblastine sulfate (Mailhes & Marchetti 1994a, Russo & Pacchierotti 1988), griseofulvin (Marchetti & Mailhes 1995, Mailhes *et al.* 1993, Tiveron *et al.* 1992) induced both meiotic delay and aneuploidy. Conversely, isobutyl-1-methylxanthine and forskolin caused meiotic delay, but not aneuploidy (Eichenlaub-Ritter 1993), while etoposide treatment resulted in aneuploidy without meiotic delay (Mailhes *et al.* 1994, Tateno & Kamiguchi 2001). Consistency among the results from different studies regarding chemically-induced meiotic delay and oocyte aneuploidy cannot necessarily be expected due to: different experimental protocols, exposure of cells to compounds with diverse and often multiple modes of action, and the numerous potential mechanisms of aneuploidy (Mailhes 1995, Pacchierotti & Ranaldi 2006).

More recent studies have combined immunocytochemical techniques with cytogenetic analyses to affirm a positive correlation between oocyte meiotic spindle abnormalities and aneuploidy (Eichenlaub Ritter *et al.* 1996, Mailhes *et al.* 1999). Additional studies employing small molecule, cell-permeable inhibitors of specific biochemical reactions during cell division showed that the proteasome and calpain inhibitor MG-132 (Mailhes *et al.* 2002), the protein phosphatase 1 and 2A inhibitor okadaic acid (Mailhes *et al.* 2003a), and the Eg5 kinesin inhibitor monastrol (Mailhes *et al.* 2004) induced aneuploidy in mouse oocytes, while the tyrosine inhibitor vanadate resulted in spontaneous oocyte activation (Mailhes *et al.* 2003b).

Considerable research is being devoted to unraveling the molecular events underlying chromosome segregation, mainly in non-mammalian somatic cells (Lee & Orr-Weaver 2001, Nasmyth 2001, Uhlmann 2003a). The complexity of understanding the multifaceted events comprising chromosome segregation is illustrated by the approximately 5,000 yeast genes involved with chromosome segregation; many of these yeast genes have also been found in humans (Yanagida 2005). Chromosome segregation requires the temporally-coordinated interaction among: topoisomerases, chiasmata resolution, chromatin condensation, protein kinase and phosphatase reactions, microtubule kinetics, centrosomes, kinetochore-microtubule attachment and bipolar tension, kinetochores and their associated proteins, anaphase promoting complex, proteasomes, and cohesion, securin, and separin proteins. Although these events generally transcend among species and cell types (Yanagida 2005), little is actually known about the molecular mechanisms of chromosome segregation in mammalian oocytes (Collins & Crosignani 2005).

Thus, it emerges that the current state of mammalian germ cell aneuploidy research is mainly descriptive with little information about the underlying molecular mechanisms. It seems that the status of aneuploidy research can be summarized by an earlier statement, “The fact is that we are really not very much nearer today to pinning down the responsible mechanisms than we were twenty years ago when the human aneuploid conditions were first identified” (Bond & Chandley 1983).

Oocyte maturation

Disarray among the numerous events that occur during OM may lead to faulty chromosome segregation. Mammalian oogenesis is controlled by FSH, LH, autocrine and paracrine signaling, and unique growth factors (Anderiesz *et al.* 2000, Hiller 2001). Meiosis begins in the fetal ovary and is later arrested postpartum at the diplotene/ dictyate stage during meiosis I. Unless human oocytes undergo atresia, they remain in diplotene for decades until meiosis resumes prior to ovulation. Following proper hormonal stimulation, oocytes undergo the transition from diplotene to metaphase II (MII). This transition represents OM and involves nuclear and cytoplasmic remodeling and reduction to the haploid state (Dekel 1988, Racowsky 1993, Schultz 1986, Schultz 1988, Schultz *et al.* 1983). Upon completing OM, mammalian oocytes remain in MII for a limited time period until fertilization, spontaneous activation, or atresia. In most mammals, MII oocytes are ovulated and primed for fertilization, which initiates anaphase II. Among marine invertebrates, amphibians, fish, and mammals, species-dependent protein modifications by kinases and phosphatases account for differences in the initiation and the orderly temporal sequence of events during OM (Yamashita *et al.* 2000).

The intraoocyte titer of cyclic adenosine monophosphate (cAMP) influences the initiation of mammalian OM. Elevated levels of cAMP favor cAMP-dependent kinase activity and the retention of oocytes in the diplotene/dictyate stage of meiotic prophase. Conversely, low cAMP levels shift the equilibrium toward cAMP-dependent phosphatase activity, which is needed for activating maturation promoting factor (MPF) and the progression of OM (Boernslaeger *et al.* 1986, Dekel 1988, Dekel 2005, Downs *et al.* 1989, Racowsky 1993, Schultz 1988, Schultz *et al.* 1983). MPF is composed of a 34 kDa catalytic subunit ($p34^{cdc2}$) that exhibits serine-threonine kinase activity and a 45 kDa cyclin B regulatory subunit. In addition to low cAMP levels, MPF activation also requires that $p34^{cdc2}$ be dephosphorylated at the tyrosine 15 residue and coupled with cyclin B. Conversely, tyrosine phosphorylation deactivates MPF (Dunphy & Kumagai 1991, Gautier *et al.* 1991, Strausfeld *et al.* 1991). MPF activity oscillates; it is highest during metaphase and lowest during anaphase (Arion *et al.* 1988, Draetta & Beach 1988), fertilization (Choi *et al.* 1991, Collas *et al.* 1993, Fulka *et al.* 1992), and partheneogenesis (Barnes *et al.* 1993, Collas *et al.* 1993, Kikuchi *et al.* 1995).

Besides MPF, other kinases and phosphatases also play significant roles during OM (Swain & Smith 2007). Mitogen-activated protein kinases (MAPKs) represent serine-threonine protein kinases that phosphorylate many of the same sites as active MPF (Fan & Sun 2004, Lee *et al.* 2000, Murray 1998, Takenaka *et al.* 1998). MAPKs mediate intracellular signal transmission in response to external stimuli, participate in assembling the first meiotic spindle, and prevent rodent oocytes from entering interphase during the interval between meiosis I and II (Gordo *et al.* 2001, Sobajima *et al.* 1993, Verlhac *et al.* 1994). Unlike MPF, MAPK activity remains high throughout OM.

Mos, the c-mos protooncogene product, represents another serine-threonine kinase that is active during OM (Paules *et al.* 1989, Sagata 1997, Singh & Arlinhgaus 1997). It helps activate the MAPK pathway (Dekel 1996) and functions as a cytostatic factor by preventing oocytes from prematurely exiting MII (Hashimoto 1996, Sagata 1996). Oocytes from c-mos deficient mice fail to arrest at MII and subsequently undergo spontaneous partheneogenic activation (Colledge *et al.* 1994, Hashimoto 1996, Hashimoto *et al.* 1994). In addition to their roles during OM, the kinases MPF, MAPKs, and Mos also have essential roles during the SAC, the anaphase promoting complex/cyclosome (APC), and the metaphase-anaphase transition (MAT) (Dekel 1996, Dorée *et al.* 1995, Hyman & Mitchison 1991, Karsenti 1991, Murray 1998).

Correct temporal and synchronous interactions between specific enzymes and their target compounds are required for OM and the MAT, and faulty kinase and phosphatase activities have been shown to lead to downstream errors resulting in chromosome missegregation. Based on their antagonistic effects, relative to the degree of tyrosine $p34^{cdc2}$ phosphorylation, unique kinase and phosphatase inhibitors have the potential for altering the rate of OM and for inducing spindle defects and aneuploidy in rodent oocytes (Jesus *et al.* 1991). Okadaic acid (OA) specifically inhibits the protein phosphatases 1 (PP1) and 2A (PP2A) that dephosphorylate serine and threonine residues (Cohen *et al.* 1990, Schönthal 1992). Following OA treatment of mouse oocytes and one-cell zygotes, hyperphosphorylation was noted in conjunction with abnormalities involving spindle fibers, multipolar spindles, kinetochores, and chromosome alignment (De Pennart *et al.* 1993, Schwartz & Schultz 1991, Vandre & Willis 1992, Zernicka-Goetz *et al.* 1993). Also, elevated frequencies of PCS and aneuploidy were found in mouse oocytes exposed to OA (Mailhes *et al.* 2003a). These effects may have been influenced by OA-induced hyperphosphorylation of microtubule organizing centers and microtubule-associated proteins (MAPs) (Schwartz & Schultz 1991, Vandre and Willis 1992) and that hyperphosphorylated MAPs have a reduced affinity for microtubules (Zernicka-Goetz *et al.* 1993).

Furthermore, the kinase inhibitor 6-dimethylaminopurine (6-DMAP) disrupts $p34^{cdc2}$ kinase and MAPK activities and prevents meiotic progression of mouse oocytes (Rime *et al.* 1989, Szollosi *et al.* 1991, 1993). Protein phosphorylation and germinal vesicle breakdown (GVBD) were repressed when dictyate mouse oocytes were exposed to 6-DMAP prior to (GVBD); conversely, expulsion of the first polar body was inhibited when oocytes were exposed after GVBD (Rime *et al.* 1989). Other data showed that 6-DMAP inhibited protein phosphorylation in activated mouse MII oocytes and resulted in premature disappearance of phosphorylated proteins coupled with abnormalities involving polar body extrusion and pronuclei formation (Szollosi *et al.* 1993). Additionally, the pattern of protein dephosphorylation events noted in postovulatory and in vitro aged oocytes was correlated with increased frequencies of spontaneous oocyte activation and PCS (Angell 1994, Dailey *et al.* 1996).

A selected list of compounds associated with the metaphase-anaphase transition (MAT) during mitosis and meiosis and their general function is presented in Tables 1A and B. Such a listing is noncomprehensive and will certainly be modified and expanded as additional data become available.

The metaphase-anaphase transition (MAT) during mitosis and meiosis

Prior to the MAT and chromosome segregation, numerous events require coordination. These include: chromatin condensation, microtubule polymerization and their capture by kinetochores, correction of erroneous microtubule-kinetochore interactions, generation of microtubule-kinetochore tension, formation of a stable bipolar spindle, satisfaction of the spindle assembly checkpoint, removal of linkages between sister chromatid arms, and temporally-coordinated removal of centromeric cohesion proteins.

Although chromosome segregation during meiosis appears to largely depend on mechanisms analogous to those of mitosis, both general cell-cycle regulators and unique proteins have been identified during meiosis (Nasmyth 2001). Three major modifications of the mitotic machinery occur during meiosis. First, synapsis and recombination (chiasmata formation) occur between homologues prior to anaphase I. Second, the two sister chromatids of each chromosome must segregate syntelically while the homologues segregate amphitelically at anaphase I. Third, the cohesion between sister chromatid centromeres must remain intact until anaphase II onset in order for sister chromatids to segregate amphitelically.

Before discussing the cytologic and biochemical changes reported in aged oocytes and their effect on chromosome segregation, an overview of the physical and chemical linkages between chromosomes, kinetochore-microtubule interactions, the spindle checkpoint assembly complex, and the metaphase-anaphase transition is presented.

Resolution of DNA catenations, chromatin condensation, and removal of cohesion arm proteins

Following DNA replication, sister chromatids are linked by DNA double-strand catenations and cohesion proteins. These physical and chemical linkages help prevent precocious separation prior to anaphase onset, which can result in aneuploidy. However, these linkages must be timely removed so that sister chromatids orient syntelically at meiotic anaphase I and undergo amphitelic orientation during meiotic anaphase II and mitotic anaphase. Most of the DNA catenations on chromosome arms are lost prior to prophase; whereas, the majority of chromosome arm cohesin proteins are removed during prophase. However, it is essential that centromeric catenations and cohesions remain intact until correct kinetochore-microtubule attachment and tension have been attained. Otherwise, premature loss of centromeric cohesion inevitably predisposes cells to abnormal chromosome segregation.

Abnormal function of proteins required for establishing and maintaining the physical linkages between sister chromatids may result in aneuploidy and apoptosis. The Spo11 protein helps initiate meiotic recombination by generating DNA double-strand breaks, and disruption of Spo11 activity in mouse spermatocytes and oocytes resulted in synaptic-deficient germ cells and apoptosis (Baudat *et al.* 2000, Romanienko *et al.* 2000). Also, the synaptonemal complex protein 3 (Sycp3) helps maintain the structural integrity of meiotic chromosome axes. Mutant Sycp3 mammalian oocytes were ineffective in repairing DNA double-strand breaks and exhibited higher frequencies of aneuploidy (Wang & Hoog 2006).

Table 1A. Selected regulators of mitosis and meiosis.

Cohesion complex subunit proteins identified during mitosis	Cohesion complex subunit proteins identified during meiosis	Spindle assembly checkpoint (SAC) proteins
Smc1 and Smc3 (structural maintenance of chromosomes) – core cohesion complex subunit proteins.	**Smc1α** – replaces mitotic Smc1.	**Mad1** (mitotic-arrest deficient) – helps recruit Mad2 to kinetochores that lack tension and attachment. Forms a complex with Cdc20, Mad2, and Mad3.
Scc1/Rad21/Mcd1 (sister chromatid cohesion) – cleaved by separase at mitotic anaphase onset.	**Smc1β** – replaces mitotic Smc3.	**Mad2** – forms a complex with Cdc20, Mad1, and Mad3 and inhibits APC^{Cdc20} activity.
Scc3 (SA1/STAG1, SA2/STAG2) – phosphorylated by Aurora B and Plk1 kinases.	**STAG3** – replaces mitotic Scc3.	**Mad3/BubR1** – helps recruit Mad1 and Mad2 to kinetochores that lack attachment and tension; forms a complex with Cdc20, Mad1, Mad2, and Bub3.
Scc2 and Scc4 – enhance the binding of Scc1 and Scc3 to kinetochores that lack attachment and tension.	**Rec8** – replaces mitotic Scc1.	**Bub1** (budding inhibited by benzimidazole) – a serine -threonine protein kinase that binds with Bub3, Mad1, Mad2, Mad3, and CenP-E and helps recruit Shugoshin proteins to kinetochores.
		Bub2/Mps1 – helps regulate APC^{Cdh1}, mitotic exit, chromosome replication, and cytokinesis.
		Bub3 – binds with Bub1 and Mad3 and helps regulate APC activity.

Table 1B (continued from **1A**). Selected regulators of mitosis and meiosis.

Other compounds	**Function**
APC/C (anaphase promoting complex/cyclosome)	A 20S multi-subunit ligase that ubiquinates specific proteins targeted for proteolysis by proteasomes. APC^{cdc20} targets securin for proteolysis at the MAT; whereas, APC^{cdh1} targets mitotic cyclins and other substrates for degradation at mitotic exit. The cdh1 protein activates the APC from late anaphase through G1.
Astrin	A microtubule and kinetochore protein that has roles involving sister chromatid adhesion, centrosome integrity, and separase activity.
Aurora B kinase-Survivin-Inner Centromeric Protein-Borelin	A chromosome passenger protein complex with multiple roles: recruits SAC proteins and CenP-E to kinetochores lacking tension, reduces the affinity of Scc1 and Scc3 to chromatin via phosphorylation, helps coordinate correct kinetochore-microtubule attachments, and cytokinesis.
Cdc20 (cell division cycle 20)	Helps activate the APC when not bound by SAC proteins, recruits substrates to the APC, and forms a complex with Mad2, Mad3, and Bub3.
Cdks (Cyclin-dependent kinases)	Enzymes composed of a kinase subunit and an activating cyclin subunit. Cdks are needed for kinase activity.
CenP-E (centromeric protein E)	A motor protein that facilitates kinetochore-microtubule stabilization, binding of SAC proteins to kinetochores, and enhanced Mad3 activity.
Dynein/Dynactin	A microtubule motor protein required for the removal of the Rod-Zw10-Zwilch complex, Mad1, Mad2, and Mad3 from properly aligned kinetochores.
Kinesin	A microtubule motor protein.
MAPK/Mps1 (mitogen-activated protein kinase)	A serine-threonine kinase that helps recruit CenP-E to kinetochores. It also interacts with Mos protein for MPF activation.
MCAK/Kip 2-3 (microtubule centromere-associated kinesin)	Depolarizes microtubules and helps correct aberrant kinetochore-microtubule attachments.
Monopolin/Mam1/CdcPlk	Facilitates amphitelic orientation of homologues and syntelic orientation of sister chromatids during meiosis I.
Mos	The protein product of the *c-mos* proto-oncogene. Mos is an active component of a cytostatic factor. In conjunction with cyclin-dependent kinase 2, Mos is required for the metaphase II arrest of mature mouse oocytes and for activating MAPK.
MPF (maturation promoting factor)	A protein kinase comprising $p34^{cdc2}$/Cdk1 and cyclin B. MPF phosphorylates and helps regulate chromosome condensation, nuclear envelope breakdown, and spindle formation.
Op18/Stathmin (oncoprotein 18)	A protein that destabilizes microtubules; it is inhibited by phosphorylation.
P31/Cmt2	A protein involved with changing the stereo-configuration of Mad2.
Plk1 (Polo-like kinase 1)	A serine-threonine kinase that phosphorylates Scc1, Scc3, and Rec8 and reduces their affinity to chromosome arms.
PP2A (protein phosphatase 2A)	Dephosphorylates Sgo1 and supports Rec8 maintenance.
Proteasomes	Proteinase complexes that degrade intracellular ubiquinated compounds.
Rod-Zw10-Zwilch	A protein complex that helps recruit dynein, Mad1, and Mad2 to unaligned kinetochores.
Securin/Pds1/Cut2p	An APC substrate that binds to and inhibits separase activity.
Separase/Esp1	A protease that is inactive when bound by securin. However, upon securin proteolysis, separase is free to cleave centromeric Scc1 cohesions at mitotic anaphase onset, Rec8 at chromosome arms at meiotic anaphase I onset, and centromeric Rec8 at meiotic anaphase II onset.
Shugoshins (Sgo1 & Sgo2)	Sgo1 is a conserved eukaryotic kinetochore protein that protects centromeric Rec8 from separase activity during meiosis I, but not during meiosis II. Sgo1 enhances dephosphorylation and cohesion removal by recruiting PP2A to kinetochores. Shugoshins also have roles in chromosome congression, kinetochore-microtubule attachment, and syntelic orientation of sister chromatids during meiotic anaphase I
Slk19p	The *Saccharomyces cervisiae* Slk19p gene product is needed for proper chromosome segregation during meiosis I.
Sororin	An APC protein substrate that interacts with Shugoshins to facilitate cohesion binding to chromatin.
Spindly	A protein that helps inactivate the APC and participates with dynactin in recruiting dynein to kinetochores.
Spo11	Helps initiate meiotic recombination.
Sycp3	Helps maintain the structural integrity of meiotic chromosome axes.
Topoisomerase II (Topo II)	An enzyme that disrupts intercalated loops of DNA and then reanneals the DNA broken ends.
UbcH10	An enzyme that ubiquinates Cdc20. This facilitates the release of Mad2 and BubR1 from Cdc20, inactivates the SAC, and helps activate the APC.
Usp44	An enzyme that deubiquinates Cdc20. This enhances the retention of Mad2 by Cdc20, promotes SAC activity, and inhibits APC activation.

Besides Spo11 and Sycp3, other proteins also participate in resolving chiasmata, condensing chromatin, and facilitating chromatid cohesion and separation. Topoisomerase II (topo II) disrupts the intercalated loops on adjacent chromatids by catalyzing a DNA double-strand break in one of the sister chromatids. This enables the other sister chromatid to pass through the broken ends followed by topo II re-annealing the broken ends (Champoux 2001, Downes *et al.* 1991, Holm *et al.* 1989, Rose *et al.* 1990, Wang 2002). Sister chromatids remained physically linked and fail to separate during anaphase in cells lacking topo II activity (Dinardo *et al.* 1984). Thus, topo II activity is required for the transition from prophase to metaphase I (MI) in mouse spermatocytes (Cobb *et al.* 1997) and for proper chromosome segregation in mammalian somatic cells (Gorbsky 1994), mouse oocytes (Mailhes *et al.* 1994), and mouse spermatocytes (Marchetti *et al.* 2001). Besides topo II, other proteins also help disentangle and condense chromatin. The structural maintenance of chromosome proteins (Smc 2 and Smc4) bind to chromatid axes and help disentangle and condense sister chromatids and homologues during prophase and prometaphase (Hagstrom *et al.* 2002, Lavoie *et al.* 2002, Ono *et al.* 2004).

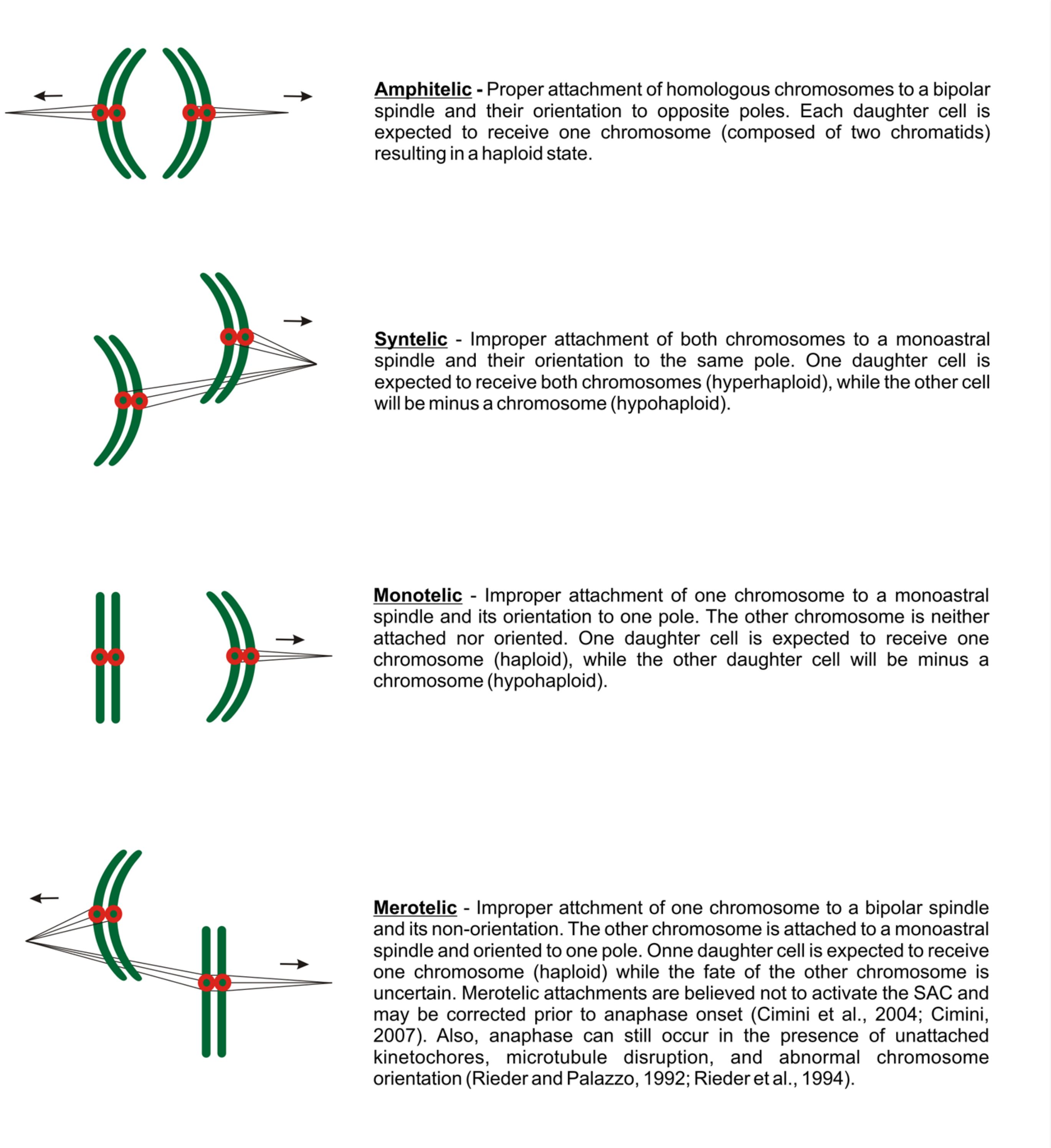
In addition to the physical linkages between sister chromatids, highly-conserved, multi-subunit protein cohesin complexes adhere to eukaryotic chromosomes and help conjoin sister chromatids and homologues (Marston & Amon 2004, Nasmyth & Schleiffer 2004). Some of the cohesin subunits differ between mitotic and meiotic cells (van Heemst & Heyting 2000). Such distinctions may reflect the need for maintaining cohesion during meiotic recombination and the requirement for sister chromatids to undergo syntelic segregation during meiotic AI and amphitelic segregation during AII (Revenkova & Jessberger 2005). Eukaryotic mitotic cells encode homologs of the Scc1/Rad21, Scc3 (SA1/STAG1, SA2/STAG2), Smc1, and Smc3 cohesion protein subunits (Haering & Nasmyth 2003, Parra *et al.* 2004, Prieto *et al.* 2002). Both Scc1 and Scc3 enhance cohesion by binding to numerous sites on chromosomes, while the core subunit proteins Smc1 and Smc3 are needed for both sister chromosome cohesion and DNA recombination (Eijpe *et al.* 2000, Haering *et al.* 2002, Lavoie *et al.* 2002, Petronczki *et al.* 2003). Scc2 and Scc4 represent a separate protein complex in yeast that facilitates the binding of cohesion proteins to centromeres and chromosome arms (Ciosk *et al.* 2000). The following differences in cohesion subunits have been found in meiotic cells: Rec8 replaces Scc1 in both budding yeast (Klein *et al.* 1999, Watanabe & Kitajima 2005) and mammals (Eijpe *et al.* 2003, Parisi *et al.* 1999); STAG3 replaces the Scc3 subunits SA1 and SA2 in mammals (Prieto *et al.* 2001, 2004); Smc1α and Smc1β replace Smc1 (Revenkova *et al.* 2001); and homologs for Smc3 have not been identified.

Cohesin proteins must remain located on centromeres until anaphase onset. Otherwise, early or nonremoval can result in PCS or nondisjunction, respectively. The retention and removal of cohesion proteins require the activities of unique kinase, phosphatase, separase, and Shugoshin proteins. During mitotic prophase-prometaphase and meiotic MII, most of the arm cohesins are lost following phosphorylation of the Scc1 and Scc3 cohesin subunits by Aurora B kinase and Polo-like kinases (PLKs) (Alexandru *et al.* 2001, Clyne *et al.* 2003, Hauf *et al.* 2005, Lee & Amon 2003, Losada *et al.* 2002, Sumara *et al.* 2002, Yu & Koshland 2005). On the other hand, loss of centromeric cohesin is mediated by separase cleavage of Scc1 during mitotic anaphase onset (Uhlmann *et al.* 2000a, Uhlmann 2001, Waizenegger *et al.* 2000). Additionally, phosphorylation by PLKs also enhances the removal of centromeric cohesins (Clarke *et al.* 2005, Dai *et al.* 2003, Goldstein 1980, Lee *et al.* 2005). Although PLK phosphorylation has been detected during meiosis in female mice and the first zygotic division, its multi-faceted role requires additional investigation (Pahlavan *et al.* 2000). As will be mentioned later, both Aurora B kinase and PLKs have additional functions during cell division.

During meiosis I, DNA catenations and chromosome arm cohesins must be removed prior to anaphase so that homologues segregate amphitelically and sister chromatids segregate syntelically. Such removal of the meiosis-specific Rec8 cohesin protein on chromosome arms during meiosis I is facilitated by separase. However, it is essential that centromeric Rec8 remain intact between sister chromatids during anaphase I so that they can undergo syntelic orientation (Pasierbek *et al.* 2001, Siomos *et al.* 2001). Rec8 displays a similar pattern of localization in mammalian oocytes and spermatocytes and yeast; it is lost from chromosome arms during the MI-AI transition and from sister centromeres at the onset of AII (Lee *et al.* 2003, 2006).

Mammalian and yeast cells that lack cohesin proteins exhibited elevated frequencies of PCS and chromosome missegregation (Hoque & Ishikawa 2002, Michaelis *et al.* 1997, Sonoda *et al.* 2001, Tanaka *et al.* 2000). The *Saccharomyces cerevisiae* Slk19p gene is required for proper chromosome segregation during meiosis I. Slk19p mutants failed to maintain Rec8 at centromeres during anaphase I and displayed elevated levels of PCS and improper amphitelic segregation of sister chromatids (Kamieniecki *et al.* 2000). Phosphorylation of Rec8 facilitates its cleavage; whereas, dephosphorylation of Rec 8 by PP2A maintains centromeric cohesion during meiosis I (Kitajima *et al.* 2006, Riedel *et al.* 2006). Okadaic acid (OA) functions as a phosphatase 1 and 2A inhibitor, and exposure of mouse oocytes to OA resulted in elevated frequencies of PCS in both MI and MII oocytes and in aneuploid MII oocytes. The higher frequencies of PCS noted in oocytes was proposed to result from an OA-induced shift in the kinase-phosphatase equilibrium that favored enhanced kinase activity (Mailhes *et al.* 2003a).

Centromeric Rec8 must be protected from separase activity during meiosis I in order to facilitate syntelic orientation of sister chromatids during AI. This is enhanced by a group of evolutionarily-conserved eukaryotic Shugoshin (Sgo) proteins and



Amphitelic - Proper attachment of homologous chromosomes to a bipolar spindle and their orientation to opposite poles. Each daughter cell is expected to receive one chromosome (composed of two chromatids) resulting in a haploid state.

Syntelic - Improper attachment of both chromosomes to a monoastral spindle and their orientation to the same pole. One daughter cell is expected to receive both chromosomes (hyperhaploid), while the other cell will be minus a chromosome (hypohaploid).

Monotelic - Improper attachment of one chromosome to a monoastral spindle and its orientation to one pole. The other chromosome is neither attached nor oriented. One daughter cell is expected to receive one chromosome (haploid), while the other daughter cell will be minus a chromosome (hypohaploid).

Merotelic - Improper attchment of one chromosome to a bipolar spindle and its non-orientation. The other chromosome is attached to a monoastral spindle and oriented to one pole. Onne daughter cell is expected to receive one chromosome (haploid) while the fate of the other chromosome is uncertain. Merotelic attachments are believed not to activate the SAC and may be corrected prior to anaphase onset (Cimini et al., 2004; Cimini, 2007). Also, anaphase can still occur in the presence of unattached kinetochores, microtubule disruption, and abnormal chromosome orientation (Rieder and Palazzo, 1992; Rieder et al., 1994).

Figure 1. Kinetochore-microtubule attachments and probable outcomes during meiosis I.

their orthologs (Katis *et al.* 2004a, Kitajima *et al.* 2004, Salic *et al.* 2004). Sgo1 in budding yeast (Kitajima *et al.* 2004) and its paralogue Sgo2 in fission yeast (Rabitsch *et al.* 2004) were initially identified and require Bub1 for proper centromeric localization (Kitajima *et al.* 2004). Subsequently, human and mouse Sgo1 and Sgo2 proteins were recognized (McGuinness *et al.* 2005, Tang *et al.* 2004, Watanabe & Kitajima 2005). During mitosis and meiosis in higher eukaryotes, Sgo1 helps maintain sister centromere cohesion by protecting centromeric Rec 8 from separase until sister chromatids undergo amphitelic segregation at anaphase II onset (Goulding & Earnshaw 2005, Kitajima *et al.* 2004, Marston *et al.* 2004, Tang *et al.* 2004, Watanabe & Kitajima 2005). In budding yeast, Sgo1disappears during anaphase I (Kitajima *et al.* 2004, Rabitsch *et al.* 2004); whereas, fission yeast Sgo2 persists until meiosis II (Katis *et al.* 2004a, Kitajima *et al.* 2004). Sgo2 in fission yeast represents a paralogue of Sgo1 and is required for chromosome congression at metaphase, proper kinetochore-microtubule attachment, and syntelic orientation of sister chromatids during AI (Kitajima *et al.* 2004, Rabitsch *et al.* 2004). Depletion of either Sgo1 (Wang & Dai 2005) or Sgo2 (Kitajima *et al.* 2004, Rabitsch *et al.* 2004) during meiosis I led to PCS and chromosome missegregation, and knock-out of Sgo1 in fission yeast resulted in chromosome missegregation (Gregan *et al.* 2005).

PP2A colocalizes with centromeric Sgo1 in human mitotic and meiotic cells. This enhances efficient PP2A dephosphorylation of Rec8, which renders it resistant to subsequent phosphorylation and cleavage. Furthermore, reduced PP2A activity resulted in loss of centromeric cohesion during mitosis and meiotic anaphase I accompanied by random sister chromatid segregation during meiotic anaphase II (Kitajima *et al.* 2006, Riedel *et al.* 2006, Tang *et al.* 2006). A human shugoshin-like protein (possibly orthologous to yeast Sgo1) localized to HeLa cell centromeres during prophase prevented phosphorylation of the Scc3 cohesin subunit. This protein normally disappears at anaphase onset, and its depletion by RNAi resulted in PCS (McGuinness *et al.* 2005).

Kinetochore-microtubule interaction, correction of faulty attachments, generation of tension and stabilization, and biorientation

Kinetochores help regulate chromosome segregation during mitosis and meiosis by mediating three main functions: attaching chromosomes to microtubules, facilitating microtubule dynamics essential for chromosome movement, and providing the site for spindle checkpoint activity. Kinetochores may initially capture microtubules by four different modes (Biggins & Walczak 2003, Cinini *et al.* 2001): (1) *Amphitelic* – sister kinetochores orientated to opposite poles of a bipolar spindle, (2) *Syntelic* – both kinetochores of sister chromatids attached to a monastral spindle, (3) *Monotelic* – only one kinetochore is orientated to a pole while the other is unattached, and (4) *Merotelic* – one kinetochore is attached to both poles (Figures 1 & 2). During metaphase of mitosis and meiosis II, amphitelic orientation of sister chromatids is needed; whereas during meiosis I, syntelic attachment of sister chromatids and amphitelic attachment of homologues are required. Persistent monotelic and merotelic attachments, if not corrected, can lead to chromosome missegregation; whereas, merotelic attachments are not detected by the spindle checkpoint (Cimini 2007, 2008, Cimini *et al.* 2001, 2004, Rieder & Maiato 2004, Salmon *et al.* 2005).

Kinetochores contain both constitutive (structural) proteins (Amor *et al.* 2004) and transient (passenger) proteins that help coordinate various events during mitosis and meiosis (Duesbery *et al.* 1997, Vagnarelli & Earnshaw 2004). The constitutive centromeric proteins **(**CENP-A, B, C, D) are involved with: microtubule capture, correcting aberrant interactions, binding of spindle checkpoint proteins, and chromosome congression to the metaphase plate (Craig *et al.* 1999, Rieder & Salmon 1998, Simerly *et al.* 1990, Vagnarelli & Earnshaw 2004). Whereas, the transient proteins reside in the nucleus during G2, associate with chromosomes during prophase, localize to centromeres during metaphase, and transfer to the spindle at anaphase onset (Earnshaw & Cooke 1991).

The Aurora A and Aurora B serine-threonine protein kinases help support mitotic spindle assembly by phosphorylating the structural and motor proteins that are essential for spindle assembly and anaphase onset (Giet *et al.* 2005, Meraldi *et al.* 2004). The biorientation of homologues during meiotic MI and that of sister chromatids during mitosis and meiotic MII resembles a state of equilibrium between sister chromatid cohesion and microtubule-kinetochore tension (Miyazaki & Orr-Weaver 1994, Tanaka *et al.* 2000, Toth *et al.* 1999). Plk1 and Aurora B kinases are also involved with a fundamental function that decreases the incidence of chromosome missegregation. These kinases help correct aberrant microtubule-kinetochore attachments by generating kinetochore-microtubule tension (Ahonen *et al.* 2005, Stern 2002, Tanaka *et al.* 2002). After proper correct microtubule-kinetochore attachment and tension have been attained, microtubule polymerization-depolymerization is minimized while centromeres bi-orient and align on the metaphase plate. In addition to Aurora B kinase, the depolymerase activity of mitotic centromere associated kinesin (MCAK) helps coordinate the release of merotelic kinetochore-microtubule attachments (Kallio *et al.* 2002, Knowlton *et al.* 2006). Also, the SPO13 protein and the monopolin protein complex found during meiosis I in fission yeast facilitate syntelic orientation of sister chromatids (Katis *et al.* 2004b, Lee *et al.* 2004).

The Aurora B-inner-centromeric protein (INCENP)-Survivin-Plk1-Borealin transient protein kinase complex is involved with several functions involving chromosome segregation and cytokinesis; these include: (1) chromatin decondensation, (2) reducing the affinity of Scc1 for chromatin at chromosome arms, (3) generating tension at kinetochores, (4) organizing a bipolar spindle, (5) targeting SAC proteins to kinetochores, (6) initiating cytokinesis, (7) inhibiting the APC, (8) sensing and correcting abnormal microtubule-kinetochore attachments, and (9) influencing spindle geometry by phosphorylating MCAK (Adams *et al.* 2001a, b, Dewar *et al.* 2004, Shang *et al.* 2003, Tanaka *et al.* 2002, Vagnarelli & Earnshaw 2004). INCENP, Survivin, and Plk1 are needed for the proper kinetochore localization of Aurora B and for correcting merotelic microtubule-kinetochore attachments (Bolton *et al.* 2002, Ditchfield *et al.* 2003, Goto *et al.* 2006, Tong *et al.* 2002). Survivin also has important roles during spindle checkpoint signaling and in correcting abnormal kinetochore-spindle fiber attachments (Carvalho *et al.* 2003, Hwang *et al.* 1998, Johnson *et al.* 2004, Lampson *et al.* 2004, Lens & Medema 2003, Taylor *et al.* 2001, 2004). Aurora B kinase activity helps to destabilize syntelic attachments of sister chromatids during meiosis II and mitosis; this enhances the re-formation of correct amphitelic orientation (Hauf *et al.* 2003, Tanaka *et al.* 2002). Furthermore, overexpression of a stable form of Aurora B in mammalian somatic cells led to aneuploidy (Nguyen *et al.* 2005).

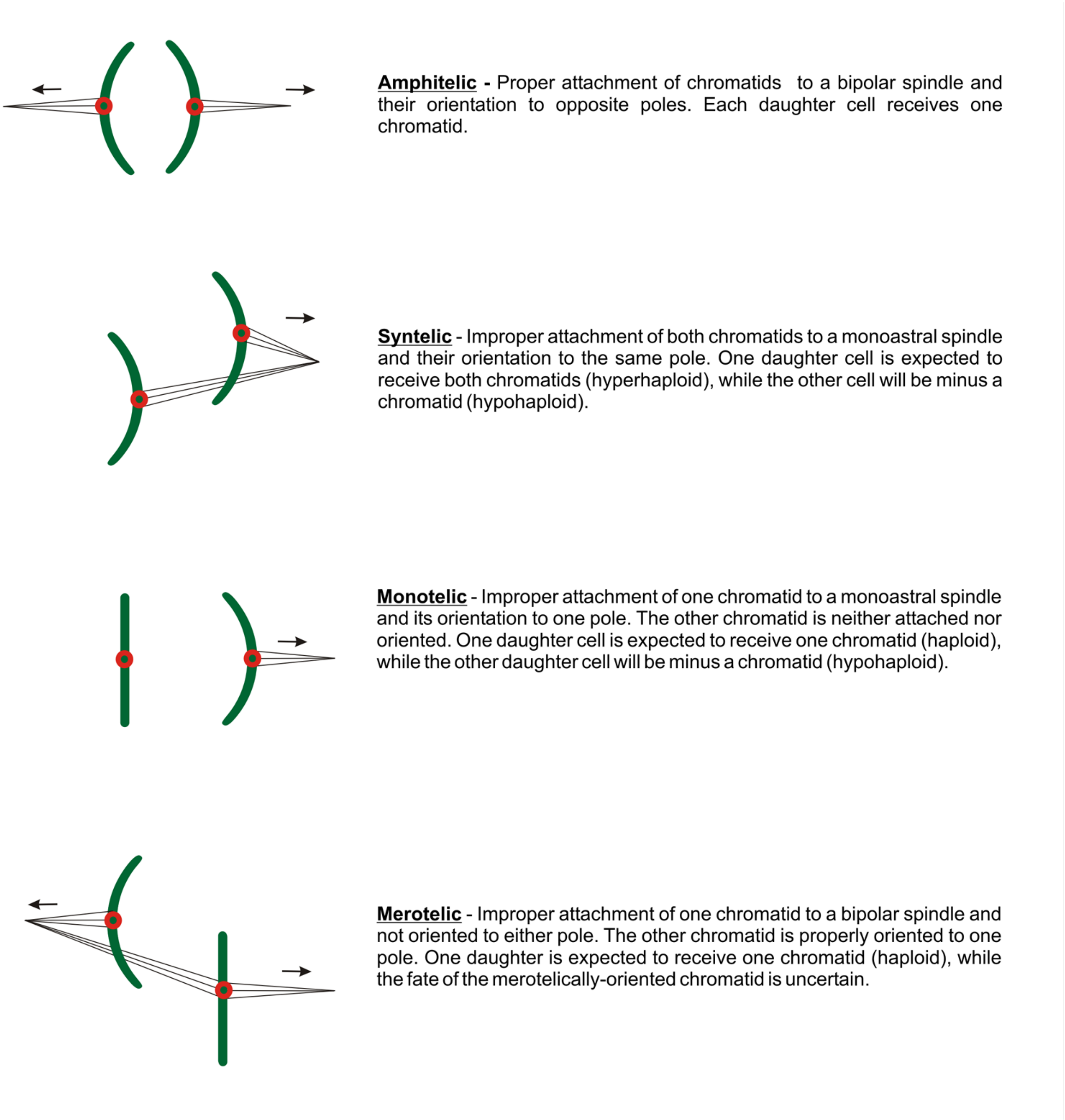
Spindle assembly checkpoint (SAC) protein complex and correction of faulty kinetochore-microtubule attachments

Chromosome segregation represents an irreversible event; orientation errors cannot be rectified after anaphase onset. In order to reduce the risk of missegregation, it is essential that a bipolar spindle be formed following correct microtubule-kinetochore attachment and tension. This is not left to chance. A transient mechanico-chemo surveillance mechanism or spindle-assembly checkpoint (SAC) protein complex helps insure that proper chromosome alignment and kinetochore-microtubule tension are attained prior to anaphase onset. However, the SAC is not foolproof; it can be overridden. Anaphase can still occur following exposure of cells to microtubule disrupting drugs, in the presence of abnormal spindle bipolarity, and in the presence of unattached kinetochores and abnormal chromosome orientation (Andreassen *et al.* 1996, Rieder & Palazzo 1992, Rieder *et al.* 1994).

Most SAC data have been derived from non-mammalian somatic cells, and although differences between mitotic and meiotic SAC proteins have been found, it appears that the basic molecular pathways are similar between mitosis and meiosis and among species (Dai *et al.* 2003a, Lee & Orr-Weaver 2001, Nasmyth 2001, Uhlmann 2001, 2003a). Three broad groups of interacting proteins comprise the SAC: (1) transport/motor proteins [dynein, Zw10, Rod] that convey unique SAC proteins from the cytoplasm to kinetochores, microtubules, and spindle poles; (2) binding proteins [Aurora B, MAPK, Mps1, Bub1, CENP-E] that bind certain SAC proteins to kinetochores; and (3) SAC proteins [Mad1, Mad2, Mad3/BubR1,Bub1, Bub3,] that transiently localize to kinetochores and temporally inhibit the MAT.

If defects in the integrity of kinetochore-spindle tension and attachment are detected, Mad1, Mad2, Mad3/ BubR1, Bub1, and Bub3 transiently associate with kinetochores by binding to Cdc20 (Fang 2002, Vigneron *et al.* 2004). Such binding inhibits APC activity and delays anaphase by blocking the ubiquination and subsequent proteolysis of securin and cyclin B by proteasomes (Bharadwaj & Yu 2004, Howell *et al.* 2004, Li & Benezra 1996, Luo *et al.* 2000, Musacchio & Hardwick 2002, Nasmyth 2005, Nicklas 1997, Rieder *et al.* 1994, Shah *et al.* 2004, Sluder & McCollum 2000, Taylor *et al.* 1998, 2004, Weiss & Winey 1996, Zhou *et al.* 2002).

Although less information is available about SAC proteins in mammalian germ cells relative to other cell types, several SAC proteins have been identified in mammalian oocytes. A functional Mad2-dependent spindle checkpoint was identified during meiosis in both mouse (Homer *et al.* 2005a, Tsurumi *et al.* 2004, Wassmann *et al.* 2003) and rat (Zhang *et al.* 2004) oocytes. Mad2 binds to unattached kinetochores and is released following proper microtubule-kinetochore tension and attachment (Homer *et al.* 2005a, Kallio *et al.* 2000, Ma *et al.* 2005, Steuerwald *et al.* 2005, Wassmann *et al.* 2003, Zhang *et al.* 2004, 2005). Mad1 helps recruit Mad2 to unattached kinetochores and was detected in mouse oocytes from the GV stage to MII (Chen *et al.* 1998, Chung & Chen 2002, Zhang *et al.* 2005). In addition to Mad1 and Mad2, Mad3/BubR1 activity was also detected in mouse oocytes (Tsurumi *et al.* 2004). Lastly, Bub1 was found on kinetochores from GVBD until early AI;



Amphitelic - Proper attachment of chromatids to a bipolar spindle and their orientation to opposite poles. Each daughter cell receives one chromatid.

Syntelic - Improper attachment of both chromatids to a monoastral spindle and their orientation to the same pole. One daughter cell is expected to receive both chromatids (hyperhaploid), while the other cell will be minus a chromatid (hypohaploid).

Monotelic - Improper attachment of one chromatid to a monoastral spindle and its orientation to one pole. The other chromatid is neither attached nor oriented. One daughter cell is expected to receive one chromatid (haploid), while the other daughter cell will be minus a chromatid (hypohaploid).

Merotelic - Improper attachment of one chromatid to a bipolar spindle and not oriented to either pole. The other chromatid is properly oriented to one pole. One daughter is expected to receive one chromatid (haploid), while the fate of the merotelically-oriented chromatid is uncertain.

Figure 2. Kinetochore-microtubule attachments and probable outcomes during meiosis II and mitosis.

then, it disappeared at late AI and re-appeared at MII (Brunet *et al.* 2003). Although SAC proteins are required for checkpoint functions during meiosis I and II in mouse oocytes, they appear non-essential for maintaining the cytostatic factor arrest during MII (Tsurumi *et al.* 2004).

Defective SAC function can lead to aneuploidy and abnormal cell cycle progression in mitotic and meiotic cells. Diminished Mad2 and Mad3/BubR1 activities resulted in PCS and aneuploidy in mammalian oocytes (Dai *et al.* 2004) and somatic cells (Michel *et al.* 2001), as well as malignant transformation in human cells (Hanks *et al.* 2004). Chromosome missegregation followed ablation of Mad2 activity during budding yeast meiosis I (Shonn *et al.* 2000), and deletion of one MAD2 allele led to faulty SAC activity, PCS, and chromosome missegregation in human cancer cells and mouse fibroblasts (Michel *et al.* 2001). Also, RNA-interference reduction of Mad2 protein levels in human somatic cells induced premature cyclin B degradation, abnormal spindles, and cell death (Michel *et al.* 2004). Knockout of Mad2 in mouse embryonic cells resulted in aneuploidy and apoptosis (Dobles *et al.* 2000). Microinjection of anti-Mad1 or anti-Mad2 into GV-stage rodent and pig oocytes induced abnormalities in spindle morphology, chromosome alignment, and chromosome segregation (Ma *et al.* 2005, Zhang *et al.* 2004, 2005). Other data from mouse oocytes showed that depletion of Mad2 protein during meiosis I resulted in premature loss of securin proteins and cyclin B and elevated levels of aneuploidy; whereas, microinjection of hMad2-GFP mRNA during meiosis I inhibited homolog segregation (Homer *et al.* 2005a). Finally, an excess of Mad2 in *Xenopus* oocytes caused in a delay of chromatid segregation during anaphase II (Peter *et al.* 2001).

Apart from alterations to Mad2, anomalies in other SAC proteins resulted in cell cycle perturbations and chromosome missegregation. Partial down regulation of Mad1 in human somatic cells led to spindle checkpoint inactivation and aneuploidy (Kienitz *et al.* 2005). Deletion of the *Bub1* gene in fission yeast led to loss of centromeric Rec8 and amphitelic segregation of sister chromatids during meiosis I (Bernard *et al.* 2001); whereas, biallelic mutations of human *BUB1B* were associated with aneuploidy and cancer (Hanks *et al.* 2004). Knockout of BubR1 alleles in mice resulted in reduced BubR1 protein expression that was correlated with elevated levels of aneuploidy in fibroblasts, spermatocytes, and oocytes (Baker *et al.* 2004).

Other data from mice showed that disruption of Bub3 led to cytogenetic anomalies and embryonic lethality (Kalitsis *et al.* 2000). Exposure of HeLa cells to 5-10 nM taxol was followed by disassociation of Mad2 and BubR1 complexes, cell-cycle delay and chromosome missegregation (Ikui *et al.* 2005). Earlier work also showed that the antineoplastic agent taxol can induce dose-response effects of maturation delay, spindle defects, and aneuploidy in mouse oocytes and one-cell zygotes (Mailhes *et al.* 1999).

Recent data have shown that oocyte aging is correlated with altered Mad2 titers and cytogenetic abnormalities. Postovulatory aging of mouse oocytes resulted in a time-dependent reduction in the number of Mad2 transcripts and a concomitant elevation in the frequencies of PCS and premature anaphase (Steuerwald *et al.* 2005). Also, in vitro aging of pig oocytes led to a reduction of Mad2 expression in conjunction with abnormal chromosome segregation (Ma *et al.* 2005). In human oocytes, hMAD2 was detected during meiosis I (Homer *et al.* 2005b), and hMAD2 mRNA titers were shown to decrease with advancing maternal age (Steuerwald *et al.* 2001). These findings suggest that altered SAC activity, as detected in oocytes aged in vivo and in vitro, represents one of many potential molecular mechanisms responsible for the genesis of aneuploidy.

Removal of centromeric cohesions and the metaphase-anaphase transition (MAT)

After proper microtubule-kinetochore tension and attachment has been attained or the SAC over-ridden, SAC proteins detach from Cdc20. This enables APC activation - a large protein complex that ubiquinates specific proteins (cyclin B, Securin, and possibly Sgo1) that are subsequently proteolyzed by proteasomes (Craig & Choo 2005, Glickman & Ciechanover 2002, Kotani *et al.* 1999, Salic *et al.* 2004). Proteasomes consist of multicatalytic 26S proteases and a 20S central core catalytic subunit bordered by two 19S components that hydrolyze C-terminal peptide bonds to acidic, basic, and hydrophobic amino-acid residues (Coux *et al.* 1996, Glickman & Ciechanover 2002, Goldberg 1995). This ubiquination and degradation of cellular proteins represent a tightly-regulated, temporally-controlled process that oversees numerous cellular processes including cell division (Glickman & Ciechanover 2002).

APC-mediated proteolysis during the somatic cell cycle depends upon both $\mathrm{APC}^{\mathrm{Cdc20}}$ and $\mathrm{APC}^{\mathrm{Cdh1}}$. $\mathrm{APC}^{\mathrm{Cdc20}}$ is active from prometaphase until the MAT; whereas, $\mathrm{APC}^{\mathrm{Cdh1}}$ becomes active during anaphase and persists until the S phase. Various regulatory pathways control $\mathrm{APC}^{\mathrm{Cdc20}}$ and $\mathrm{APC}^{\mathrm{Cdh1}}$ activities. Phosphorylation of APC subunits by Cdk1 and Plk1 facilitate Cdc20 binding and APC activation (Glover *et al.* 1998, Sumara *et al.* 2004). Conversely, Emi1 inhibits Cdc20 binding to APC. Prior to mitosis, phosphorylation by Cdk1 and Cdk2 kinases inactivates Cdh1. However, as cells exit mitosis following cyclin B proteolysis, Cdh1 is dephosphorylated and $\mathrm{APC}^{\mathrm{Cdh1}}$ mediates the proteolysis of Cdc20 and Plk1 (Peters 2002, Zachariae & Nasmyth 1999). $\mathrm{APC}^{\mathrm{Cdc20}}$ targets cyclin B for degradation, which leads to Cdk1 inactivation. Also, $\mathrm{APC}^{\mathrm{Cdc20}}$ activity leads to securin inactivation, which liberates separase upon satisfaction of the SAC.

Prior to normal chromatid segregation, the securin proteins, which inhibit separase activity, are ubiquinated by the APC and subsequently proteolyzed by proteasomes (Cohen-Fix *et al.* 1996, Uhlmann *et al.* 1999). Securin (Pds1p in budding yeast) activity is abrogated after each meiotic anaphase onset (Salah & Nasmyth 2000). In human somatic cells, D-box mutants of securin that were not degraded during metaphase resulted in chromosome missegregation (Hagting *et al.* 2002). This proteolysis of securin liberates the cysteine protease separase, which cleaves centromeric Scc1 during mitotic anaphase onset (Nasmyth

2002, Uhlmann *et al.* 1999, Waizenegger *et al.* 2002), Rec8 from chromosome arms during anaphase I (Agarwal & Cohen-Fix 2002, Buonomo *et al.* 2000, Jallepalli *et al.* 2001, Uhlmann 2003b), and Rec8 from centromeres during anaphase II (Waizenegger *et al.* 2000). Similar to mitosis, both APC and separase activities have been shown essential for proteolyzing securin and cyclin B prior to homolog segregation in mouse oocytes (Herbert *et al.* 2003, Terret *et al.* 2003).

Following inactivation or overriding of the SAC, the MAT represents a point-of-no-return. The temporal coordination of the MAT is directed by the interaction of unique biochemical events (kinases, phosphatases, proteolysis, topoisomerases, and motor proteins) with cellular organelles (kinetochores, centromeres, centrosomes, spindle fibers) (Dorée *et al.* 1995, Kirsch-Volders *et al.* 1998). During mitosis, the positive ends of microtubules are embedded in kinetochores and the negative ends are lodged in centrosomes. In conjunction with motor proteins, chromosome movement towards centrosomes arises from depolymerization of both the minus and positive ends of microtubules. Even though the MAT appears straightforward from a cytogenetic viewpoint, it is actually a complex series of events involving the coordination of independent processes that depend on prior checkpoint release and APC activation.

Separation of sister chromatids occurs by two independent processes: removal of cohesins from chromosomes and microtubule-dependent movement of chromatids to opposite poles. Chromatid arm separation and centromere separation (anaphase A) are independent events with different mechanisms (Rieder & Salmon 1998, Sluder & Rieder 1993), and chromatid separation does not initiate poleward movement of chromatids (anaphase B) (Zhang & Nicklas 1996). Also, sister chromatid separation does not directly depend on spindle formation because chromatids can separate in the absence of spindle attachment (Nasmyth *et al.* 2000, Rieder & Palazzo 1992) and even when MPF activity is elevated (Sluder & Rieder 1993).

Mishaps can occur during the MAT. If sister chromatids separate too early, they may both segregate to the same pole resulting in aneuploidy. Conversely, if sister chromatids fail to segregate, the outcome can range from aneuploidy to diploid gametes. In order to reduce the occurrence of such cytogenetic abnormalities, the MAT is not left to chance alone; it normally depends on satisfaction of the SAC and APC activation.

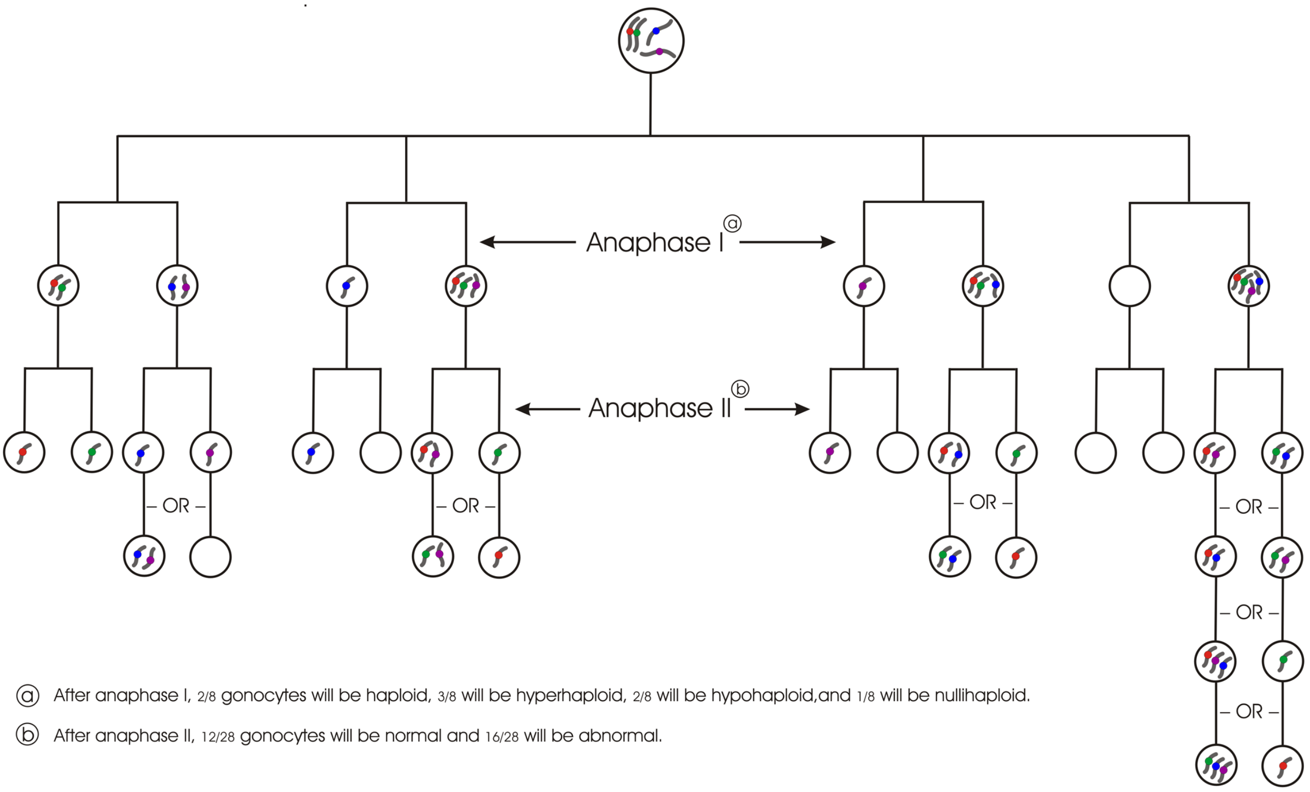
Premature Centromere Separation (PCS)

PCS and nondisjunction represent the major cytogenetic errors that lead to aneuploidy (Angell 1994, Dailey *et al.* 1996, Fragouli *et al.* 2006, Lim *et al.* 1995, Pellestor *et al.* 2005, Plachot 2003, Vialard *et al.* 2006, Wolstenholme & Angell 2000). PCS denotes the separation of sister chromatids or homologues prior to anaphase; whereas, nondisjunction results from the failure of chromatids or homologues to properly separate during anaphase. The link between PCS and aneuploidy during meiosis is that if homologues or sister chromatids separate prior to anaphase I, each of the homologues or sister chromatids may undergo random segregation (Figure 3). Also, PCS of sister chromatids prior to anaphase II onset can result in random segregation instead of amphitelic segregation. Experimental data have demonstrated a positive correlation between time postovulation and elevated frequencies of PCS in MII oocytes and aneuploidy in one-cell mouse zygotes (Mailhes *et al.* 1998).

The degree of PCS should be noted when considering the possible chromosome segregation patterns of a primary oocyte with PCS and the probability of aneuploidy. This can range from only the sister chromatids of one dyad to complete separation of all chromatids (Mailhes *et al.* 2003a). Considering the most elementary situation whereby the sister chromatids of one homologous chromosome separate prematurely and the other homologues segregate normally, three potential events may occur during anaphase I: (1) both of the disjoined sisters may segregate to the secondary oocyte, while the homologue segregates to the first polar body or *vice versa;* (2) both sisters may segregate along with its homologue to the secondary oocyte; and (3) both sisters may segregate to the first polar body along with its homologue. Thus, following anaphase I, the latter two outcomes would result in aneuploid secondary oocytes. Now, considering the case of a MII oocyte with two single chromatids (PCS of one dyad), three possible outcomes may occur during anaphase II: (1) one sister may segregate to the oocyte pronucleus, while the other goes to the second polar body; (2) both sisters may segregate to the oocyte pronucleus; or (3) both sisters may segregate to the second polar body. Again, the latter two segregation possibilities would result in aneuploidy. A noteworthy finding is that a bipolar spindle is not required for PCS because both chromatid arm and centromere separation can occur in the absence of a spindle (Rieder & Palazzo 1992, Sluder 1979).

The occurrence of PCS is not new. Rodman (1971) noted that postovulatory-aged mouse oocytes had higher frequencies of PCS than freshly ovulated

Figure 3. Possible segregation patterns during meiosis I and II when one homologue undergoes PCS prior to anaphase I.



ⓐ After anaphase I, 2/8 gonocytes will be haploid, 3/8 will be hyperhaploid, 2/8 will be hypohaploid,and 1/8 will be nullihaploid.

ⓑ After anaphase II, 12/28 gonocytes will be normal and 16/28 will be abnormal.

oocytes. Subsequently, several groups found a positive correlation between postovulatory and in vitro oocyte aging with elevated levels of PCS in human (Angell 1991, 1994, Cupisti *et al.* 2003, Dailey *et al.* 1996, Pellestor *et al.*, 2002, 2003, Rosenbusch 2004), rodent (Mailhes *et al.* 1997b, 1998, Yin *et al.* 1998), and Drosophila oocytes (Jeffreys *et al.* 2003). Also, experimental data have supported a correlation between chemically-induced PCS in MII oocytes and aneuploidy in one-cell mouse zygotes (Mailhes *et al.* 1997b).

The molecular events underlying PCS are receiving considerable attention. The precocious loss of cohesin proteins from sister chromatids and homologues during mitosis and meiosis has been shown to result in PCS (Hoque & Ishikawa 2002, Sonada *et al.* 2001, Uhlmann 2003a). Mutants of the *ord* and *Mei-S322* Drosophila proteins, which help hold sister chromatids together prior to anaphase, exhibited higher frequencies of PCS and aneuploidy (Kerrebrock *et al.* 1992, Miyazaki & Orr-Weaver 1992). Also, abnormalities in other proteins involved with chromosome cohesion, such as SMC1 beta in mice (Hodges *et al.* 2005) and the yeast Pds5 protein (Hartman *et al.* 2000, Panizza *et al.* 2000) can alter normal segregation patterns. Recent results with HeLa cells showed that depletion of the microtubule and kinetochore protein astrin resulted in checkpoint arrested cells with PCS (Thein *et al.* 2007). Although specific proteins have central roles in sister chromatid and homologue cohesion, other compounds also appear to be involved. Both culture media and the follicular fluid-meiosis-activating sterol were reported to affect the incidence of PCS in mouse oocytes in vitro (Cukurcam *et al.* 2003).

In addition to defects in cohesion proteins, PCS and aneuploidy can also result from abnormal SAC protein activity. Deficient Mad2 activity resulted in MPF degradation, APC activation, loss of sister chromatid cohesion, and PCS in both *Xenopus* oocytes (Peter *et al.* 2001) and aged mammalian oocytes (O'Neill & Kaufman 1988). Other data indicated that as time postovulation increased in mouse oocytes, the frequencies of PCS and premature anaphase (PA) increased, while the intraoocyte titer of MAD2 transcripts decreased (Steuerwald *et al.* 2005). Elevated PCS levels was also reported following the exposure of mouse oocytes to propylene glycol (Mailhes *et al.* 1997b) and tamoxifen (London & Mailhes 2001). Furthermore, when mouse oocytes were exposed to the phosphatase 1 and 2A inhibitor OA prior to metaphase I, complete separation of homologues into 80 chromatids and elevated levels of aneuploidy in MII oocytes were found (Mailhes *et al.* 2003a). A possible explanation for the elevated levels of PCS found in OA-exposed oocytes may involve protein hyperphosphorylation, as noted in hepatocytes (Cohen *et al.* 1990) and rat oocytes (Zernicka-Goetz & Maro 1993) following OA treatment. During mitosis and meiosis, phosphorylation of cohesins facilitates their removal prior to anaphase onset (Alexandru *et al.* 2001, Hoque & Ishikawa 2001, Lee & Amon 2003, Losada *et al.* 2000, Tomonaga *et al.* 2000, Yu & Koshland 2005). Finally, PP2A is found at yeast centromeres during mitosis and meiosis, and decreased PP2A activity led to loss of centromeric cohesion at anaphase I and random segregation of chromatids during anaphase II (Kitajima *et al.* 2006, Riedel *et al.* 2006, Tang *et al.* 2006).

Postovulatory and In Vitro Oocyte Aging

The broad focus of this review is that postovulatory or in vitro oocyte aging leads to a progressive and functional deterioration of the biochemical and cellular organelles required for accurate chromosome segregation, normal fertilization, and embryonic development (Austin 1967, 1970, Wilcox *et al.* 1998). Some of these age-related changes may serve as models for studying the numerous potential mechanisms of aneuploidy.

Mature mammalian oocytes remain capable of fertilization for a longer period of time than their time for expressing optimal gamete physiology. The fertilizable lifespan of mammalian oocytes ranges from 12 to 24 h (Hafez 1993). Although the fertilizable average lifespan for both induced-and naturally-ovulated mouse oocytes is approximately 15 h postovulation, their optimal time for fertilization lies between 4 to 6 h postovulation (Edwards & Gates 1959, Lewis & Wright 1935, Marston & Chang 1964). After ovulation, time-dependent intraoocyte changes occur that can lead to apoptosis (Exley *et al.* 1999, Gordo *et al.* 2002, Morita & Tilly 1999, Perez *et al.* 1999) and nuclear fragmentation (Gordo *et al.* 2002). Also, the time from insemination to fertilization, the rate of pronuclear formation, and the first cleavage division were shorter in postovulatory aged mouse oocytes than in freshly ovulated oocytes (Fraser 1979, Boerjan & de Boer 1990).

Most mammals, excluding humans and induced-ovulators, ovulate during or shortly after the estrus period of their estrous cycle; this facilitates fertilization of freshly ovulated oocytes (Hafez 1993). Since this situation does not occur in humans, a probability exists that postovulatory aged oocytes will be fertilized. Indeed, several groups have proposed that fertilization of postovulatory aged oocytes (delayed fertilization) represents a predisposition to aneuploidy (Blazak 1987, Hecht & Hecht 1987, Juberg 1983, Mailhes 1987, Pellestor 1991, Zenzes & Casper 1992). Two human epidemiologic studies offered support for an association between delayed fertilization and early embryonic failure (Wilcox *et al.* 1998) and trisomic offspring (Juberg 1983).

Chemical Alterations in Aged Oocytes

Although freshly-ovulated and postovulatory aged oocytes appear morphologically similar, differences exist among certain cellular organelles and biochemical activities. Some of these dissimilarities resemble those found following fertilization or partheneogenic activation (Tarin *et al.* 1996, Xu *et al.* 1997), while others involve alterations to cellular organelles and biochemical events that can affect chromosome segregation.

Mammalian oocytes possess a time- and species-dependent predisposition to spontaneous activation if fertilization does not occur within a limited time following ovulation or in vitro culture. Numerous studies have shown that the incidence of spontaneous oocyte activation in mice begins to increase four hours postovulation (Homa *et al.* 1993, Kaufman 1983, Kubiak 1989, Moses & Masui 1994, Nagai 1987, Whittingham & Siracusa 1978, Winston *et al.* 1991, Yanagimachi & Chang 1961). Aged oocytes also had lower ATP levels at fertilization (Igrashi *et al.* 2005), higher sensitivities to: oxidative stress (Boerjan & deBoer 1990, Takahashi *et al.* 2003, Tarin *et al.* 1996), calcium ionophores (Fulton & Whittingham 1978, McConnell *et al.* 1995, Vincent *et al.* 1992), partheneogenetic activation following chemical or mechanical stimuli (Cutherbertson & Cubbold 1985, Kaufman 1983, Kline & Kline 1992, Kubiak 1989, Nagai 1987), and spontaneous calcium release (Beatrice *et al.* 1984, Orrenius *et al.* 1992, Tombes *et al.* 1992). The higher titers of calcium found in aged oocytes were proposed to inhibit both tubulin polymerization and the depolymerization of existing microtubules (Kosower & Kosower 1978).

Relative to fresh oocytes, aged oocytes displayed higher calmodulin-dependent protein kinase II activities, but lower activities of MPF and MAPKs (Lorca *et al.* 1993, Moos *et al.* 1995, Verlhac *et al.* 1994). The diminished MPF activity (resulting from phosphorylation and conversion to pre-MPF) in aged porcine (Kikuchi *et al.* 1995, 2000) and bovine oocytes (Liu *et al.* 1998) and of MAPKs in both aged mouse (Xu *et al.* 1997) and porcine oocytes in vitro (Ma *et al.* 2005) were proposed to lead to spontaneous activation, abnormal chromosome segregation, and apoptosis. Furthermore, it was shown that the levels of active and inactive MPF could be regulated by exposing porcine oocytes to certain phosphatase and kinase inhibitors (Kikuchi *et al.* 2000). Such exogenous manipulation of phosphorylation-dephosphorylation events appear to offer another venue for investigating the events associated with oocyte aging and chromosome segregation. Additionally, both MPF and MAPK titers were reported to decrease more rapidly in oocytes cultured from biologically aged mice than those from young mice (Tatone *et al.* 2006).

Differences in kinase and phosphatase activities, protein synthesis, and maternal mRNA recruitment were also noted between fresh and aged bovine oocytes (Liu *et al.* 1998). Mos kinase (the product of the c-mos protooncogene) is needed for stabilizing MPF during the MII arrest of mouse oocytes (Gabrielli *et al.* 1993, Sagata 1996, 1997) and for microtubule spindle assembly (Sagata 1996, Wang *et al.* 1994, Zhao *et al.* 1991), and in vitro aging of bovine oocytes was shown to reduce the activity of Mos kinase (Wu *et al.* 1997).

When immature porcine oocytes were cultured for 40 to 72h in vitro, the levels of tubulin and the centromere protein B (CENP-B) remain unchanged as oocytes aged; whereas, the expressions of the Mad2 spindle checkpoint protein, the BCL2 antiapoptotic protein, and the mitogen-activated protein kinase (MAPK) decreased as culture time increased. Also, the proportions of oocytes with abnormal spindles and chromosomes increased with oocyte aging (Ma *et al.* 2005). Other data have shown that postovulatory aging of mouse oocytes resulted in a time-dependent reduction in the number of Mad2 transcripts and a concomitant elevation in the frequencies of PCS and PA (Steuerwald *et al.* 2005). A recent report utilized bisulfite sequencing and COBRA methods to evaluate the DNA methylation status of differentially methylated regions (DMRs) of two maternally imprinted genes – *Snrpn* and *Peg1/Mest.* Mouse oocytes aged in vivo for 29 h post-hCG exhibited demethylation of *Snrpn* DMRs. However, no change in the methylation status of *Peg1/Mest* was found at 29 h (Liang *et al.* 2008).

Histone deacetylase inhibitors are powerful anti-proliferative compounds undergoing clinical studies as antitumor drugs. Enhanced acetylation of lysines on histone H3 and H4 occurs during postovulatory oocyte aging, and the histone deacetylase inhibitor trichostatin A (TSA) can accelerate the rate of in vivo aging in mouse oocytes (Huang *et al.* 2007). Also, mouse oocytes cultured in the presence of TSA exhibited elevated levels of aneuploidy and early embryonic death (Akiyama *et al.* 2006). Another study found that exposure of HeLa cells to TSA led to loss of the Mad2 SAC protein from kinetochores and elevated levels of PCS (Magnaghi-Jaulin *et al.* 2007).

Cytologic and Cytogenetic Alterations in Aged Oocytes

Numerous cytologic and cytogenetic alterations have been described in aged mammalian oocytes. Relative to freshly ovulated oocytes, aged oocytes displayed alterations in cortical granule exocytosis and the zona pellucida (Cascio & Wassarman 1982, Diaz & Esponda 2004, Gianfortoni & Gulyas 1985, Howlett 1986, Longo 1981, Szollosi 1975, Xu *et al.* 1997, Yanagimachi & Chang 1961) and elevated levels of cytoplasmic asters and spindle anomalies (Eichenlaub-Ritter *et al.* 1986, 1988, George *et al.* 1996, Kim *et al.* 1996, Pickering *et al.* 1988, Segers *et al.* 2008). Furthermore, aged oocytes displayed higher frequencies of premature extrusion of the second polar body and apoptosis (Fissore *et al.* 2002, Gordo *et al.* 2000).

When exogenous calcium was added to *Xenopus* egg extracts, elevated frequencies of PCS and PA were detected (Shamu & Murray 1992). Others proposed that an excess of intracellular calcium, as found in aged oocytes, triggers a cascade of events resulting in PCS, PA, and chromosome missegregation (Fissore *et al.* 2002, Gordo *et al.* 2000, Tarin *et al.* 1996). Both PCS and PA have been proposed to represent cytogenetic manifestations of spontaneous activation in aged oocytes (Mailhes *et al.* 1997a, 1998). Aged oocytes displayed higher frequencies of chromosome displacement from the metaphase plate (Saito *et al.* 1993, Webb *et al.* 1986), and the levels of PCS and PA were higher in postovulatory and in vitro aged mammalian oocytes (Angell 1991, Cupisti *et al.* 2003, Dailey *et al.* 1996, Mailhes *et al.* 1997b, 1998, Pellestor *et al.* 2002, 2003, Rosenbusch 2004, Yin *et al.* 1998). Fertilization of aged oocytes was correlated with higher frequencies of fragmented female pronuclei (Fissore *et al.* 2002, Kikuchi *et al.* 2000, Szollosi 1971), decreased fertilization rates (Smith & Lodge 1987, Wolf *et al.* 1996), and embryonic viability (Ekins & Shaver 1975, Sakai & Endo 1988, Wilcox *et al.* 1998). Also, the frequencies of polyploidy (Austin 1967, Ishikawa & Endo 1995, Juetten & Bavister 1983, Shaver & Carr 1967, Vickers 1969) and aneuploidy (Mailhes *et al.* 1998, Plachot *et al.* 1988, Rodman 1971, Sakurada *et al.* 1996, Yamamoto & Ingalls 1972) were higher following delayed fertilization of mammalian oocytes.

Although most studies found a positive correlation between postovulatory aged oocytes and cytological and cytogenetic abnormalities, two studies reported that aneuploidy was not elevated in aged oocytes. Although an increase in aneuploidy was not detected when mouse oocytes were aged in vivo for 0 -14 hrs prior to in vitro fertilization, only 1 and 2 zygotes were analyzed from the 14 and 10 hr aged groups, respectively (Zackowski & Martin-Deleon 1988). Another study involving in vivo aging of mouse oocytes and cytogenetic analysis of single pronuclear haploid partheneogenones reported no association between oocyte ageing and aneuploidy (O'Neill & Kaufman 1988). However, these findings may be compromised by the difficulty of distinguishing between MII oocyte chromosomes and partheneogenome chromosomes as well as that between a first and a second polar body. It is noted that analysis of MII chromosomes cannot detect aneuploidy in postovulatory aged oocytes because an intervening cell division is needed between the induction and expression of aneuploidy.

When the developmental potential of a limited number of aged, failed-to-fertilize human oocytes were compared with fresh, ovulation-induced oocytes, higher levels of aneuploidy, aberrant spindles, and cleavage failure were noted in the aged oocytes (Hall *et al.* 2007). Also, human embryos resulting from in vitro maturation and delayed intracytoplasmic sperm injection exhibited higher levels of aneuploidy when compared with control embryos (Emery *et al.* 2005).

Conclusion

At each stage of mitosis and meiosis, the correct order and temporal interaction among various chemical reactions and cellular organelles are needed to preserve genomic integrity. Considerable experimental data and human epidemiological studies have shown that the probability of successful chromosome segregation and zygotic development are compromised when oocytes undergo in vivo or in vitro aging prior to fertilization. These biochemical and cytological changes reported in aged oocytes offer unique models for studying some of the numerous molecular aspects of aneuploidy.

Several innovative technologies have been used to study the molecular aspects of mitosis and meiosis. High-density oligonucleotide microarrays and PCR microarrays (Schlecht & Primig 2003) can be used to identified loci that regulate the cell cycle in eukaryotes, including mice and humans. Also, double-strand RNA-mediated post-transcriptional gene silencing (RNA interference) offers promise for investigating the pathways controlling cell cycle progression and chromosome segregation (Bettencourt-Dias *et al.* 2004, Prawitt *et al.* 2004). RNA silencing/knockdown has been used to alter the expression of Mos mRNA (Stein *et al.* 2003) and Mad2 (Homer *et al.* 2005a) in order to study the role of genes involved with oocyte maturation and chromosome segregation in mouse oocytes. When employing RNA interference technologies, the possibility of off-target effects and the efficiency of gene silencing should be considered. Gene knockout strategies for genes upregulated during yeast meiosis showed that deletion of specific genes required for maintaining centromeric cohesion during anaphase I resulted in chromosome missegregation (Gregan *et al.* 2005, Marston *et al.* 2004). Furthermore, genomic and proteomic analyses have the ability to expand our knowledge about gene expression. Analyses of cancer cells showed that a subset of genes are universally activated in most cancers (Rhodes *et al.* 2004), and that overexpression of cell division regulatory genes were linked with chromosome aberrations and neoplastic progression (Rajagopalan & Lengauer 2004). Finally, the use of unique chemical inhibitors that block a specific pathway during chromosome segregation are helping to advance our knowledge about aneuploidy (Dorer *et al.* 2005, Mailhes *et al.* 2003a, 2004).

The present and future challenge will be to understand the complex molecular mechanisms of aneuploidy and genomic instability and to apply such knowledge to reducing the incidence of human genetic disease and cancer.

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