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.

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 decantation, chiasmata resolution, chromatin condensation, microtubule kinetics, centromeres, 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 cerevisiae), fission yeast (Schizosaccharomyces pombe), and other eukaryotes (Yanagida 2005).

Numerous reports have described biochemical and cellular organelle changes in aged oocytes. How-
ever, 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 & Chandlely 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 & Chandlely 1983, Chandlely 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 & Chandlely 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, Nicolaides & 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).

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), g里斯ofulvin (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 & Ka-miguchi 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 immuno-cytochemical 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 treatment (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 temporarily-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 (Andersiesz et al. 2000, Hiller 2001). Meiosis begins in the fetal ovary and is later arrested postpar-tum at the diplote/ 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 meta-phase 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 (p34cdc2) 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 p34cdc2 be dephosphorylated at the tyrosine 15 residue and coupled with cyclin B. Conversely, tyrosine phosphorylation deactivates MPF (Dunphy & Kumagai 1993, Collas et al. 1993). 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, Fukai et al. 1992), and parthenogenesis (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, Soba-jima 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 & Ar-linhaps 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 parthenogenetic 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 p34cdc2 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-DMP) disrupts p34cdc2 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-DMP 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-DMP 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).
comprehensive 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 centromeres, and temporally-coordinate 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 (chiasma formation) occur between homologues prior to anaphase I. Second, the two sister chromatids of each chromosome must segregate amphitely at anaphase I. Third, the cohesion between sister chromatid centromeres must remain intact until anaphase II onset in order for sister chromatids to segregate amphitely.

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 cohesion proteins are removed during prophase. However, it is essential that centromeric catenations and cohesion 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.**

<table>
<thead>
<tr>
<th>Cohesion complex subunit proteins identified during mitosis</th>
<th>Cohesion complex subunit proteins identified during meiosis</th>
<th>Spindle assembly checkpoint (SAC) proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smc1 and Smc3 (structural maintenance of chromosomes) – core cohesion complex subunit proteins.</td>
<td>Smc1u – replaces mitotic Smc1.</td>
<td>Mad1 (mitotic-arrest deficient) – helps recruit Mad2 to kinetochores that lack tension and attachment. Forms a complex with Cdc20, Mad2, and Mad3.</td>
</tr>
<tr>
<td>Scc1/Rad21/Mcd1 (sister chromatid cohesion) – cleaved by separase at mitotic anaphase onset.</td>
<td>Scc1β – replaces mitotic Scc1.</td>
<td>Mad2 – forms a complex with Cdc20, Mad1, and Mad3 and inhibits APC(^{Cdc20}) activity.</td>
</tr>
<tr>
<td>Sce3 (SA1/STAG1, SA2/STAG2) – phosphorylated by Aurora B and Plk1 kinases.</td>
<td>STAG3 – replaces mitotic Scc3.</td>
<td>Mad3/BubR1 – helps recruit Mad1 and Mad2 to kinetochores that lack attachment and tension; forms a complex with Cdc20, Mad1, Mad2, and Bub3.</td>
</tr>
<tr>
<td>Sce2 and Sce4 – enhance the binding of Sce1 and Sce3 to kinetochores that lack attachment and tension.</td>
<td>Rec8 – replaces mitotic Sce1.</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bub2/Mps1 – helps regulate APC(^{Cdc20}), mitotic exit, chromosome replication, and cytokinesis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bub3 – binds with Bub1 and Mad3 and helps regulate APC activity.</td>
</tr>
<tr>
<td>Other compounds</td>
<td>Function</td>
<td></td>
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<tr>
<td>-----------------</td>
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<td></td>
</tr>
<tr>
<td>APC/C (anaphase promoting complex/cyclosome)</td>
<td>A 20S multi-subunit ligase that ubiquinates specific proteins targeted for proteolysis by proteasomes. APC&lt;sup&gt;cdc20&lt;/sup&gt; targets securin for proteolysis at the MAT; whereas, APC&lt;sup&gt;cdh1&lt;/sup&gt; targets mitotic cyclins and other substrates for degradation at mitotic exit. The cdh1 protein activates the APC from late anaphase through G1.</td>
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<tr>
<td>Astrin</td>
<td>A microtubule and kinetochore protein that has roles involving sister chromatid adhesion, centrosome integrity, and separase activity.</td>
<td></td>
</tr>
<tr>
<td>Aurora B kinase-Survivin-Inner Centromeric Protein-Borelin</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Cdc20 (cell division cycle 20)</td>
<td>Helps activate the APC when not bound by SAC proteins, recruits substrates to the APC, and forms a complex with Mad2, Mad3, and Bub3.</td>
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</tr>
<tr>
<td>Cdk5 (Cyclin-dependent kinases)</td>
<td>Enzymes composed of a kinase subunit and an activating cyclin subunit. Cdk5s are needed for kinase activity.</td>
<td></td>
</tr>
<tr>
<td>CenP-E (centromeric protein E)</td>
<td>A motor protein that facilitates kinetochore-microtubule stabilization, binding of SAC proteins to kinetochores, and enhanced Mad3 activity.</td>
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<tr>
<td>Dynein/Dynactin</td>
<td>A microtubule motor protein required for the removal of the Rod-Zw10-Zwilch complex, Mad1, Mad2, and Mad3 from properly aligned kinetochores.</td>
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<tr>
<td>Kinesin</td>
<td>A microtubule motor protein.</td>
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<tr>
<td>MAPK/Mps1 (mitogen-activated protein kinase)</td>
<td>A serine-threonine kinase that helps recruit CenP-E to kinetochores. It also interacts with Mos protein for MPF activation.</td>
<td></td>
</tr>
<tr>
<td>MCAK/Kip 2-3 (microtubule centromere-associated kinesin)</td>
<td>Depolarizes microtubules and helps correct aberrant kinetochore-microtubule attachments.</td>
<td></td>
</tr>
<tr>
<td>Monopolin/Mam1/CdcPlk</td>
<td>Facilitates amphitelic orientation of homologues and syntenic orientation of sister chromatids during meiosis I.</td>
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<tr>
<td>Mos</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>MPF (maturation promoting factor)</td>
<td>A protein kinase comprising p34&lt;sup&gt;cdc28&lt;/sup&gt;/Cdk1 and cyclin B. MPF phosphorylates and helps regulate chromosome condensation, nuclear envelope breakdown, and spindle formation.</td>
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<tr>
<td>Op18/Stathmin (oncoprotein 18)</td>
<td>A protein that destabilizes microtubules; it is inhibited by phosphorylation.</td>
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<tr>
<td>P31/Cmt2</td>
<td>A protein involved with changing the stereo-configuration of Mad2.</td>
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<tr>
<td>Plk1 (Polo-like kinase 1)</td>
<td>A serine-threonine kinase that phosphorylates Scc1, Scc3, and Rec8 and reduces their affinity to chromosomes.</td>
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<tr>
<td>PP2A (protein phosphatase 2A)</td>
<td>Dephosphorylates Sgo1 and supports Rec8 maintenance.</td>
<td></td>
</tr>
<tr>
<td>Proteasomes</td>
<td>Proteinase complexes that degrade intracellular ubiquinated compounds.</td>
<td></td>
</tr>
<tr>
<td>Rod-Zw10-Zwilch</td>
<td>A protein complex that helps recruit dynein, Mad1, and Mad2 to unaligned kinetochores.</td>
<td></td>
</tr>
<tr>
<td>Securin/Pds1/Cut2p</td>
<td>An APC substrate that binds to and inhibits separase activity.</td>
<td></td>
</tr>
<tr>
<td>Separase/Esp1</td>
<td>A protease that is inactive when bound by securin. However, upon securin proteolysis, separase is free to cleave centromeric Scc1 cohesion at mitotic anaphase onset, Rec8 at chromosome arms at meiotic anaphase I onset, and centromeric Rec8 at meiotic anaphase II onset.</td>
<td></td>
</tr>
<tr>
<td>Shugoshins (Sgo1 &amp; Sgo2)</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Slk19p</td>
<td>The <em>Saccharomyces cerevisiae</em> Slk19p gene product is needed for proper chromosome segregation during meiosis I.</td>
<td></td>
</tr>
<tr>
<td>Sororin</td>
<td>An APC protein substrate that interacts with Shugoshins to facilitate cohesion binding to chromatin.</td>
<td></td>
</tr>
<tr>
<td>Spindly</td>
<td>A protein that helps inactivate the APC and participates with dynactin in recruiting dynein to kinetochores.</td>
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<tr>
<td>Spo11</td>
<td>Helps initiate meiotic recombination.</td>
<td></td>
</tr>
<tr>
<td>Sycp3</td>
<td>Helps maintain the structural integrity of meiotic chromosome axes.</td>
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</tr>
<tr>
<td>Topoisomerase II (Topo II)</td>
<td>An enzyme that disrupts intercalated loops of DNA and then reanneals the DNA broken ends.</td>
<td></td>
</tr>
<tr>
<td>UbcH10</td>
<td>An enzyme that ubiquinates Cdc20. This facilitates the release of Mad2 and BubR1 from Cdc20, inactivates the SAC, and helps activate the APC.</td>
<td></td>
</tr>
<tr>
<td>Usp44</td>
<td>An enzyme that deubiquinates Cdc20. This enhances the retention of Mad2 by Cdc20, promotes SAC activity, and inhibits APC activation.</td>
<td></td>
</tr>
</tbody>
</table>
Besides Spo11 and Syce3, 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 AI (Revenkova & Jessberger 2005). Eukaryotic mitotic cells encode homologs of the Scc1/Rad21, Scc3 (SA1/STAG1, SA2/STAG2), Scc1, and Scc3 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 Scc1 and Scc3 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); Sme1α and Sme1β replace Smc1 (Revenkova et al. 2001); and homologs for Smc3 have not been identified.

Cohesin proteins must remain located on chromosomes until anaphase onset. Otherwise, early or non-removal can result in PCS or nondisjunction, respectively. The retention and removal of cohesin 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 amphitetically and sister chromatids segregate syntetically. 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 (Pasierebek 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). Phos-
phorylation of Rec8 facilitates its cleavage; whereas, dephosphorylation of Rec8 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

**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 parologue 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 Rec8 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, Sgo1 disappears 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**

Kinetochore helps 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. Kinetochore may initially capture microtubules by four different modes (Biggins & Waleczak 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, Cinini et al. 2001, 2004, Rieder & Maito 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 de-
polymerase 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 (Rattis 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.


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;
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 cy-

**Figure 2.** Kinetochore-microtubule attachments and probable outcomes during meiosis II and mitosis.
clin 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 a delay in chromatic 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 multicyclic 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 APC<sup>Cdc20</sup> and APC<sup>Cdh1</sup>. APC<sup>Cdc20</sup> is active from prometaphase until the MAT; whereas, APC<sup>Cdh1</sup> becomes active during anaphase and persists until the S phase. Various regulatory pathways control APC<sup>Cdc20</sup> and APC<sup>Cdh1</sup> 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 APC<sup>Cdh1</sup> mediates the proteolysis of Cdc20 and Plk1 (Peters 2002, Zachariae & Nasmyth 1999). APC<sup>Cdc20</sup> targets cyclin B for degradation, which leads to Cdk1 inactivation. Also, APC<sup>Cdc20</sup> 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 ubiquininated 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 (Hagsing et al. 2002). This proteolysis of securin liberates the cysteine protease separase, which cleaves centromeric Scc1 during mitotic anaphase onset (Nasmyth...**
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...

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-5322 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 hyperphosphory-
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, Kubia 1989, Takahashi et al. 1996, Yamanagami & Chang 1961). Aged oocytes also had lower ATP levels at fertilization (Igrashi et al. 2005), higher sensitivities to: oxidative stress (Boerjan & de Boer 1990, Takahashi et al. 2003, Tarin et al. 1996), calcium ionophores (Fulton & Whittingham 1978, McBevill et al. 1995, Vincent et al. 1992), partheneogenetic activation following chemical or mechanical stimuli (Cuthbertson & Cubbold 1985, Kaufman 1983, Kline & Kline 1992, Kubia 1989, Nagai 1987), and spontaneous calcium release (Beatrice et al. 1984, Orenius et al. 1992, Tames 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 (Li 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 MI 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. 2006). 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


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 parthenogenones reported no association between oocyte aging and aneuploidy (O’Neill & Kaufman 1988). However, these findings may be compromised by the difficulty of distinguishing between MII oocyte chromosomes and parthenogenone 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 identify 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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