Histol Histopathol (2006) 21: 873-886

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Mad2 and spindle assembly checkpoint function during meiosis I in mammalian oocytes

H.A. Homer

Newcastle Fertility Centre at Life, BioSciences Centre, International Centre for Life, Times Square, Newcastle upon Tyne, UK and School of Surgical and Reproductive Sciences, The Medical School, Framlington Place, University of Newcastle, Newcastle upon Tyne, UK

Summary. During mammalian mitosis, a proofreading network called the spindle assembly checkpoint (SAC) is indispensable for ensuring the fidelity of chromosome segregation. An inhibitory SAC signal is deputed to inhibits mitotic cell-cycle progression in response to misaligned chromosomes until such imperfections are rectified thereby ensuring equitable chromosome partitioning to daughter cells. Amongst the cast of SAC proteins, mitotic arrest deficient 2 (Mad2) plays a leading role in transducing the SAC signal. The aneuploidy and cancer predispositions of individuals who harbour genetic mutations in SAC genes emphasise the in vivo significance of this surveillance mechanism. In humans, congenital aneuploidies such as Down's syndrome demonstrate an exponential increase with advancing female age. Although largely the result of female meiosis I errors, the molecular entities that succumb with age in oocytes remain elusive. Declining oocyte SAC function could plausibly contribute to such errors. Until recently however, convincing evidence for a functional SAC in mammalian oocytes during meiosis I was unforthcoming. Here I review the evidence regarding the SAC in female mammalian meiosis I and how our understanding of this system has evolved in recent years. This review will focus on Mad2 as this is the SAC protein that has been most comprehensively investigated.

Key words: Nosdisjunction, Mammals, Oocyte maturation, Homologue disjunction, Aneuploidy

Introduction

Meiosis represents a specialized form of cell division which fulfils two important functions in sexually reproducing organisms: (1) the halving of the chromosome compliment in preparation for sexual reproduction, and; (2) the generation of genetic diversity through reciprocal recombination. As discussed in greater detail later, meiosis is comprised of two nuclear divisions referred to as meiosis I and meiosis II. During meiosis I, recombined homologous chromosomes segregate or disjoin so that errors in this process are, somewhat confusingly, referred to as nondisjunction.

Chromosome mis-segregation produces catastrophic sequelae by generating cells containing abnormal numbers of chromosomes, a condition referred to as aneuploidy. Aneuploidy resulting from errant mitoses sews the seeds for cancer (Draviam et al., 2004) whilst birth defects and miscarriage are the consequences when meiosis goes awry (Hassold and Hunt, 2001). Among meiotic divisions, meiosis I in human oocytes is notoriously error-prone and becomes increasingly faulty with advancing female age (Hassold and Hunt, 2001). Such miscreant female meiosis I divisions are of immense importance to human reproduction as the resulting aneuploidies are the single largest genetic cause of mental retardation and make a substantial contribution to human miscarriage (Hassold and Hunt, 2001). Why does aging increase the rates of nondisjunction in human oocytes? It is generally accepted that molecular components required for faithfully segregating homologues degrade or else become dysfunctional towards the twilight of a woman's reproductive lifetime (Lamb et al., 1996; Hassold and Hunt, 2001). The outstanding question pertains to the identity of these hitherto mysterious molecular components.

Great strides have been made in understanding the regulation of mitosis in many species, including humans, and thus the molecular basis for cancer. An essential element of mitotic regulation is a proofreading mechanism called the spindle assembly checkpoint (SAC) which is indispensable for maintaining ploidy during mitotic divisions; SAC malfunction results in aneuploidy which in turn can contribute to tumorigenesis (Bharadwaj and Yu, 2004; Draviam et al., 2004). In keeping with this, it has recently been demonstrated that the increased susceptibility to aneuploidy and cancer in patients with the mosaic variegated aneuploidy (MVA)

Offprint requests to: Dr. Hayden A. Homer, Department of Obstetrics & Gynaecology, Cumberland Infirmary, Carlisle, Cumbria CA2 7HY, UK. email: h.a.homer@ncl.ac.uk

syndrome is related to the presence of germline biallelic mutations in the *BUB1B* gene which encodes the SAC component BubR1 (Hanks et al., 2004).

Unlike mitosis, our knowledge regarding the molecular regulation of chromosome segregation during meiosis I is polarised in favour of lower eukaryotes with data from the yeast model contributing the bulk of our current understanding (for review see Marston and Amon, 2004). Although the overall scheme of meiotic events is conserved, additional dimensions of complexity not replicated in models such as yeast are encountered by the meiotic machinery in mammalian oocytes. Unlike meiosis in model organisms such as yeast, female mammalian meiosis is a discontinuous multi-stage process which commences during fetal life and is not completed until postnatal life, an interval which could last decades in humans. Other unique features include a dynamic cross-talk between oocyte and follicular somatic cell compartment that is important for oocyte growth (Matzuk et al., 2002); asymmetric divisions (Maro and Verlhac, 2002) essential for ensuring that the mature egg retains the bulk of cytoplasm to sustain early embryonic development; unique meiotic genes expressed in vertebrates but not in lower eukaryotes (Lefebvre et al., 2002; Libby et al., 2003); and a relatively large genome in mammals (the budding yeast *Saccharomyces cerevesiae* possesses 16 chromosome pairs and 12 MB of DNA as compared with 2500 MB over 19 autosome pairs in the mouse *Mus musculus*) (Svetlanov and Cohen, 2004). In addition to the invaluable insight provided by studies in lower eukaryotes, it will therefore be important to dissect the regulatory pathways involved in coordinating meiosis I in mammalian oocytes in order to refine our insight into the origins of human aneuploidy.

It is conceivable that SAC deficiencies in oocytes of older women could contribute to human aneuploidy (Steuerwald et al., 2001; Shonn et al., 2003; Homer et al., 2005a). Although lagging behind investigations into other meiosis I model organisms, robust evidence regarding SAC function in mammalian oocytes is beginning to emerge. At the molecular level, greatest strides have been made in elucidating the function of the Mad2 SAC protein. In this review, I discuss evidence pertinent to SAC function in mammalian oocytes with particular emphasis on Mad2.

Mitosis and the spindle assembly checkpoint

Much of our current understanding of meiosis has been derived from an in depth knowledge of the molecular players involved in mitosis. It will therefore be useful at this stage to outline some of the fundamental aspects of mitotic regulation prior to delving further into meiosis I.

Overview of mitosis

During mitosis, a single round of DNA replication during S-phase produces two identical DNA copies (referred to as sister chromatids) which undergo a single round of chromosome segregation during M-phase thus generating two identical daughter cells (see Fig. 2). In order to achieve this, sister chromatids generated during S-phase are held together by a phenomenon called cohesion. At the molecular level, cohesion is conferred by a highly conserved protein complex called cohesin which is comprised of four subunits, SMC1, SMC3, Scc3 and Scc1 (reviewed in Haering and Nasmyth, 2003; Uhlmann, 2003). Sister chromatids attach to spindle microtubules via proteinaceous structures assembled on centromeric DNA called kinetochores (reviewed in Cleveland et al., 2003; Hauf and Watanabe, 2004). Cohesion is essential for enabling sister chromatids to become attached to opposing poles of the spindle, a configuration referred to as amphitelic attachment and a pre-requisite for accurate segregation of the genome (Fig. 1A).

After ensuring that all chromosome pairs have become amphitelically attached, it is then deemed safe to initiate anaphase which requires that cohesion along both chromosome arms and centromeres be resolved. In vertebrates, the bulk of arm cohesins are removed during prometaphase by a cleavage-independent mechanism mediated by polo-like kinase and aurora B kinase (Fig. 2) (Losada et al., 2002; Sumara et al., 2002; Giménez-Abián et al., 2004). However, complete resolution of cohesion, and hence anaphase onset, is ultimately dependent upon cleavage of the Scc1 subunit of cohesin by the thiol protease, separase (Fig. 2) (Waizenegger et al., 2000; Hauf et al., 2001). Separase activity is negatively regulated by its association with an inhibitory

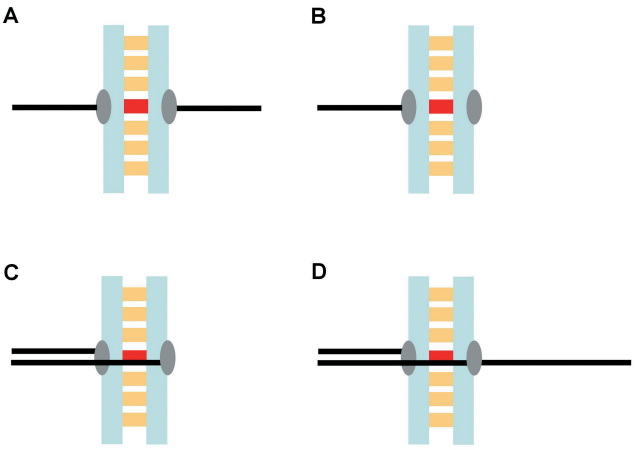

Fig. 1. Kinetochore-microtubule attachment configurations during mitosis. **A.** Amphitelic. One sister kinetochore (grey) attached by microtubules (black) to a single spindle pole and the other kinetochore attached to the opposite pole. **B.** Monotelic. One sister kinetochore attached to one pole. **C.** Syntelic. Both sister kinetochores attached to the same pole. **D.** Merotelic. One kinetochore attached to two poles. Orange, arm cohesion; red, centromeric cohesion.

chaperone called securin and by phosphorylation induced by the major cell-cycle kinase, cyclin-dependent kinase 1 (Cdk1) (Stemmann et al., 2001). Conversely, destruction of securin and cyclin B, the latter resulting in Cdk1 inactivation, liberates separase to engage Scc1. The destruction of securin and cyclin B at the metaphase-to-anaphase transition is brought about by a multi-subunit ubiquitin ligase called the anaphase-promoting complex or cyclosome (APC/C) in combination with its activating subunit, Cdc20 (Fig. 2) (reviewed in Peters, 2002; Castro et al., 2005). The APC/C^{Cdc20} ubiquitinates securin and cyclin B thereby consigning them to recognition and subsequent destruction by the 26S proteasome. APC/C^{Cdc20} activity must be tightly regulated as anaphase initiation under conditions of imperfect chromosome alignment can irremediably alter ploidy with disastrous consequences.

The mitotic spindle assembly checkpoint

The principal network for regulating the APC/C^{Cdc20} in response to kinetochore-microtubule attachment status is the SAC (Fig. 2) (reviewed in Musacchio and Hardwick, 2002; Taylor et al., 2004). The SAC is comprised primarily of members of the Mad (for mitotic arrest-deficient) and Bub (budding uninhibited by benzimidazole) protein families along with, among others, Mps1 and CENP-E (Musacchio and Hardwick, 2002; Taylor et al., 2004). One of the currently favoured models regarding SAC function posits that in response to kinetochores which lack microtubule attachment and/or tension, the primary downstream effect of SAC activation is the generation of a conformation of Mad2 capable of sequestering cellular Cdc20 thereby preventing APC/C activation and hence cell cycle progression (Fig. 2) (De Antoni et al., 2005; Nasmyth, 2005). Although the mechanism of SAC signalling in response to improperly attached kinetochores remains incompletely resolved, the net effect is indisputably APC/C^{Cdc20} inhibition for which Mad2 is of central importance.

In vertebrate somatic cells, spindle microtubules nucleated from centrosomes engage kinetochores by a process of "search and capture" (Kirschner and Mitchison, 1986). In this model, dynamically unstable microtubules probe the space ("search") and become stabilized when they contact a kinetochore ("capture"). Because of the stochastic nature of this process, it is possible for a number of mis-attachment configurations to be generated during prometaphase of any given mitosis. These configurations include monotelic (one kinetochore attached to one spindle pole); syntelic (both kinetochores attached to one pole) and; merotelic (one kinetochore attached to both poles) attachments (Fig. 1B-D). It is essential that anaphase is prevented in the presence of inappropriate attachments by SAC-mediated inhibition of the APC/C^{Cdc20}. Incidentally, merotelic configurations are largely invisible to the SAC and are thought to be a leading cause of aneuploidy in mammalian somatic cells (Cimini et al., 2001, 2004).

It is unresolved whether the SAC monitors microtubule occupancy or tension or both, a debate which is difficult to resolve given the mutual dependency between the two phenomena (King and Nicklas, 2000; Pinsky and Biggins, 2005). Nevertheless, there is evidence to suggest that attachment and tension might be monitored by separate arms of the SAC (Waters et al., 1998; Skoufias et al., 2001).

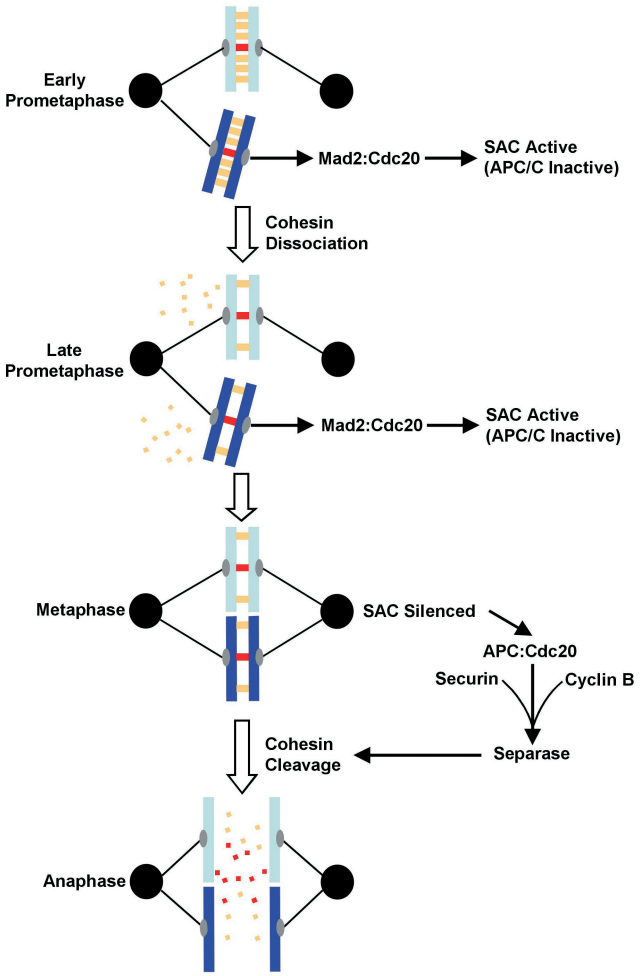

Fig. 2. Summary of SAC signalling during mitosis. During early prometaphase, misaligned chromosomes (dark blue) activate the SAC by generating a kinetochore (grey)-based inhibitory signal involving Mad2 which sequesters Cdc20 thereby preventing APC/C activation. As prometaphase proceeds, the bulk of arm cohesins (orange) dissociate so that by late prometaphase, chromatids (light and dark blue) are united primarily by centromeric cohesion (red) although small residual levels of arm cohesins remain. Once all chromosomes attain amphitelic attachments at metaphase, the SAC signal is abrogated releasing Cdc20 to activate the APC/C, in turn leading to securin and cyclin B degradation. The resulting activation of separase is required for anaphase onset by cleaving the remaining cohesins tethering sister chromatids together.

Blueprint for investigating SAC activity

Broadly speaking, in vertebrate cells, SAC activity is evident in two situations. The first situation arises when microtubule-kinetochore interactions are artificially perturbed as occurs when cells are treated with spindle poisons. At the molecular level, mitotic arrest following spindle disruption is the consequence of APC/C^{Cdc20} inhibition by SAC proteins such as Mad2 which in turn results in stabilisation of securin and cyclin B (Li and Benezra, 1996; Meraldi et al., 2004; Michel et al., 2004; Morrow et al., 2005). In contrast, under these same conditions, SAC-compromised cells are incapable of restraining APC/C activity and cannot therefore sustain a mitotic arrest (Li and Benezra, 1996; Dobles et al., 2000).

The second situation arises in unperturbed cells in which the SAC is required for setting the timing of anaphase onset and hence the duration of mitosis (for the purposes of this review, the term unperturbed refers to cells that have not been treated with spindle poisons). A corollary of this is that when the SAC is compromised, anaphase is initiated in the presence of chromosomes that have not become properly attached to the spindle. This is reflected in premature APC/C^{Cdc20}-mediated destruction of securin and cyclin B culminating in a reduction in the total duration of mitosis and ultimately, in aneuploidy (Gorbsky et al., 1998; Geley et al., 2001; Hagting et al., 2002; Kops et al., 2004; Michel et al., 2004).

The logic of meiosis

Unlike mitosis in which one nuclear division follows S-phase, halving the chromosome compliment during meiosis requires that two consecutive nuclear divisions, meiosis I and meiosis II, are executed on the heels of a single round of DNA replication (reviewed in Petronczki et al., 2003; Marston and Amon, 2004; Watanabe, 2004). Unlike meiosis II and mitosis in which sister chromatids are partitioned, during meiosis I, pairs of homologous chromosomes are segregated, each homologue consisting of a pair of sister chromatids (Fig. 3).

The theme of meiosis is firstly to segregate homologous chromosome pairs during meiosis I (reductional-type division) and secondly to segregate sister chromatids during meiosis II (equational-type division). Given that the overall objective of mitosis and meiosis II is similar, that is to segregate sister chromatids, meiosis II shares many features in common with mitosis. In contrast, the segregation of homologous chromosomes requires unique mechanistic tweaking. Firstly, homologous pairs need to be connected, a feat which is usually achieved during prophase I by reciprocal recombination. It is outside the scope of this review to detail the events involved in reciprocal recombination for which the interested reader is directed to recent excellent reviews on the subject (Page and Hawley, 2003; Svetlanov and Cohen, 2004; Gerton and Hawley, 2005). Recombination produces homologous pairs that are united distal to cross-over sites by arm cohesion, the conjoined homologues being referred to as a bivalent (Fig. 3) (Hauf and Watanabe, 2004; Watanabe, 2004). Secondly, homologous sister chromatid pairs (as opposed to sister chromatids as in mitosis and meiosis II) need to be attached to opposite poles (Fig. 3). Therefore, unlike mitotis in which syntelic attachments are abhorred and thus actively eliminated (Lampson et al., 2004), such attachments are an important facet of normal meiosis I. This is achieved in part by a side-by-side kinetochore geometry, otherwise known as mono-orientation of kinetochores (Hauf and Watanabe, 2004). Thirdly, only arm cohesion must be targeted during meiosis I leaving centromeric cohesion in tact for proper execution of meiosis II (Fig. 3) (Watanabe, 2004). During meiosis, cohesion is conferred by a cohesin complex in which Scc1 is largely replaced by a meiosis-specific isoform called Rec8 (Uhlmann, 2003; Watanabe, 2004). Evidence from budding yeast demonstrates that separase-mediated cleavage of Rec8 along chromosome arms is responsible for homologue disjunction (Buonomo et al., 2000). This mechanism for reductional meiotic division appears conserved as immunolocalisation studies in male and female mammals indicate that Rec8 is lost from chromosome arms during the meiosis I-to-meiosis II transition (Eijpe et al., 2003; Lee et al., 2003; Kouznetsova et al., 2005). As discussed below, other evidence involving securin and separase in mammalian oocytes also lend support to this notion.

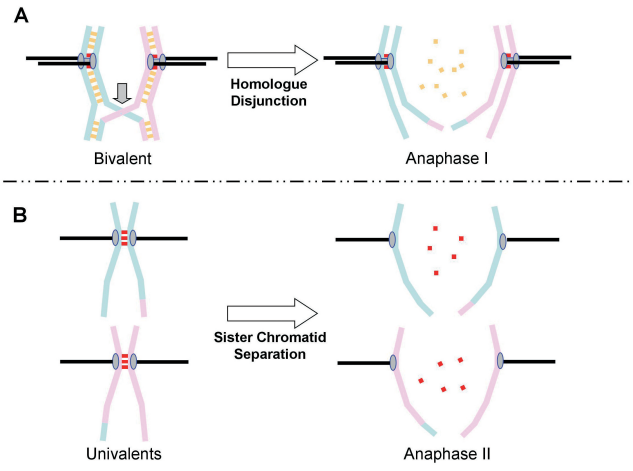

Fig. 3. Mechanics of meiotic chromosome segregation. **A**. Meiosis I. During meiosis I homologous chromosome pairs (blue and pink) are united by arm cohesion (orange) distal to chiasmata (grey arrow), the cytological manifestations of cross-overs. Sister kinetochores (grey ovals) are constrained in a side-by-side geometry thereby facilitating orientation towards one pole (mono-orientation). Homologue disjunction is mediated by selective loss of arm cohesion whilst retaining centromeric cohesion (red). **B** Meiosis II. At metaphase II sister chromatids are united by residual centromeric cohesion. Note the back-to-back sister kinetochore geometry facilitating amphitelic attachment. At anaphase II sister chromatids separate following cleavage of residual centromeric cohesion.

In mammalian oocytes, distinct morphological changes accompany the archetypal meiotic events described above. Fully-grown oocytes arrested at the dictyate stage of prophase I are identifiable by the presence of an intact nucleus (referred to as a germinal vesicle, or GV) (Fig. 4). Resumption of meiosis I is marked by GV breakdown (GVBD) and is followed by assembly of a bipolar spindle during prometaphase I. Following alignment of bivalents at the spindle equator, homologue disjunction ensues (anaphase I) and is followed very closely by first polar body extrusion (PBE) (Brunet et al., 1999) subsequent to which oocytes enter meiosis II and arrest at metaphase II awaiting fertilisation (Fig. 4). Thus, in mammalian oocytes, meiosis I is represented by the period between GVBD and PBE, the duration of which is both species- and, among model animals, strain-specific.

The APC/C in female mammalian meiosis I

During mitosis, SAC activity converges on the APC/C^{Cdc20} (Musacchio and Hardwick, 2002; Peters, 2002; Taylor et al., 2004; Castro et al., 2005; Nasmyth, 2005). Knowledge of whether the APC/C regulates meiotic progression in mammalian oocytes is therefore germane to the issue of SAC function. As detailed above, the APC/C^{Cdc20} is responsible for initiating mitotic anaphase through the ubiquitination of securin and cyclin B which earmarks them for subsequent degradation by the 26S proteasome (Peters, 2002; Castro et al., 2005) thereby releasing active separase for cleavage of cohesin (Fig. 2). Current evidence indicates that every one of these steps, and by extension APC/C activity, is also important for homologue disjunction in mammalian oocytes.

In mammalian oocytes, degradation of securin and cyclin B are required for anaphase I and exit from meiosis I. The decline of Cdk1 activity on exit from meiosis I in mouse oocytes was shown by pulse-chase experiments to be the consequence of cyclin B degradation (Hampl and Eppig, 1995; Winston, 1997), a fact later confirmed using real-time analysis of a green fluorescent protein (GFP)-labelled cyclin B construct (Ledan et al., 2001; Herbert et al., 2003; Tsurumi et al., 2004; Homer et al., 2005b,c). It contrast to the deluge of reports pertaining to Cdk1 activity and cyclin B during meiosis I in mammalian oocytes, reports investigating securin are relative newcomers. Mouse oocytes were shown to express endogenous securin and, using fluorescently labelled chimerae, securin was shown to turnover in a similar manner to cyclin B, being synthesised from GVBD onwards and later degraded on exit from meiosis I (Herbert et al., 2003; Tsurumi et al., 2004; Homer et al., 2005b,c). Furthermore, inhibition of either securin or cyclin B degradation is sufficient to inhibit homologue disjunction and exit from meiosis I (Herbert et al., 2003).

The APC/C^{Cdc20} targets substrates for polyubiquitination via an RXXL motif called the destruction box (D-box) (Pfleger and Kirschner, 2000; Peters, 2002; Castro et al., 2005). Apart from Cdc20, the APC/C possesses another co-activator called Cdh1 which targets substrates via either a D-box or another consensus sequence called the KEN-box (Pfleger and Kirschner, 2000; Peters, 2002; Castro et al., 2005). Indeed, Cdc20 lacks a D-box and its degradation in late mitosis and G1 is conferred by a KEN-box (Pfleger and Kirschner, 2000). As cyclin B possesses a single APC/C degron (a D-box), mutating or deleting this sequence is sufficient to prevent the destruction of cyclin B during vertebrate mitosis (Brandeis and Hunt, 1996; Clute and Pines, 1999; Pfleger and Kirschner, 2000; Hagting et al., 2002). In contrast, human securin possesses both D- and KEN-boxes and can thus be degraded by both APC/C^{Cdc20} and APC/C^{Cdh1} during mitosis (Zur and Brandeis, 2001; Hagting et al., 2002).

The requirement for D-box mediated destruction in meiosis I mouse oocytes has been examined using timelapse imaging of two mutant constructs fused C-terminally to GFP. One mutant was a truncated form of cyclin B lacking the D-box-containing N-terminal 90 amino acids (Δ90 cyclin B1; Fig. 5) (Glotzer et al., 1991) while the other was a securin D-box mutant (securin^{dm}) in which the D-box was mutated (RXXL to AXXA). Both of these mutants were resistant to degradation, and expression of either one inhibited

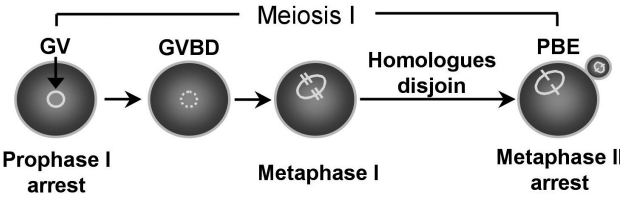

Fig. 4. Schematic of meiosis I in mammalian oocytes. Oocytes arrested at prophase I are identifiable by the presence of a GV. GVBD marks the resumption of meiosis I following which homologues are segregated. PBE marks the conclusion of meiosis I after which oocytes arrest at metaphase II.

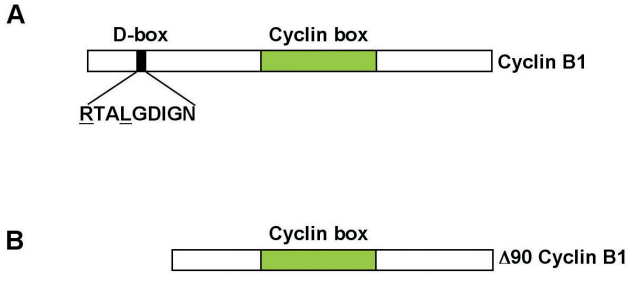

Fig. 5. Schematic model of full-length cyclin B1 **(A)** and Δ90 cyclin B1 **(B)**. Δ90 cyclin B1 lacks the N-terminal 90 amino-acids containing the D-box but retains the ability to activate Cdk1 by virtue of the cyclin box (green).

homologue disjunction and PBE (Herbert et al., 2003) thus strongly implicating the APC/C in progression through meiosis I. Given that securin possesses both D- and KEN-boxes, it is interesting to note that securin*dm*-GFP was stable in mouse oocytes. This implies that although endogenous Cdc20 and Cdh1 are present in mouse oocytes during meiosis I and APC/C^{Cdh1} is active shortly after exit from meiosis II (Chang et al., 2004), APC/C^{Cdh1} is not active during meiosis I.

A number of other pieces of evidence are also consistent with a role for the APC/C in mammalian oocytes. During mitosis, overexpression of securin induces a mitotic delay at metaphase (Hagting et al., 2002) due to saturation of the APC/C. Like mitosis, overexpression of securin induces a metaphase I arrest in mouse oocytes (Terret et al., 2003). Saturation of a putative destruction machinery is further corroborated by the fact that overexpression of exogenous securin also inhibited the destruction of endogenous cyclin B resulting in sustained Cdk1 activity (Terret et al., 2003). Consistent with a requirement for securin destruction (and hence APC/C activity) mouse oocytes require separase activity for proper homologue disjunction as an inhibitor of separase induced defects in homologue disjunction including failure of homologues to segregate and a 15% "cut" phenotype in which chromosomes become ensnared between the oocyte and the first polar body (Terret et al., 2003). Preventing the formation of a multi-ubiquitin chain with methylated ubiquitin induces a meiosis I arrest in rat oocytes (Dekel, 2005) indicating that progression through meiosis I is dependent upon polyubiquitination. In keeping with this, endogenous expression of the proteasome has been demonstrated in rat oocytes and the proteasome inhibitor, MG132, arrests rat and mouse oocytes in meiosis I with metaphase I spindles and high Cdk1 activity, the latter secondary to inhibition of cyclin B destruction (Josefsberg et al., 2000; Terret et al., 2003).

In summary therefore, the degradation of securin and cyclin B are requirements for the metaphase I-to-anaphase I transition and exit from meiosis I in mammalian oocytes. Moreover, protein degradation depends on the APC/C-specific D-box and is inhibited by overexpression of APC/C substrates implying sensitivity to APC/C saturation. Finally disrupting the polyubiquitination process or inhibiting the 26S proteasome negatively impacts meiosis I progression. Taken together, these data strongly implicate the APC/C as an important orchestrator of homologue disjunction and meiosis I exit in mammalian oocytes.

The SAC in mammalian oocytes

Evaluating the SAC using spindle poisons

SAC competence in mitosis is characterised by mitotic arrest and stabilisation of securin and cyclin B upon spindle disruption (see above). Two types of microtubule-kinetochore defects are observed following pharmacological treatment. In the first type, usually induced by high doses of nocodazole, the spindle is depolymerised thereby depriving all kinetochores of both attachment and tension. The second type of defect is classically associated with taxol but may be produced by low doses of spindle depolymerising agents and is associated with an intact spindle which primarily lacks tension although there may also be minor changes in microtubule occupancy status (Waters et al., 1998; Skoufias et al., 2001; Pinsky and Biggins, 2005). The mitotic arrests induced by both types of spindle defects are robust and perdure indefinitely in many mammalian cells in culture (Skoufias et al., 2001).

Evidence from spindle depolymerisation studies

Hashimoto and Kishimoto (1988) depolymerised the spindle in mouse oocytes using colcemid and found that PBE rates were reduced in drug-treated compared to untreated oocytes (10.9% versus 69%) after 14-16 hours of incubation. Furthermore, this was accompanied by stabilisation of an MPF activity (for maturation or M-phase promoting factor activity; synonymous with Cdk1 activity) (Hashimoto and Kishimoto, 1988). Another indirect assay for MPF activity based on the protein synthesis inhibitor, puromycin, also suggested that 4 hours of spindle depolymerisation stabilised MPF activiy (Brunet et al., 2003). More recently, the absence of a spindle was demonstrated to stabilise Cdk1 activity measured directly by histone H1 kinase assays (Homer et al., 2005c); in mouse oocytes, all of the histone H1 kinase activity represents Cdk1 activity (Hampl and Eppig, 1995). Together these experiments demonstrate that spindle depolymerisation induces a meiosis I arrest in which Cdk1 activity is stabilised.

In mouse oocytes, Cdk1 inactivation at the meiosis I-to-meiosis II transition is due to cyclin B degradation (Hampl and Eppig, 1995; Winston, 1997). Thus, stabilisation of Cdk1 activity upon spindle depolymerisation suggested that this was due to inhibition of cyclin B destruction. However, given that rat embryos and pig oocytes can regulate Cdk1 activity independently of cyclin B (Josefsberg et al., 2001; Takakura et al., 2005), this was not a foregone conclusion. In support of cyclin B stabilisation, micromolar concentrations of nocodazole which depolymerise the spindle in mouse oocytes were found to prevent the usual disappearance of a 62 kDa phosphoprotein at the meiosis I-to-meiosis II transition as determined by pulse-chase experiments (Kubiak et al., 1992). Although the authors speculated that the 62kDa protein could be cyclin B, this was not demonstrated nor was it known whether maintenance of the phosphoprotein in nocodazole represented inhibition of dephosphorylation or of protein destruction (Kubiak et al., 1992). Formal proof that cyclin B was stabilised by spindle depolymerisation was provided by immunoblotting oocytes for cyclin B after 3 hours incubation in nocodazole (Lefebvre et al., 2002). More

recently, Cdk1 and cyclin B stabilisation upon spindle depolymerisation were demonstrated together in a single report thereby confirming that Cdk1 stabilisation in the absence of a spindle is due to inhibition of cyclin B degradation (Homer et al., 2005c). Moreover, the latter paper extended previous reports by showing that cyclin B could be stabilised for prolonged periods (>18 hours) following spindle depolymerisation (Homer et al., 2005c).

In addition to cyclin B, the other principal downstream target of the SAC is securin. The obvious question therefore was whether spindle depolymerisation also stabilised securin. Spindle depolymerisation for 14-16 hours was found to prevent homologue disjunction as assessed by chromosome spreads (Soewarto et al., 1995). More recently, DNA staining also suggested that a 4 hour duration of spindle depolymerisation inhibited homologue disjunction (Brunet et al., 2003). Given that securin destruction and separase activity are required for homologue disjunction (Herbert et al., 2003; Terret et al., 2003), the extrapolation of these data is that spindle depolymerisation inhibited securin destruction. Formal proof of this was later provided by timelapse fluorescence imaging which showed that securin was stabilised by spindle depolymerisation and, like cyclin B, could be stabilised for prolonged periods (>18 hours) (Homer et al., 2005c).

It was also shown that prolonged periods of securin stabilisation were accompanied by inhibition of homologue disjunction, the latter assessed by chromosome spreads (Homer et al., 2005c). This is an important finding pertinent to the regulation of arm cohesion in mammalian oocytes. In vertebrate somatic cells, the bulk of cohesin (95%) is removed from chromosome arms in a cleavage-independent manner prior to securin destruction and separase activation (see Fig. 2) (Waizenegger et al., 2000; Losada et al., 2002; Sumara et al., 2002; Giménez-Abián et al., 2004). Although not sufficient to separate chromosome arms within the timeframe of an unperturbed mitosis, prolongation of prometaphase by incubation in nocodazole enables the non-cleavage pathway to completely resolve arm cohesion (Giménez-Abián et al., 2004). Given that homologue disjunction in meiosis I is mediated by resolution of arm cohesion (Buonomo et al., 2000), there was a suspicion that a non-cleavage pathway might be sufficient for segregating homologues. Notably, mitosis has to be prolonged beyond the usual limits observed in wild-type cells for the non-cleavage pathway to become cytologically evident (Giménez-Abián et al., 2004). Studies in which mouse oocytes were incubated in nocodazole for periods which were not in excess of the wild-type meiosis I duration could not therefore adequately address this issue (Soewarto et al., 1995; Brunet et al., 2003). However, when meiosis I is prolonged almost 2-fold by nocodazole, homologues did not disjoin thus demonstrating that a non-cleavage pathway is insufficient on its own for resolving arm cohesion in mammalian oocytes (Homer et al., 2005c). This is in keeping with a requirement for securin degradation and separase activity for homologue disjunction (Herbert et al., 2003; Terret et al., 2003). From this we can conclude that unlike mitosis, the stabilisation of securin in meiosis I mammalian oocytes is associated with the persistence of arm cohesion.

Was the meiosis I arrest and protein stabilisation following spindle depolymerisation mediated by the SAC? Alternatively, could this phenotype merely be due to the absence of a spindle as was suggested could be the case under such experimental conditions (Wassmann et al., 2003a)? This was addressed by examining the role of the SAC protein, Mad2, under conditions in which the spindle was depolymerised (Homer et al., 2005c). Somatic cells depleted of the majority of Mad2 using the reverse genetic approach of RNA interference (RNAi) are unable to sustain a mitotic arrest upon spindle depolymerisation and prematurely degrade securin and cyclin B (Kops et al., 2004; Michel et al., 2004). In line with this paradigm, in oocytes in which the majority of Mad2 was depleted using morpholino antisense technology (see later discussions), securin and cyclin B were unstable following spindle depolymerisation whereas mock-depleted oocytes phenocopied wild-type oocytes by sustaining high levels of securin and cyclin B for several hours (Homer et al., 2005c). Furthermore, although PBE was completely inhibited in control oocytes treated with nocodazole, 15% of Mad2-depleted oocytes extruded polar bodies which, due to the absence of a spindle, were devoid of DNA (Homer et al., 2005c). Moreover, protein destabilisation in Mad2-depleted oocytes was the result of unrestrained APC/C activity as Δ90 cyclin B was stable following Mad2 knockdown (Homer et al., 2005c).

From these data, we can conclude that in response to spindle depolymerisation, mouse oocytes mount a sustained SAC-mediated response which arrests meiosis I by inhibiting the destruction of securin and cyclin B, likely by inhibiting APC/C activity. The biochemical nature of this meiosis I arrest is reminiscent of the response of mammalian somatic cells to spindle depolymerisation and indicates that the molecular players involved in the SAC are conserved between mitosis and meiosis I. In spite of this similarity however, mammalian somatic cells and oocytes are divergent when it comes to the degree of dependency on separase for the resolution of arm cohesion.

Evidence from studies with drugs which do not depolymerise the spindle

Nanomolar concentrations of nocodazole which leave an intact spindle in mouse oocytes were shown to inhibit PBE and to stabilise Cdk1 activity during a 3 hour period of drug exposure (Wassmann et al., 2003a). In keeping with this, a 4 hour exposure to paclitaxel which stabilises microtubules without depolymerising the spindle, also inhibited homologue disjunction and PBE and stabilised Cdk1 activity, the latter assayed

indirectly using puromycin (Brunet et al., 2003). Consistent with this, histone H1 kinase assays demonstrate that Cdk1 is stabilised in taxol-treated oocytes (Homer, H, unpublished data).

The role of Mad2 under these conditions has been addressed using a mutant form of Mad2 harbouring serine to aspartic acid substitutions at positions 170, 178 and 195 (3S-D Mad2) (Wassmann et al., 2003a). 3S-D Mad2 acts as a dominant negative in human somatic cells by impairing the ability of endogenous Mad2 to form ternary complexes with APC/C^{Cdc20} (Wassmann et al., 2003b). In mouse oocytes cultured in low doses of nocodazole, it was found that 3S-D Mad2 was associated with of Cdk1 destabilisation, homologue disjunction and PBE (Wassmann et al., 2003a). Assuming that 3S-D Mad2 also exhibits dominant negative properties in mouse oocytes, this indicates that Mad2 is required for the meiosis I delay in response to low doses of nocodazole.

During mitosis, treatment with taxol or low doses of spindle depolymerising agents is associated with the persistence of an intact spindle in which tension is reduced as determined objectively using inter-kinetochore distance measurements (Waters et al., 1998; Skoufias et al., 2001; Pinsky and Biggins, 2005). Experiments in mouse oocytes have not formally measured inter-kinetochore distances. However, given the similar types of pharmacological treatments and the persistence of an intact spindle in both mammalian somatic cells (Waters et al., 1998; Skoufias et al., 2001; Pinsky and Biggins, 2005) and oocytes (Brunet et al., 2003; Wassmann et al., 2003a), a reasoned assumption is that these drug treatments also induce a tension defect in female meiosis I. Therefore, and in combination with the molecular characterisation of the meiosis I arrest of mouse oocytes induced by spindle depolymerisation (Homer et al., 2005c), these data could be interpreted as prima facie evidence that tension defects activate a Mad2-dependent SAC response which targets securin and cyclin B. This would be consistent with the requirement for Mad2 in sensing tension defects in other meiosis I systems including maize (Yu et al., 1999) and budding yeast (Shonn et al., 2000). Interestingly, Mad2 may not be involved in the tension-mediated SAC response in mitosis (Skoufias et al., 2001).

Importantly, extending the duration of culture in nanomolar concentrations of nocodazole was associated with substantial rates of PBE (40-60%), Cdk1 inactivation and homologue disjunction (Wassmann et al., 2003a; Shen et al., 2005). Therefore, unlike the response to spindle depolymerisation (Homer et al., 2005c), drug treatment which leaves an intact spindle induces only a transient Mad2-mediated meiosis I arrest. One interpretation of this is that the complete absence of attachment and tension activates a robust SAC response whereas in the absence of tension alone, the SAC is susceptible to "slippage" with time. In contrast, somatic cells mount an equally robust mitotic arrest following loss-of-tension as they do after spindle depolymerisation (Skoufias et al., 2001). Alternatively, rather than there being a qualitative difference in SAC activation signal accounting for inconsistent effects, such effects may instead reflect quantitative signal differences: in contrast to the signal when all kinetochores are unattached and lacking in tension, the signal produced from subtle defects induced by low doses of nocodazole may simply be too weak to induce sustained SAC activation. Another interesting observation is that homologues can disjoin in the absence of PBE when oocytes are treated with nanomolar concentrations of nocodazole (Shen et al., 2005). This suggests that securin degradation can be uncoupled from cyclin B degradation during meiosis I in mouse oocytes. However, this could also represent a failure of PBE in spite of cyclin B degradation as can occur in the presence of high exogenous levels of cyclin B (Ledan et al., 2001).

The SAC in unperturbed mammalian oocytes

Experiments involving spindle poisons constitute and in vitro means for demonstrating the existence or not of an SAC and for helping to dissect the molecular details of the SAC circuitry. However, such experimentation does not define the interdependence between SAC activity and chromosome segregation fidelity in unperturbed cells. For instance, although the SAC is required for cell survival after spindle disruption during yeast mitosis, under normal growth conditions chromosomes can be segregated reasonably accurately in the absence of Mad2 (Li and Murray, 1991). In contrast to yeast mitosis, Mad2 is essential in both drug-treated as well as unperturbed mammalian somatic cells (Gorbsky et al., 1998; Dobles et al., 2000; Michel et al., 2001).

Mad2 expression and localisation in mammalian oocytes

Western blotting demonstrates that Mad2 is endogenously expressed in unperturbed rat and mouse oocytes during meiosis I (Zhang et al., 2004; Homer et al., 2005b). Furthermore, in mouse oocytes, the concentration of Mad2 is ~200nM by mid-meiosis I (Homer et al., 2005b), similar to the estimated Mad2 concentration of 230nM in mammalian somatic cells (Fang, 2002). Notably, and unlike mitosis in which Mad2 levels remain stable (Fang, 2002), Mad2 levels increase during progression through meiosis I in mouse oocytes so that relative to levels at the GV-stage, Mad2 increases ~2-fold by mid-meiosis I and ~10-fold by metaphase II (Homer et al., 2005b). Thus, Mad2 is present in mammalian oocytes at levels that are consistent with SAC activity.

Immunolocalisation studies in mammalian mitosis indicate that Mad2 localises primarily to unattached kinetochores (Li and Benezra, 1996; Waters et al., 1998; Skoufias et al., 2001) consistent with the notion that mis-attached kinetochores are the source of a Mad2-based

signal important for SAC activation (Li and Benezra, 1996; Waters et al., 1998; Howell et al., 2000; Skoufias et al., 2001; De Antoni et al., 2005). In keeping with this, Mad2 immunostaining in mouse and rat oocytes reveals kinetochore localisation during early prometaphase I which gradually declines as meiosis I progresses becoming undetectable at metaphase I (Wassmann et al., 2003a; Zhang et al., 2004). This suggests that Mad2 dissociates from kinetochores during meiosis I as they accumulate microtubules. Moreover, spindle depolymerisation at metaphase I induced Mad2 to rebind kinetochores in mouse and rat oocytes (Wassmann et al., 2003a; Zhang et al., 2004) providing further evidence that Mad2 responds to kinetochore attachment status in mammalian oocytes. Thus, Mad2 is expressed in mammalian oocytes and dynamically localises to unattached kinetochores.

Evidence from gain-of-function studies

In somatic cells with wild-type levels of Mad2, improperly attached kinetochores act as a platform for amplifying Mad2-based Cdc20 inhibition (Howell et al., 2000; De Antoni et al., 2005; Nasmyth, 2005). Following Mad2 over-expression however, it has been proposed that Cdc20 is sequestered by a mass action effect rendering unattached kinetochores superfluous for signal-amplification and APC/C inhibition (De Antoni et al., 2005). Thus, in the presence of high levels of Mad2, although chromosome congression is unaffected, mitosis does not progress beyond metaphase (Howell et al., 2000; De Antoni et al., 2005) even though kinetochores possess their full compliment of microtubules. This effect of exogenous Mad2 is dose-dependent as a 10-fold excess of Mad2 over endogenous levels arrests mitotic progression whereas a 2-fold excess has no effect on mitosis (Howell et al., 2000).

Like mitosis, the response of mouse oocytes to Mad2 over-expression is graded as 4-fold excess levels have no effect on meiosis I, 15-fold excess levels induce a partial arrest and 35-fold excess levels arrest meiosis I completely (Homer et al., 2005b). Oocytes induced to arrest by Mad2 over-expression demonstrate an equatorial distribution of chromosomes on a bipolar spindle, sustained Cdk1 activity and intact bivalents consistent with being at metaphase I (Wassmann et al., 2003a; Homer et al., 2005b,d). Furthermore, oocytes not arrested by moderate Mad2 over-expression complete meiosis I with normal kinetics implying that excess Mad2 does not induce a delay; meiosis I is either arrested or proceeds at a normal rate when Mad2 is over-expressed (Homer et al., 2005b). Overall therefore, in response to Mad2 over-expression, mouse oocytes exhibit a dose-dependent arrest at metaphase I reminiscent of mitosis. This suggests that Mad2 over-expression constitutively activates the SAC in mammalian oocytes. Moreover, this provides another line of evidence in support of a role for the APC/C in mammalian oocytes.

Evidence from loss-of-function studies: timing is everything

During mitosis, the role of Mad2 has been comprehensively defined using a number of approaches which disable Mad2. These include, among others, function-blocking agents (antibodies and dominant negative-acting mutants) and reverse genetic approaches which deplete cellular Mad2 (Gorbsky et al., 1998; Howell et al., 2000; Canman et al., 2002; Wassmann et al., 2003b; Kops et al., 2004; Michel et al., 2004; De Antoni et al., 2005). Based on studies such as these, it has emerged that one of the essential functions of Mad2 is to furnish sufficient time for chromosomes to become properly aligned prior to anaphase onset (Gorbsky et al., 1998; Kops et al., 2004; Meraldi et al., 2004; Michel et al., 2004). In the absence of Mad2, mitosis is accelerated to a rate that is deleterious to chromosome alignment thereby culminating in aneuploidy. In contrast to mammalian somatic cells, it appears that the intrinsic mitotic timing machinery in mitotic yeast cells provides sufficient time for chromosome alignment to be completed so making SAC-imposed delays redundant (see Dobles et al., 2000).

Initial attempts to define Mad2 function in unperturbed mouse oocytes utilising dominant negative mutants produced conflicting results. Although 3S-DMad2 demonstrated that Mad2 was required for the meiosis I arrest in response to nanomolar concentrations of nocodazole, no noticeable effect was observed in the absence of drug treatment (Wassmann et al., 2003a). Subsequently, in a study primarily designed to examine the role of the SAC in meiosis II in mouse oocytes, a Mad2 mutant lacking its C-terminal 10 amino acids (Mad2ΔC) was used (Tsurumi et al., 2004). Mad2ΔC produces a dominant negative effect in mitotic cells due to its inability to adopt the closed conformation required for sequestering Cdc20 whilst maintaining its ability to be recruited to kinetochores thereby out-competing endogenous Mad2 (Luo et al., 2000; De Antoni et al., 2005). In contrast to 3S-DMad2, Mad2ΔC was observed to shorten the duration of meiosis I by ~2 hours (Tsurumi et al., 2004). It was therefore unclear at this stage whether or not Mad2 contributed to the timing of meiosis I in mammalian oocytes.

More recently, experiments using morpholinos helped to resolve this discrepancy in dominant negative data. Morpholinos target the mRNA START region and reduce target protein levels by sterically inhibiting translation initiation (Heasman, 2002). A Mad2-targeting morpholino (Mad2MO) was shown by Western blotting and immunofluorescence to consistently and specifically deplete the majority of endogenous Mad2 in mouse oocytes (Homer et al., 2005b,c). Timelapse imaging of mouse oocytes revealed that Mad2 depletion reduced the duration of meiosis I from ~11 hours to ~9 hours (Homer et al., 2005b). These results are therefore consistent with the effects of Mad2ΔC and together define a role for Mad2 in determining the timing of meiosis I in

mammalian oocytes. It is not known why 3S-DMad2 did not produce an observable effect in unperturbed mouse oocytes but possible explanations have been explored previously (Homer et al., 2005a). Mad2-depleted oocytes also underwent an increased rate of aberrant PBE (~60%) compared with wild-type oocytes producing either excessively large or multiple PBs (Homer et al., 2005b). This phenotype was not due to aberrant spindle morphology (Homer et al., 2005b) but may be a consequence of disrupting the 2 hour period during the latter stages of meiosis I when the spindle normally migrates to the cortex (Verlhac et al., 2000).

The accelerated progression through meiosis I with Mad2MO and Mad2ΔC suggested that the APC/C was prematurely activated secondary to deficient Mad2-based Cdc20 inhibition. In line with premature APC/C activation, securin and cyclin B destruction occur ~2 hours earlier in Mad2-depleted oocytes (Homer et al., 2005b). Further evidence that premature APC/C activation was due to inefficient Cdc20 inhibition was derived from experiments involving a phosphorylation-resistant Cdc20 mutant (Tsurumi et al., 2004). Cdc20 phosphorylation at residues 50, 64, 68 and 79 increases its affinity for Mad2 upon activation of the SAC (Chung and Chen, 2003). Consequently, a phosphorylation-resistant Cdc20 mutant (Cdc20-4AV) binds less avidly to Mad2 resulting in unrestrained APC/C activity and reduced SAC competence (Chung and Chen, 2003). In unperturbed mouse oocytes, Cdc20-4AV shortened the duration of meiosis I to a similar degree as Mad2MO and Mad2ΔC (Tsurumi et al., 2004; Homer et al., 2005b). Thus, disrupting Mad2 function produces similar effects to a Mad2-resistant Cdc20 implying that in the former case, the observed effects were due to defective Cdc20 inhibition. Interestingly, partial Cdc20 de-phosphorylation in mitosis secondary to MAPK inactivation may initiate a cascade which leads to full APC/C^{Cdc20} activation and hence anaphase and mitotic exit (Chung and Chen, 2003). In mouse oocytes however, MAPK is not inactivated upon exit from meiosis I (Verlhac et al., 1993; Polanski et al., 1998), suggesting either that MAPK-dependent Cdc20 phosphorylation may be dispensable in mouse oocytes for regulating SAC activity or else that Cdc20 dephosphorylation can proceed independently of MAPK inactivation.

Does the modest reduction (~20%) in meiosis I duration consequent upon disrupted Mad2 function affect the fidelity of homologue disjunction? Oocytes depleted of Mad2 still exhibited a comparatively long meiosis I transit time of ~9 hours (Homer et al., 2005b) whereas similar degrees of Mad2 depletion reduced mitosis on average to a mere 60 minutes (Meraldi et al., 2004; Michel et al., 2004). Since intrinsic timing alone might be adequate for accurate chromosome segregation (see above), it was important to determine whether or not the modest reduction in meiosis I consequent upon loss of Mad2 function conferred a disadvantage to homologue disjunction. Analysis of chromosome spreads revealed that homologue disjunction fidelity did indeed suffer as aneuploidy rates increased dramatically from ~2% in wild-type oocytes to ~32% following Mad2 depletion (Homer et al., 2005b). From this we can conclude that Mad2 is indispensable for accurate homologue disjunction. The degree of dependency on Mad2 for accurate homologue disjunction appears disproportionate to its effect on meiosis I timing and suggests that other facets of Mad2 function may be important in safeguarding homologue disjunction. In this respect it is noteworthy that although loss of Mad2 does not produce a detectable change in meiosis I timing in budding yeast, Mad2 is nonetheless important for accurate homologue disjunction which may be related to its role in actively bi-orienting bivalents (Shonn et al., 2000, 2003; Cheslock et al., 2005).

Concluding remarks and future prospects

From these data, it is evident that the basic scheme of mitotic SAC signalling is conserved during meiosis I in mammalian oocytes. Thus in both systems, securin and cyclin B are important downstream targets of Mad2 likely via the APC/C as intermediary (see Fig. 2). Although this represents a significant advancement regarding our understanding of female meiosis I regulation, it is important to acquiesce that these data provide only a bare bones appreciation for SAC function in mammalian oocytes. Several other aspects of SAC signalling in oocytes remain to be elucidated. For instance, Bub1 and Mad1 localise to kinetochores during meiosis I in mammalian oocytes (Brunet et al., 2003; Zhang et al., 2005) and dominant negative mutants of BubR1 and Bub1 accelerate progression through meiosis I (Tsurumi et al., 2004). Are SAC proteins such as Mad1, Bub1 and BubR1 also required for averting nondisjunction in mammalian oocytes and might they potentiate one another's function?

Bub1 and BubR1 may have functions apart from classical SAC signalling that are of particular relevance to human nondisjunction. In humans, one of the predisposing factors for nondisjunction is absent recombination between homologous chromosomes (Lamb et al., 1996; Hassold and Hunt, 2001). As a species, humans are not singular in this respect as the presence of a non-exchange chromosome also renders homologue disjunction error-prone in budding yeast and mouse oocytes (LeMaire-Adkins et al., 1997; Cheslock et al., 2005). However, budding yeast appear to possess a so-called distributive system based on a centromere-pairing step in prophase I which mitigates against (although does not completely eliminate) random segregation of achiasmate chromosomes (Kemp et al., 2004). It will be important to determine whether the mammalian oocyte possesses an equivalent weapon within its arsenal for fending off the mis-segregation of non-exchange chromosomes. In this regard, BubR1 is potentially important as recent data indicates that Mad3 (the yeast homologue of vertebrate BubR1) is important

for the distributive system in budding yeast (Cheslock et al., 2005).

Premature sister chromatid separation (PSCS) makes a significant contribution to human aneuploidy (Angell, 1991; Sandalinas et al., 2002). In yeast meiosis I, Bub1 has been shown to be important for preventing PSCS by influencing centromeric cohesion through its effect on shugoshin (Sgo1), a protector of centromeric cohesion (Bernard et al., 2001; Katis et al., 2004; Kitajima et al., 2004; Marston et al., 2004; Rabitsch et al., 2004). The recent demonstration that Bub1 is also required for Sgo1-mediated protection of centromeric cohesion during mammalian mitosis (Kitajima et al., 2005) encourages speculation that Bub1 might be important for preventing PSCS in mammalian oocytes.

Another important issue relates to SAC function within the unique cellular context of the oocyte. For instance, does the relatively large oocyte volume negatively impact the efficiency of SAC signal transduction? The average volume of a mouse oocyte (~270pl) is about 45-fold greater than that of a PtK1 cell (~6pl) (Howell et al., 2000), an archetypal mammalian somatic cell. A single unattached kinetochore is sufficient for inhibiting mitotic progression in PtK1 cells (Rieder et al., 1995). Would a single unattached kinetochore be capable of generating an SAC signal sufficient to inhibit all of the APC/C within the much larger mammalian oocyte? Indirect evidence suggests that oocytes may not possess this capacity. Although one or a few misaligned chromosomes often persist in oocytes monosomic for the X chromosome (XO oocytes), theses oocytes do not exhibit a delay in meiosis I (LeMaire-Adkins et al., 1997). In contrast, meiosis I progression is effectively inhibited when the majority of chromosomes are misaligned due to defective recombination in oocytes from MLH1$^{-/-}$ and MEIL1$^{-/-}$ knockout strains (Woods et al., 1999; Libby et al., 2003). Also, oocytes sustain a prolonged SAC-mediated meiosis I arrest when all kinetochores are unattached and lacking in tension (Homer et al., 2005c) whereas more subtle defects induce only a transient arrest (Wassmann et al., 2003a; Shen et al., 2005). Taken together, one interpretation of these data is that oocytes respond less efficiently to weak inhibitory SAC signals. Systematic studies will be required to explore this supposition further.

There is now compelling evidence that Mad2 (and hence the SAC) is indispensable for accurate homologue disjunction in mammalian oocytes. The trend towards a decline in transcripts encoding SAC proteins in human oocytes suggests that declining SAC function is a feature of the aging process (Steuerwald et al., 2001). Indeed, in mice, ovarian BubR1 levels have been shown to decrease with age suggesting that impairment of SAC function might be a universal feature of mammalian aging (Baker et al., 2004). Furthermore, declining levels of BubR1 in mice have not only been linked to age-related rises in aneuploidy but also with subfertility, implicating the SAC in wider aspects of mammalian reproduction (Baker et al., 2004). Although human oocytes have been shown to express SAC transcripts and proteins (Steuerwald et al., 2001; Homer et al., 2005b,d), the burning question is whether or not they replicate their rodent counterparts in requiring these proteins for enacting faithful homologue disjunction. The answer to this question is undoubtedly on the horizon as RNAi has recently been shown to be a feasible approach for down-regulating Mad2 expression in human oocytes during meiosis I (Homer et al., 2005d).

Acknowledgements. This work was supported by a WellBeing Research Training Fellowship (RTF/387) along with grants from Newcastle University Hospitals Special Trustees and Wellcome. I am grateful to Mary Herbert, Alex McDougall, Mark Levasseur, Alison Murdoch and Michael Whitaker.

References

Angell R. (1991). Predivision in human oocytes at meiosis I: a mechanism for trisomy formation in man. Hum. Genet. 86, 383-387.

Baker D., Jeganathan K., Cameron J., Thompson M., Juneja S., Kopecka A., Kumar R., Jenkins R., de Groen P., Roche P. and van Deursen J. (2004). BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. Nat. Genet. 36, 744-749.

Bernard P., Maure J.F. and Javerzat J.P. (2001). Fission yeast Bub1 is essential in setting up the meiotic pattern of chromosome segregation. Nat. Cell Biol. 3, 522-526.

Bharadwaj R. and Yu H. (2004). The spindle checkpoint, aneuploidy, and cancer. Oncogene 23, 2016-2027.

Brandeis M. and Hunt T. (1996). The proteolysis of mitotic cyclins in mammalian cells persists from the end of mitosis until the onset of S phase. EMBO J. 15, 5280-5289.

Brunet S., Santa Maria A., Guillaud P., Dujardin D., Kubiak J.Z. and Maro B. (1999). Kinetochore fibers are not involved in the formation of the first meiotic spindle of mouse oocytes, but control the exit from the first meiotic M phase. J. Cell Biol. 146, 1-12.

Brunet S., Pahlavan G., Taylor S.S. and Maro B. (2003). Functionality of the spindle checkpoint during the first meiotic division of mammalian oocytes. Reproduction 126, 443-450.

Buonomo S.B., Clyne R.K., Fuchs J., Loidl J., Uhlmann F. and Nasmyth K. (2000). Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell 103, 387-398.

Canman J.C., Salmon E.D. and Fang G. (2002). Inducing precocious anaphase in cultured mammalian cells. Cell Motil. Cytoskeleton 52, 61-65.

Castro A., Bernis C., Vigneron S., Labbé J. and Lorca T. (2005). The anaphase-promoting complex: a key factor in the regulation of cell cycle. Oncogene 24, 314-325.

Chang H., Levasseur M. and Jones K. (2004). Degradation of APCcdc20 and APCcdh1 substrates during the second meiotic division in mouse eggs. J. Cell Sci. 117, 6289-6296.

Cheslock P., Kemp B., Boumil R. and Dawson D. (2005). The roles of MAD1, MAD2 and MAD3 in meiotic progression and the segregation of nonexchange chromosomes. Nat. Genet. 37, 756-760.

Chung E. and Chen R. (2003). Phosphorylation of Cdc20 is required for

its inhibition by the spindle checkpoint. Nat. Cell Biol. 5, 748-753.

Cimini D., Howell B., Maddox P., Khodjakov A., Degrassi F. and Salmon E. (2001). Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells. J. Cell Biol. 153, 517-527.

Cimini D., Cameron L. and Salmon E. (2004). Anaphase spindle mechanics prevent mis-segregation of merotelically oriented chromosomes. Curr. Biol. 14, 2149-2155.

Cleveland D.W., Mao Y. and Sullivan K.F. (2003). Centromeres and kinetochores: from epigenetics to mitotic checkpoint signalling. Cell 112, 407-421.

Clute P. and Pines J. (1999). Temporal and spatial control of cyclin B1 destruction in metaphase. Nat. Cell Biol. 1, 82-87.

De Antoni A., Pearson C., Cimini D., Canman J., Sala V., Nezi L., Mapelli M., Sironi L., Faretta M., Salmon E. and Musacchio A. (2005). The Mad1/Mad2 complex as a template for Mad2 activation in the spindle assembly checkpoint. Curr. Biol. 15, 214-225.

Dekel N. (2005). Cellular, biochemical and molecular mechanisms regulating oocyte maturation. Mol. Cell. Endocrinol. 234, 19-25.

Dobles M., Liberal V., Scott M.L., Benezra R. and Sorger P.K. (2000). Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2. Cell 101, 635-645.

Draviam V., Xie S. and Sorger P. (2004). Chromosome segregation and genomic stability. Curr. Opin. Genet. Dev. 14, 120-125.

Eijpe M., Offenberg H., Jessberger R., Revenkova E. and Heyting C. (2003). Meiotic cohesin REC8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1beta and SMC3. J. Cell Biol. 160, 657-670.

Fang G. (2002). Checkpoint protein BubR1 acts synergistically with Mad2 to inhibit anaphase-promoting complex. Mol. Biol. Cell 13, 755-766.

Geley S., Kramer E., Gieffers C., Gannon J., Peters J.M. and Hunt T. (2001). APC/C-dependent proteolysis of human cyclin A starts at the beginning of mitosis and is not subject to the spindle assembly checkpoint. J. Cell Biol. 153, 137-148.

Gerton J. and Hawley R. (2005). Homologous chromosome interactions in meiosis: diversity amidst conservation. Nat. Rev. Genet. 6, 477-487.

Giménez-Abián J., Sumara I., Hirota T., Hauf S., Gerlich D., de la Torre C., Ellenberg J. and Peters J. (2004). Regulation of sister chromatid cohesion between chromosome arms. Curr. Biol. 14, 1187-1193.

Glotzer M., Murray A.W. and Kirschner M.W. (1991). Cyclin is degraded by the ubiquitin pathway. Nature 349, 132-138.

Gorbsky G.J., Chen R.H. and Murray A.W. (1998). Microinjection of antibody to Mad2 protein into mammalian cells in mitosis induces premature anaphase. J. Cell Biol. 141, 1193-1205.

Haering C. and Nasmyth K. (2003). Building and breaking bridges between sister chromatids. Bioessays 25, 1178-1191.

Hagting A., den Elzen N., Vodermaier H.C., Waizenegger I.C., Peters J.M. and Pines J. (2002). Human securin proteolysis is controlled by the spindle checkpoint and reveals when the APC/C switches from activation by Cdc20 to Cdh1. J. Cell Biol. 157, 1125-1127.

Hampl A. and Eppig J.J. (1995). Analysis of the mechanism(s) of metaphase I arrest in maturing mouse oocytes. Development 121, 925-933.

Hanks S., Coleman K., Reid S., Plaja A., Firth H., Fitzpatrick D., Kidd A., Méhes K., Nash R., Robin N., Shannon N., Tolmie J., Swansbury J., Irrthum A., Douglas J. and Rahman N. (2004). Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. Nat. Genet. 36, 1159-1161.

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

Hassold T. and Hunt P. (2001). To err (meiotically) is human: the genesis of human aneuploidy. Nat. Rev. Genet. 2, 280-291.

Hauf S., Waizenegger I.C. and Peters J.M. (2001). Cohesin cleavage by separase required for anaphase and cytokinesis in human cells. Science 293, 1320-1323.

Hauf S. and Watanabe Y. (2004). Kinetochore orientation in mitosis and meiosis. Cell 119, 317-327.

Heasman J. (2002). Morpholino oligos: Making sense of antisense. Dev. Biol. 243, 209-214.

Herbert M., Levasseur M., Homer H.A., Yallop K., Murdoch A. and McDougall A. (2003). Homologue disjunction in mouse oocytes requires proteolysis of securin and cyclin B1. Nat. Cell Biol. 5, 1023-1025.

Homer H.A., McDougall A., Levasseur M. and Herbert M. (2005a). Restaging the spindle assembly checkpoint in female mammalian meiosis I. Cell Cycle 4, 650-653.

Homer H.A., McDougall A., Levasseur M., Yallop K., Murdoch A. and Herbert M. (2005b). Mad2 prevents aneuploidy and premature proteolysis of cyclin B and securin during meiosis I in mouse oocytes. Genes Dev. 19, 202-207.

Homer H.A., McDougall A., Levasseur M., Murdoch A. and Herbert M. (2005c). Mad2 is required for inhibiting securin and cyclin B degradation following spindle depolymerisation in meiosis I mouse oocytes. Reproduction 130, 829-843.

Homer H.A., McDougall A., Levasseur M., Murdoch A. and Herbert M. (2005d). RNA iterference in human oocytes: towards an understanding of human aneuploidy. Mol. Hum. Reprod. 11, 397-404.

Howell B.J., Hoffman D.B., Fang G., Murray A.W. and Salmon E.D. (2000). Visualization of Mad2 dynamics at kinetochores, along spindle fibers, and at spindle poles in living cells. J. Cell Biol. 150, 1233-1249.

Josefsberg L., Galiani D., Dantes A., Amsterdam A. and Dekel N. (2000). The proteasome is involved in the first metaphase-to-anaphase transition of meiosis in rat oocytes. Biol. Reprod. 62, 1270-1277.

Josefsberg L., Kaufman O., Galiani D., Kovo M. and Dekel N. (2001). Inactivation of M-phase promoting factor at exit from first embryonic mitosis in the rat is independent of cyclin B1 degradation. Biol. Reprod. 64, 871-878.

Katis V., Galova M., Rabitsch K., Gregan J. and Nasmyth K. (2004). Maintenance of cohesin at centromeres after meiosis I in budding yeast requires a kinetochore-associated protein related to MEI-S332. Curr. Biol. 14, 560-572.

Kemp B., Boumil R., Stewart M. and Dawson D. (2004). A role for centromere pairing in meiotic chromosome segregation. Genes Dev. 18, 1946-1951.

King J. and Nicklas R. (2000). Tension on chromosomes increases the number of kinetochore microtubules but only within limits. J. Cell Sci. 113, 3815-3823.

Kirschner M. and Mitchison T. (1986). Beyond self-assembly: from microtubules to morphogenesis. Cell 45, 329-342.

Kitajima T., Kawashima S. and Watanabe Y. (2004). The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. Nature 427, 510-517.

Kitajima T., Hauf S., Ohsugi M., Yamamoto T. and Watanabe Y. (2005). Human Bub1 defines the persistent cohesion site along the mitotic chromosome by affecting Shugoshin localization. Curr. Biol. 15, 353-359.

Kops G., Foltz D. and Cleveland D. (2004). Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. Proc. Natl. Acad. Sci. USA. 101, 8699-8704.

Kouznetsova A., Novak I., Jessberger R. and Höög C. (2005). SYCP2 and SYCP3 are required for cohesin core integrity at diplotene but not for centromere cohesion at the first meiotic division. J. Cell Sci. 118, 2271-2278.

Kubiak J., Weber M., Géraud G. and Maro B. (1992). Cell cycle modification during the transitions between meiotic M-phases in mouse oocytes. J. Cell Sci. 102, 457-467.

Lamb N.E., Freeman S.B., Savage-Austin A., Pettay D., Taft L., Hersey J., Gu Y., Shen J., Saker D., May K.M., Avramopoulos D., Peterson M.B., Hallberg A., Mikkelsen M., Hassold T.J. and Sherman S.L. (1996). Susceptible chiasmate configurations of chromosome 21 predispose to non-disjunction in both maternal meiosis I and meiosis II. Nat. Genet. 14, 400-405.

Lampson M., Renduchitala K., Khodjakov A. and Kapoor T. (2004). Correcting improper chromosome-spindle attachments during cell division. Nat. Cell Biol. 6, 232-237.

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

Lee J., Iwai T., Yokota T. and Yamashita M. (2003). Temporally and spatially selective loss of Rec8 protein from meiotic chromosomes during mammalian meiosis. J. Cell Sci. 116, 2781-2790.

Lefebvre C., Terret M.E., Djiane A., Rassinier P., Maro B. and Verlhac M.H. (2002). Meiotic spindle stability depends on MAPK-interacting and spindle-stabilizing (MISS), a new MAPK substrate. J. Cell Biol. 157, 603-613.

LeMaire-Adkins R., Radke K. and Hunt P.A. (1997). Lack of checkpoint control at the metaphase/anaphase transition: a mechanism of meiotic nondisjunction in mammalian females. J. Cell Biol. 139, 1611-1619.

Li R. and Murray A.W. (1991). Feedback control of mitosis in budding yeast. Cell 66, 519-531.

Li Y. and Benezra R. (1996). Identification of a human mitotic checkpoint gene: hsMAD2. Science 274, 246-248.

Libby B., Reinholdt L. and Schimenti J. (2003). Positional cloning and characterization of Mei1, a vertebrate-specific gene required for normal meiotic chromosome synapsis in mice. Proc. Natl. Acad. Sci. USA 100, 15706-15711.

Losada A., Hirano M. and Hirano T. (2002). Cohesin release is required for sister chromatid resolution, but not for condensin-mediated compaction, at the onset of mitosis. Genes Dev. 16, 3004-3016.

Luo X., Fang G., Coldiron M., Lin Y., Yu H., Kirschner M. and Wagner G. (2000). Structure of the Mad2 spindle assembly checkpoint protein and its interaction with Cdc20. Nat. Struct. Biol. 7, 224-229.

Maro B. and Verlhac M. (2002). Polar body formation: new rules for asymmetric divisions. Nat. Cell Biol. 4, E281-283.

Marston A. and Amon A. (2004). Meiosis: cell-cycle controls shuffle and deal. Nat. Rev. Mol. Cell Biol. 5, 983-997.

Marston A., Tham W., Shah H. and Amon A. (2004). A genome-wide screen identifies genes required for centromeric cohesion. Science 303, 1367-1370.

Matzuk M., Burns K., Viveiros M. and Eppig J. (2002). Intercellular communication in the mammalian ovary: oocytes carry the conversation. Science 296, 2178-2180.

Meraldi P., Draviam V. and Sorger P. (2004). Timing and checkpoints in the regulation of mitotic progression. Dev. Cell 7, 45-60.

Michel L., Liberal V., Chatterjee A., Kirchwegger R., Pasche B., Gerald W., Dobles M., Sorger P.K., Murty V.V. and Benezra R. (2001). MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. Nature 409, 355-359.

Michel L., Diaz-Rodriguez E., Narayan G., Hernando E., Murty V. and Benezra R. (2004). Complete loss of the tumor suppressor MAD2 causes premature cyclin B degradation and mitotic failure in human somatic cells. Proc. Natl. Acad. Sci. USA 101, 4459-4464.

Morrow C., Tighe A., Johnson V., Scott M., Ditchfield C. and Taylor S. (2005). Bub1 and aurora B cooperate to maintain BubR1-mediated inhibition of APC/CCdc20. J. Cell Sci. 118, 3639-3652.

Musacchio A. and Hardwick K.G. (2002). The spindle checkpoint: structural insights into dynamic signalling. Nat. Rev. Mol. Cell Biol. 3, 731-741.

Nasmyth K. (2005). How do so few control so many? Cell 120, 739-746.

Page S. and Hawley R. (2003). Chromosome choreography: the meiotic ballet. Science 301, 785-789.

Peters J. (2002). The Anaphase-promoting complex: Proteolysis in mitosis and beyond. Mol. Cell 9, 931-943.

Petronczki M., Siomos M. and Nasmyth K. (2003). Un ménage à quatre: the molecular biology of chromosome segregation in meiosis. Cell 112, 423-440.

Pfleger C.M. and Kirschner M.W. (2000). The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1. Genes Dev. 14, 655-665.

Pinsky B. and Biggins S. (2005). The spindle checkpoint: tension versus attachment. Trends Cell Biol. 15, 486-493.

Polanski Z., Ledan E., Brunet S., Louvet S., Verlhac M.H., Kubiak J.Z. and Maro B. (1998). Cyclin synthesis controls the progression of meiotic maturation in mouse oocytes. Development 125, 4989-4997.

Rabitsch K., Gregan J., Schleiffer A., Javerzat J., Eisenhaber F. and Nasmyth K. (2004). Two fission yeast homologs of Drosophila Mei-S332 are required for chromosome segregation during meiosis I and II. Curr. Biol. 14, 287-301.

Rieder C.L., Cole R.W., Khodjakov A. and Sluder G. (1995). The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. J. Cell Biol. 130, 941-948.

Sandalinas M., Márquez C. and Munné S. (2002). Spectral karyotyping of fresh, non-inseminated oocytes. Mol. Hum. Reprod. 8, 580-585.

Shen Y., Betzendahl I., Sun F., Tinneberg H. and Eichenlaub-Ritter U. (2005). Non-invasive method to assess genotoxicity of nocodazole interfering with spindle formation in mammalian oocytes. Reprod. Toxicol. 19, 459-471.

Shonn M., McCarroll R. and Murray A.W. (2000). Requirement of the spindle checkpoint for proper chromosome segregation in budding yeast meiosis. Science 289, 300-303.

Shonn M., Murray A.L and Murray A.W (2003). Spindle checkpoint component Mad2 contributes to biorientation of homologous chromosomes. Curr. Biol. 13, 1979-1984.

Skoufias D.A., Andreassen P.R., Lacroix F.B., Wilson L. and Margolis R.L. (2001). Mammalian mad2 and bub1/bubR1 recognize distinct spindle-attachment and kinetochore-tension checkpoints. Proc. Natl. Acad. Sci. USA. 98, 4492-4497.

Soewarto D., Schmiady H. and Eichenlaub-Ritter U. (1995). Consequences of non-extrusion of the first polar body and control of the sequential segregation of homologues and chromatids in mammalian oocytes. Hum. Reprod. 10, 2350-2360.

Stemmann O., Zou H., Gerber S.A., Gygi S.P. and Kirschner M.W. (2001). Dual inhibition of sister chromatid separation at metaphase. Cell 107, 715-726.

Steuerwald N., Cohen J., Herrera R.J., Sandalinas M. and Brenner C.A. (2001). Association between spindle assembly checkpoint expression and maternal age in human oocytes. Mol. Hum. Reprod. 7, 49-55.

Sumara I., Vorlaufer E., Stukenberg P.T., Kelm O., Redemann N., Nigg E.A. and Peters J.M. (2002). The dissociation of cohesin from chromosomes in prophase is regulated by Polo-like kinase. Mol. Cell 9, 515-525.

Svetlanov A. and Cohen P. (2004). Mismatch repair proteins, meiosis, and mice: understanding the complexities of mammalian meiosis. Exp. Cell Res. 296, 71-79.

Takakura I., Naito K., Iwamori N., Yamashita M., Kume S. and Tojo H. (2005). Inhibition of mitogen activated protein kinase activity induces parthenogenetic activation and increases cyclin B accumulation during porcine oocyte maturation. J. Reprod. Dev. 51, 617-626.

Taylor S., Scott M. and Holland A. (2004). The spindle checkpoint: a quality control mechanism which ensures accurate chromosome segregation. Chromosome Res. 12, 599-616.

Terret M., Wassmann K., Waizenegger I., Maro B., Peters J. and Verlhac M. (2003). The meiosis I-to-meiosis II transition in mouse oocytes requires separase activity. Curr. Biol. 13, 1797-1802.

Tsurumi C., Hoffmann S., Geley S., Graeser R. and Polanski Z. (2004). The spindle assembly checkpoint is not essential for CSF arrest of mouse oocytes. J. Cell Biol. 167, 1037-1050.

Uhlmann F. (2003). Chromosome cohesion and separation: from men and molecules. Curr. Biol. 13, R104-114.

Verlhac M.H., de Pennart H., Maro B., Cobb M.H. and Clarke H.J. (1993). MAP kinase becomes stably activated at metaphase and is associated with microtubule-organizing centers during meiotic maturation of mouse oocytes. Dev. Biol. 158, 330-340.

Verlhac M.H., Lefebvre C., Guillaud P., Rassinier P. and Maro B. (2000). Asymmetric division in mouse oocytes with or without Mos. Curr. Biol. 10, 1303-1306.

Waizenegger I.C., Hauf S., Meinke A. and Peters J.M. (2000). Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. Cell 103, 399-410.

Wassmann K., Niault T. and Maro B. (2003a). Metaphase I arrest upon activation of the MAD2-dependent spindle checkpoint in mouse oocytes. Curr. Biol. 13, 1596-1608.

Wassmann K., Liberal V. and Benezra R. (2003b). Mad2 phosphorylation regulates its association with Mad1 and the APC/C. EMBO J. 22, 797-806.

Watanabe Y. (2004). Modifying sister chromatid cohesion for meiosis. J. Cell Sci. 117, 4017-4023.

Waters J.C., Chen R.H., Murray A.W. and Salmon E.D. (1998). Localization of Mad2 to kinetochores depends on microtubule attachment, not tension. J. Cell Biol. 141, 1181-1191.

Winston N.J. (1997). Stability of cyclin B during meiotic maturation and the first meiotic cell division in mouse oocytes. Biol. Cell 89, 211-219.

Woods L., Hodges C., Baart E., Baker S., Liskay M. and Hunt P. (1999). Chromosomal influence on meiotic spindle assembly: abnormal meiosis I in female Mlh1 mutant mice. J. Cell Biol. 145, 1395-1406.

Yu H., Muszynski M.G. and Dawe R.K. (1999). The maize homologue of the cell cycle checkpoint protein MAD2 reveals kinetochore substructure and contrasting mitotic and meiotic localization patterns. J. Cell Biol. 145, 425-435.

Zhang D., Ma W., Li Y., Hou Y., Li S., Meng X., Sun X., Sun Q. and Wang W. (2004). Intra-oocyte localization of MAD2 and its relationship with kinetochores, microtubules, and chromosomes in rat oocytes during meiosis. Biol. Reprod. 71, 740-748.

Zhang D., Li M., Ma W., Hou Y., Li Y., Li S., Sun Q. and Wang W. (2005). Localization of mitotic arrest deficient 1 (MAD1) in mouse oocytes during the first meiosis and its functions as a spindle checkpoint protein. Biol. Reprod. 72, 58-68.

Zur A. and Brandeis M. (2001). Securin degradation is mediated by fzy and fzr, and is required for complete chromatid separation but not for cytokinesis. EMBO J. 20, 792-801.

Accepted January 9, 2006