The spindle checkpoint protein Mad2 regulates APC/C activity during prometaphase and metaphase of meiosis I in *Saccharomyces cerevisiae*

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ABSTRACT **In many eukaryotes, disruption of the spindle checkpoint protein Mad2 results in an increase in meiosis I nondisjunction, suggesting that Mad2 has a conserved role in ensuring faithful chromosome segregation in meiosis. To characterize the meiotic function of Mad2, we analyzed individual budding yeast cells undergoing meiosis. We find that Mad2 sets the duration of meiosis I by regulating the activity of APC^{Cdc20}. In the absence of Mad2, most cells undergo both meiotic divisions, but securin, a substrate of the APC/C, is degraded prematurely, and prometaphase I/metaphase I is accelerated. Some *mad2*Δ cells have a misregulation of meiotic cell cycle events and undergo a single aberrant division in which sister chromatids separate. In these cells, both APC^{Cdc20} and APC^{Ama1} are prematurely active, and meiosis I and meiosis II events occur in a single meiotic division. We show that Mad2 indirectly regulates APC^{Ama1} activity by decreasing APC^{Cdc20} activity. We propose that Mad2 is an important meiotic cell cycle regulator that ensures the timely degradation of APC/C substrates and the proper orchestration of the meiotic divisions.**

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INTRODUCTION

The cell cycle is precisely controlled to ensure a specific order and timing of events. Cell cycle regulators promote the correct sequence of events, and checkpoint mechanisms monitor specific events, delaying the cell cycle if those events have not been completed. Cell cycle proteins have been characterized extensively in mitosis; however, less is known about the activity of the proteins that promote progression through meiosis. There are sequential steps required to ensure that, in meiosis I, homologous chromosomes segregate, and in meiosis II, sister chromatids separate (reviewed in Brar and Amon, 2008). Characterization of the regulation of the two meiotic divisions is necessary to understand how meiotic errors occur.

For faithful chromosome segregation in meiosis I, homologous chromosomes pair, recombine, and attach to spindle microtubules. Once chromosomes are properly attached to microtubules emanating from opposite spindle poles, a ubiquitin ligase called the anaphase-promoting complex/cyclosome (APC/C), bound by the regulatory subunit Cdc20, targets substrates for proteasomal degradation (Pesin and Orr-Weaver, 2008). One substrate, securin (Pds1 in budding yeast), sequesters separase, the protease that cleaves Rec8, the meiotic cohesin (Klein *et al.*, 1999; Buonomo *et al.*, 2000; Kitajima *et al.*, 2003; Kudo *et al.*, 2006). When securin is degraded, separase is active, and chromosomes segregate. A meiosis-specific activator of the APC/C, Ama1, also targets securin and other substrates for degradation (Cooper *et al.*, 2000; Oelschlaegel *et al.*, 2005; Penkner *et al.*, 2005). Although Ama1 is not required for meiosis I, APC^{Ama1} activity does promote the timely degradation of APC/C substrates (Cooper *et al.*, 2000; Oelschlaegel *et al.*, 2005; Penkner *et al.*, 2005).

For faithful segregation of chromosomes in meiosis II, the cohesins around the centromere are protected from cleavage in metaphase I. Sgo1, an orthologue of the *Drosophila* MEI-S332 protein, is required for the protection of cohesins to ensure that sister chromatids stay together until meiosis II (Kerrebrock *et al.*, 1995; Katis *et al.*, 2004; Kitajima *et al.*, 2004; Marston *et al.*, 2004). After meiosis I,

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Abbreviations used: APC/C, anaphase-promoting complex/cyclosome; *cdc20-mn*, Cdc20 meiotic null; GFP-LacI, green fluorescent protein–lactose repressor fusion protein; LacO, lactose operator.

securin reaccumulates and sequesters separase, and Sgo1 is inactivated and degraded. The APC/C will again target securin for degradation, and in the absence of Sgo1, separase will cleave the remaining cohesins, allowing sister chromatids to separate.

In mitosis and meiosis, the attachment of chromosomes to spindle microtubules is monitored at the metaphase-to-anaphase transition by the spindle assembly checkpoint (Musacchio and Salmon, 2007). Improperly attached kinetochores send a signal to delay the cell cycle by inhibiting the APC/C, allowing the cells additional time to correct the error in attachment. In mammalian cells, the spindle checkpoint protein Mad2 also has a kinetochore-independent role as a cell cycle timer. Depletion of Mad2 results in the premature loss of APC/C substrates, a faster cell cycle, and an increase in chromosome missegregation in both mitosis and meiosis (Geley *et al.*, 2001; Wassmann *et al.*, 2003; Meraldi *et al.*, 2004; Michel *et al.*, 2004; Tsurumi *et al.*, 2004; Homer *et al.*, 2005a, 2005b). In contrast, in budding yeast, the spindle checkpoint proteins are not essential in mitosis (Hoyt *et al.*, 1991; Li and Murray, 1991; Hardwick *et al.*, 1999). Deletion of Mad2 results in only a modest increase in mitotic chromosome missegregation unless cells are challenged with genetic or chemical perturbations that disrupt microtubule–kinetochore attachments. In meiosis, however, Mad2 is essential for chromosome segregation; deletion of Mad2 results in an increase in meiosis I nondisjunction (Shonn *et al.*, 2000). The molecular basis for this phenotype was previously unknown.

Here we investigate Mad2's role in meiotic cell cycle regulation in budding yeast to understand how Mad2 functions to promote proper chromosome segregation. We find that, similar to mammalian oocytes, in budding yeast, Mad2 serves as a "meiotic timer" to set the duration of meiosis I by regulating the activity of APC^{Cdc20}. To further understand the consequence of premature APC/C activity, we investigated the timing of other meiotic cell cycle events in *mad2*Δ cells. We find that Mad2 has a role in coordinating chromosome segregation with the regulation of the meiotic cell cycle. In the absence of Mad2, premature APC^{Cdc20} can lead to premature APC^{Ama1} activity in some cells, resulting in a single aberrant division in which sister chromatids separate inappropriately because the chromosome segregation cycle is uncoupled from other cell cycle events. Our results indicate that Mad2 has a role in meiosis I separate from its role in monitoring chromosome attachment: ensuring the timely degradation of APC/C substrates and the proper execution of the meiotic divisions.

RESULTS

Mad2 affects the timing of the meiotic cell cycle

To investigate the role of Mad2 in meiosis, we analyzed sporulation in wild-type and *mad2*Δ cells. We used the W303 budding yeast strain because the mitotic and meiotic phenotypes of *mad2*Δ were previously characterized in this background (Hwang *et al.*, 1998; Shonn *et al.*, 2000, 2003). The process of sporulation, which includes meiosis and spore formation, can be induced through nutrient starvation of diploid budding yeast cells. Sixty-five percent of wild-type W303 cells sporulate, and of those, 96% package the four products of meiosis into four spores, forming a tetrad. Sixty-five percent of *mad2*Δ cells sporulate as well. However, sporulation of *mad2*Δ cells results in two major populations of spores: 1) 58% form tetrads, and 2) 34% form dyads, or asci containing two spores (Figure 1A). A small fraction of wild-type and *mad2*Δ cells form triads (2 and 6%, respectively). We reasoned that investigating the differences between meiotic cell cycle events in wild-type cells, *mad2*Δ cells that form dyads, and *mad2*Δ cells that form tetrads might uncover a role of Mad2 in regulating the meiotic divisions.

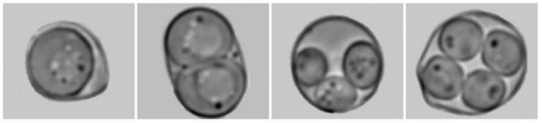
We first asked whether Mad2 is required for the proper timing of the meiotic divisions. Past studies did not detect a difference in timing of *mad2*Δ cells, but these studies analyzed fixed cells and, due to asynchrony in meiotic induction, may not have detected small changes in specific phases of the meiotic cell cycle (Shonn *et al.*, 2000). To analyze more carefully the timing of the meiotic divisions, we used time-lapse microscopy to measure the duration of meiotic phases in individual wild-type and *mad2*Δ cells. We expressed two green fluorescent protein (GFP)–tagged proteins, ZIP1-GFP and TUB1-GFP, to follow progression through meiosis. Zip1 is a component of the synaptonemal complex that assembles in zygotene and disassembles in diplotene, and it serves as a visual marker for prophase I (Sym *et al.*, 1993; Scherthan *et al.*, 2007). TUB1-GFP encodes a tagged α-tubulin, permitting observation of spindle formation and breakdown in meiosis I and meiosis II (Carminati and Stearns, 1997; Straight *et al.*, 1997). Although we used the same fluorescent tag for both proteins, they are distinguishable because they are both morphologically and temporally different during the meiotic cell cycle (Figure 1B). Time-lapse images taken during sporulation allow us to measure the duration of each stage. We define the cell cycle stages based on disappearance of Zip1 and spindle morphology (Figure 1B).

The time-lapse microscopy indicates that the *mad2*Δ cells that form dyads undergo only one meiotic division, but the *mad2*Δ cells that form tetrads undergo both meiotic divisions (Figure 1, B and C). We will refer to the *mad2*Δ cells that undergo one meiotic division and form dyads as "1 division *mad2*Δ cells" and the *mad2*Δ cells that undergo both divisions and form tetrads as "2 division *mad2*Δ cells." The small fraction (6%) of *mad2*Δ cells that form triads also undergo two divisions; however, there is an error in spore formation, and only three of the four products of meiosis are packaged into spores. Therefore, 64% of sporulating cells undergo both divisions; in our analysis of the 2 division *mad2*Δ cells, we only include those that form tetrads since we are unsure about the underlying cause of the spore formation error resulting in a triad.

Observation of the 2 division *mad2*Δ cells shows that these cells have a faster prometaphase I/metaphase, 23 ± 8 min, compared with 41 ± 12 min in wild-type cells (Figure 1B). This difference is highly significant ($p < 0.0001$; unpaired Student's *t* test). This change in cell cycle timing only occurs in meiosis I; the duration of each stage in meiosis II of 2 division *mad2*Δ cells is similar to that of wild-type cells. The shorter duration of prometaphase I/metaphase I may cause the increase in chromosome missegregation that occurs in 2 division *mad2*Δ cells.

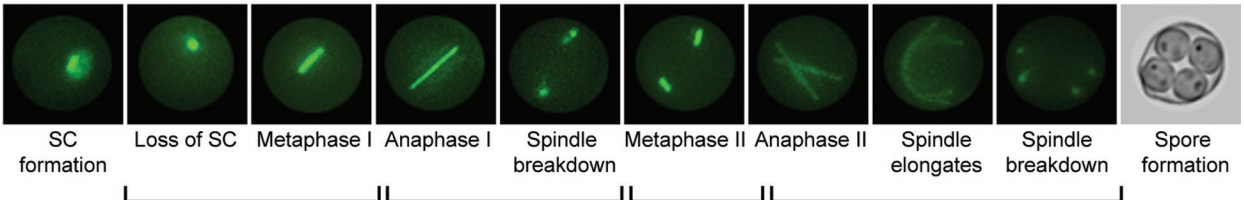
In contrast, the 1 division *mad2*Δ cells have an extended prometaphase I/metaphase I at 59 ± 16 min, compared with 41 ± 12 min in wild-type cells (Figure 1C). The difference in the duration of prometaphase I/metaphase I between wild-type and 1 division *mad2*Δ cells is highly statistically significant ($p < 0.0001$; unpaired Student's *t* test). The duration of anaphase I in 1 division *mad2*Δ cells is 69 min ± 13 min, substantially greater than the 13 ± 5 min in wild-type cells but much more similar to anaphase II in wild-type cells (76 ± 9 min). In the 1 division *mad2*Δ cells, the anaphase I spindle elongates and bends around the cell, which is a characteristic of anaphase II spindles but not anaphase I spindles in wild-type cells (Figure 1, B and C). In summary, the 2 division *mad2*Δ cells have a faster prometaphase I/metaphase I, and the 1 division *mad2*Δ cells have a longer prometaphase I/metaphase I, but the entire meiotic cell cycle is shorter than in wild-type cells.

A



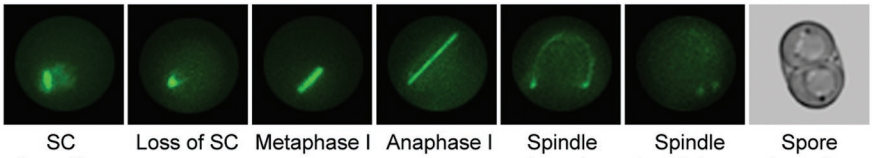
	Monad	Dyad	Triad	Tetrad
Wildtype	0%	2%	2%	96%
*mad2*Δ	2%	34%	6%	58%

B



Wildtype	41 ± 12 min	13 ± 5 min	32 ± 9 min	76 ± 9 min
*mad2*Δ (2 divisions)	23 ± 8 min	16 ± 5 min	31 ± 12 min	78 ± 16 min

C



*mad2*Δ (1 division)	59 ± 16 min	69 ± 13 min

FIGURE 1: Mad2 affects the duration of the meiotic cell cycle. (A) Wild-type and *mad2*Δ/*mad2*Δ sporulated cells were counted for the number of spores in each ascus. Nine hundred sporulated cells were counted in three biological replicates. (B, C) Synaptonemal complex (SC) formation and loss, and spindle assembly and disassembly, were visualized by expressing Zip1-GFP and TUB1-GFP and monitored using time-lapse fluorescence microscopy. The stages of meiosis were determined based on loss of Zip1 and spindle morphology, and the time of each stage was calculated (in minutes ± SD). (B) Still images from a representative movie of wild-type cells. One hundred cells of each genotype were counted. (C) Still images from a representative movie of *mad2*Δ cells that form dyads. Fifty *mad2*Δ cells that formed dyads were counted.

The 1 division mad2Δ cells undergo an aberrant meiotic division in which the homologous chromosomes pair and recombine, but sister chromatids separate inappropriately

To determine the role of Mad2 in meiosis, we further analyzed the 1 division *mad2*Δ cells. We monitored the segregation of chromosome IV by placing a lactose operator (LacO) array near the centromere and expressing a GFP–lactose repressor fusion protein (GFP-LacI), targeting GFP to the chromosome (Straight *et al.*, 1996; Shonn *et al.*, 2000). When both homologous chromosomes have GFP targeted near CEN4, 97% of the *mad2*Δ dyads have two GFP-marked chromosomes in each spore, suggesting that the spores are diploid (Figure 2A). As a further confirmation that the spores are diploid, we dissected the dyad spores and found that 78% were viable and able to sporulate.

The formation of two diploid spores after a single division in *mad2*Δ cells could be the result of either 1) segregating homologous chromosomes and ending the cell cycle after meiosis I or 2) separating sister chromatids inappropriately. To determine how the chromosomes separated in the 1 division *mad2*Δ cells, we labeled one of the two homologous chromosomes with a LacO array near the centromere of chromosome IV. If homologous chromosomes separate, one of the two spores will have two copies of the marked chromosome, and the other spore will have two copies of the unmarked chromosome. If sister chromatids separate, each

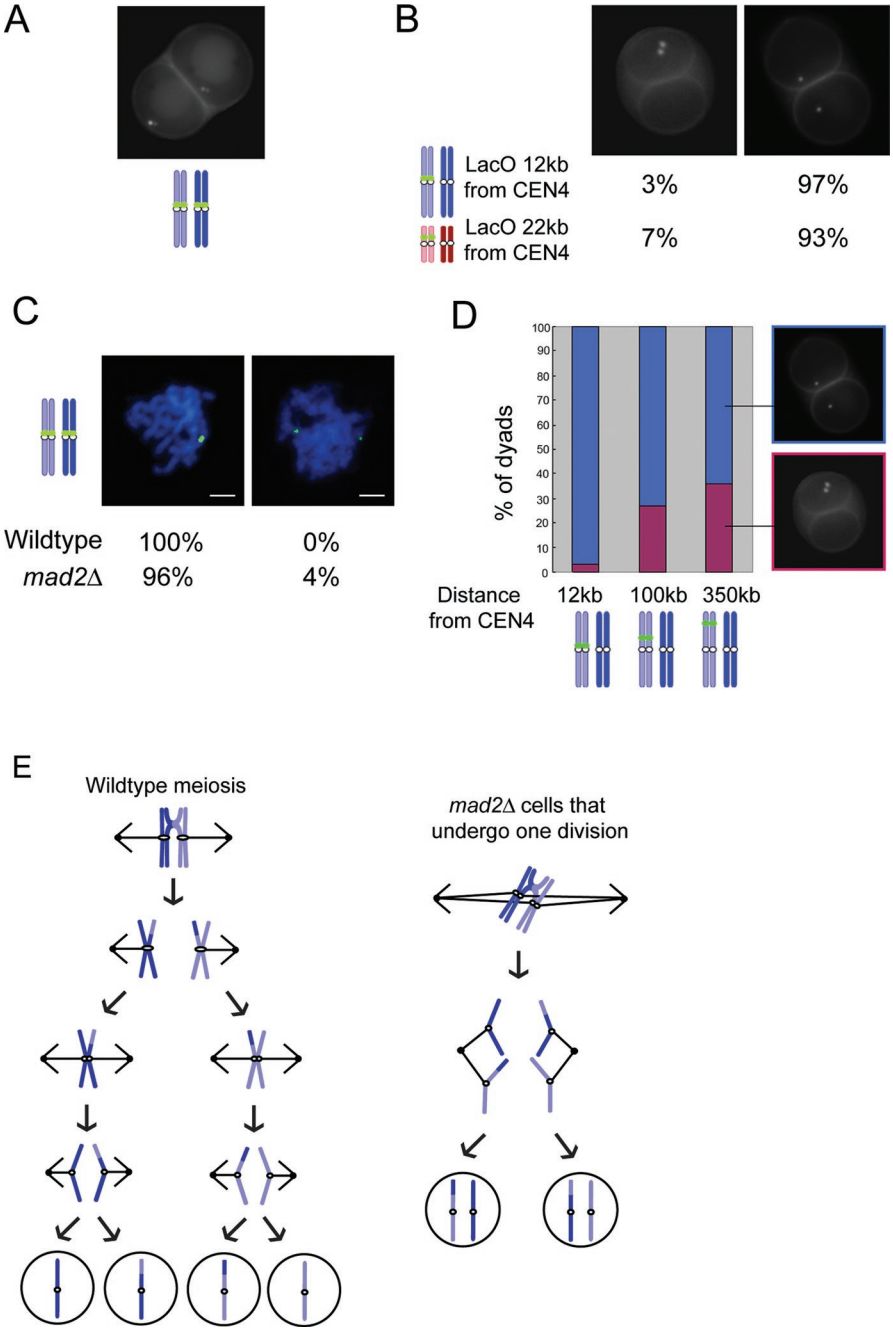


FIGURE 2: The *mad2*Δ dyad spores are the result of an aberrant meiotic division in which homologous chromosomes pair and recombine and then sister chromatids separate. (A) *mad2*Δ/*mad2*Δ cells with both chromosome IV's marked with a LacO array near CEN4 were analyzed for the segregation of the marked chromosome. Three hundred dyads were counted in three experiments. (B) Dyads from *mad2*Δ/*mad2*Δ cells with only one of the two homologous chromosome marked with a LacO array were analyzed for the segregation of the marked chromosome. Strains contain a LacO array either 12 kb from CEN4 or 22 kb from CEN3. Three hundred dyads from each genotype were counted in three experiments. (C) Wild-type and *mad2*Δ/*mad2*Δ cells with both homologous chromosome IV's marked with a LacO array 12 kb from CEN4 were prepared for chromosome spreads. Fifty spreads from each genotype were counted. Scale bar, 2 μm. (D) *mad2*Δ/*mad2*Δ dyads with only one of the two homologous chromosomes marked with a LacO array were analyzed for the segregation of the marked chromosome. Strains contain the LacO array 12, 100, or 350 kb from CEN4. The percentage of dyads with one marked chromosome in each spore is shown in blue. The percentage of dyads with two marked chromosomes in one spore is shown in pink. Three hundred dyads from each genotype were counted in three experiments. (E) Diagram showing a wild-type meiosis compared with the aberrant meiosis in *mad2*Δ cells that results in the separation of sister chromatids in a single division.

spore will have one marked chromosome. When the LacO array is placed at the TRP1 locus, approximately 12 kb from CEN4, 97% of the dyads contain one marked chromosome in each spore (Figure 2B). We verified that chromosome III also segregates sister chromatids by placing the LacO array at the LEU2 locus, approximately 22 kb away from CEN3. Ninety-three percent of *mad2*Δ dyads contained one marked chromosome in each spore (Figure 2B). Surprisingly, the data show that sister chromatids separate inappropriately in the single meiotic division. These results suggest that in the 1 division *mad2*Δ cells, meiosis II events occur in the single meiotic division.

To investigate whether other events of meiosis I were perturbed in the 1 division *mad2*Δ cells, we examined whether the cells initiate meiosis correctly by pairing and recombining homologous chromosomes in prophase I. To monitor pairing, we examined spread meiotic nuclei of *mad2*Δ cells in the pachytene stage of prophase. In pachytene, homologous chromosomes have paired, synapsed, and initiated recombination. We marked both homologous chromosomes with a LacO array near CEN4 and expressed GFP-LacI. Chromosomes that are paired will have two GFP marked chromosomes in close proximity. One hundred percent of wild-type and 96% of *mad2*Δ cells have paired homologous chromosomes (Figure 2C). Using this assay, we cannot determine which cells will form dyads, but the proportion of cells with paired chromosomes is so great that it must include the *mad2*Δ cells that undergo one division, showing that there is not a defect in pairing in *mad2*Δ cells.

To determine whether crossovers occur in the 1 division *mad2*Δ cells, we monitored the segregation pattern of a LacO array placed at different locations along one of the homologous chromosome IVs. As shown earlier, if a LacO array is placed only 12 kb from the centromere on one of the homologous chromosomes, the sister centromeres split, and 97% of the dyads have one chromosome with the array in each spore. Only 3% of the dyads have two chromosomes with the array in one spore. We figured that if an array is placed further from the centromere, a crossover could occur between the array and the centromere, and the dyads would inherit two marked chromosomes in one spore. Indeed, with an array located approximately 100 kb from the centromere, 27% of the dyads have two marked chromosomes in one spore. With an array located 350 kb from the centromere, 36% of the dyads have two marked chromosomes in one spore (Figure 2D). In summary, the *mad2*Δ dyads undergo a single meiotic division in which homologous chromosomes pair, recombine, and separate sister chromatids inappropriately (Figure 2E).

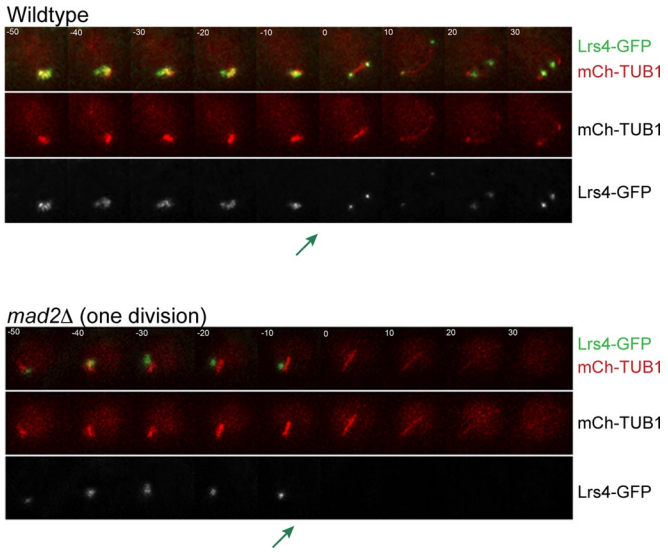


FIGURE 3: In the *mad2*Δ cells that undergo one meiotic division, the monopolin complex does not bind sister kinetochores. (A) Time lapse images of meiosis in wild-type and 1 division *mad2*Δ/*mad2*Δ cells. Both strains are expressing Lrs4-GFP and Tub1-mCherry. The green arrow shows the point in which Lrs4-GFP leaves the nucleolus. One hundred of the wild-type cells, 100 of the 2 division *mad2*Δ cells, and 50 of the 1 division *mad2*Δ cells were analyzed.

In the 1 division mad2Δ cells, kinetochores are not clamped together, and cohesin does not remain protected around the centromere

Our results suggest that in the 1 division *mad2*Δ cells, the paired homologous chromosomes attach sister chromatids to opposite spindle poles in metaphase I instead of homologous chromosomes (Figure 2E). In wild-type cells, sister chromatids do not attach to opposite spindle poles because the monopolin complex holds the sister chromatids' kinetochores together, assembling one microtubule-binding site (Toth *et al.*, 2000; Rabitsch *et al.*, 2003; Winey *et al.*, 2005; Petronczki *et al.*, 2006; Monje-Casas *et al.*, 2007). To determine whether monopolin localization is disrupted in 1 division *mad2*Δ cells, we monitored the localization of the monopolin component Lrs4 tagged with GFP, using time-lapse microscopy (Figure 3). In wild-type cells, Lrs4 normally resides in the nucleolus until the end of prophase I. Then Lrs4 leaves the nucleolus and binds to the kinetochores until the end of anaphase I (Rabitsch *et al.*, 2003). In 2 division *mad2*Δ cells, Lrs4-GFP behaves similarly to wild-type cells (unpublished data). In 1 division *mad2*Δ cells, Lrs4-GFP leaves the nucleolus but does not bind to kinetochores (Figure 3).

The chromosome segregation pattern of 1 division *mad2*Δ cells is quite distinct from that in monopolin mutants. In monopolin mutants, sister chromatids cannot separate in meiosis I due to the protected centromeric cohesins. However, the spindle poles will separate, and the cells will undergo meiosis II, making four mostly inviable spores (Toth *et al.*, 2000; Rabitsch *et al.*, 2003). However, in the 1 division *mad2*Δ cells, sister chromatids separate in the single division, making two viable diploid spores, suggesting that centromeric cohesins are not protected.

We monitored the meiotic cohesin Rec8 to determine whether centromeric cohesin is lost prematurely in the 1 division *mad2*Δ cells. In wild-type cells, previous reports showed that there is stepwise cleavage of Rec8; the Rec8 along chromosome arms is cleaved in meiosis I, and the centromeric Rec8 is cleaved in meiosis II (Klein *et al.*, 1999; Buonomo *et al.*, 2000; Kitajima *et al.*, 2003, 2004). To monitor cohesin cleavage during the meiotic cell cycle, we tagged Rec8 with GFP in cells also expressing mCherry-TUB1. As expected, wild-type and 2 division *mad2*Δ cells show a stepwise loss of Rec8. The majority of Rec8 is cleaved concurrently with anaphase I spindle assembly, leaving a fraction of Rec8 until meiosis II (Figure 4A). In the 1 division *mad2*Δ cells, there is still a stepwise loss of cohesin, except that the first cleavage occurs prematurely, 45 ± 12 min prior to anaphase I.

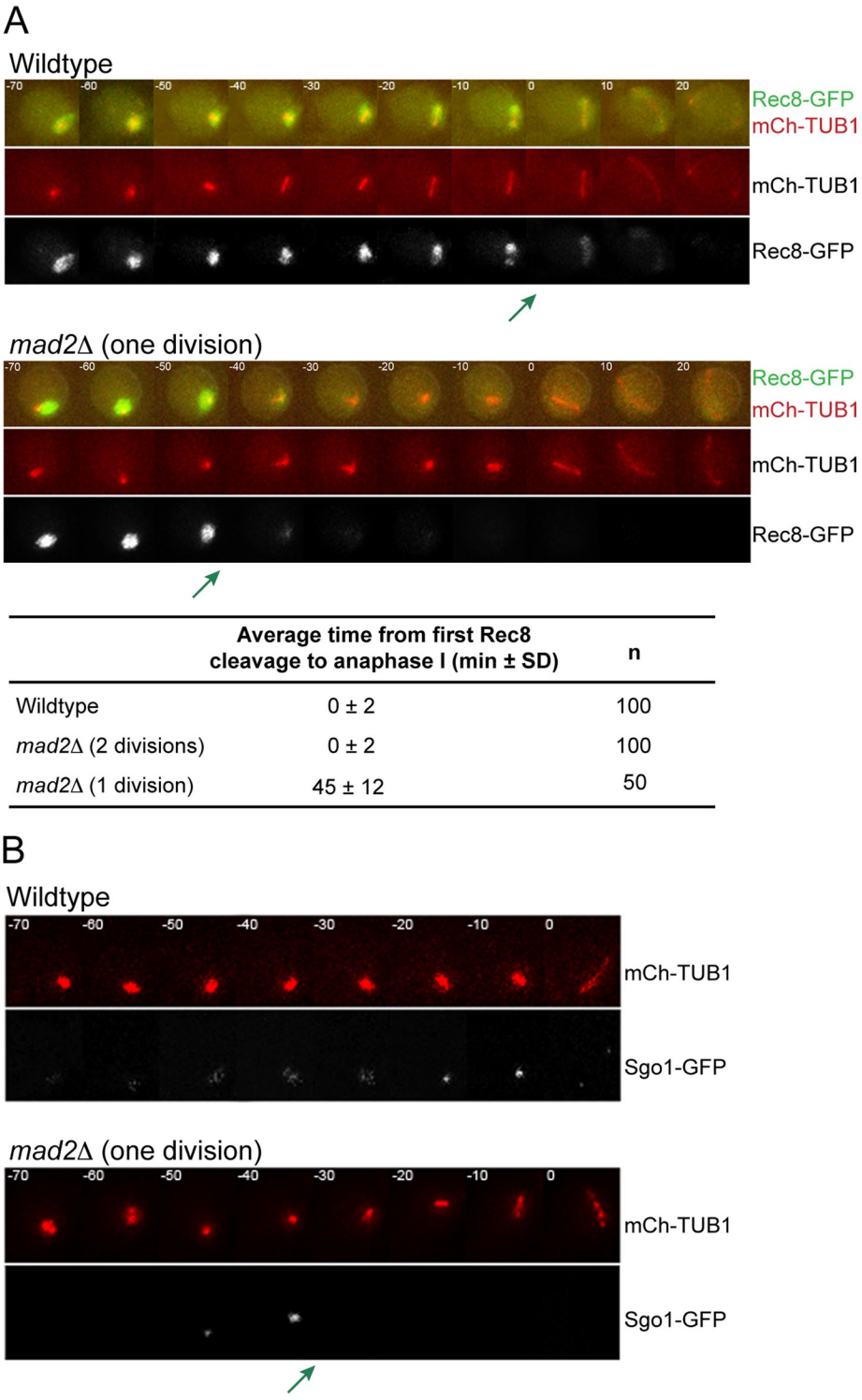
Our results suggest that in the 1 division *mad2*Δ cells, centromeric cohesins do not remain protected in anaphase I, allowing the cells to separate sister chromatids. We used time-lapse microscopy to monitor Sgo1, one of the factors required for protection of centromeric cohesins (Kerrebrock *et al.*, 1995; Katis *et al.*, 2004; Kitajima *et al.*, 2004; Marston *et al.*, 2004). We made an Sgo1-GFP fusion protein and expressed mCherry-TUB1 in wild-type and *mad2*Δ cells. As expected, Sgo1-GFP associates with chromosomes throughout meiosis I in wild-type and in 2 division *mad2*Δ cells (Figure 4B). In contrast, in 1 division *mad2*Δ cells, Sgo1 is lost 38 ± 7 min prior to anaphase I. The loss of Sgo1 most likely results in the cleavage of centromeric Rec8 (Kitajima *et al.*, 2004; Marston *et al.*, 2004). Therefore, our data show that in the 1 division *mad2*Δ cells, sister chromatids separate because sister kinetochores are not clamped together by monopolin, Sgo1 is lost prematurely, and centromeric cohesins are cleaved in the single meiotic division. Clamping of sister kinetochores by monopolin is independent of cohesin and cohesin protection (Monje-Casas *et al.*, 2007), suggesting that the phenotype seen in *mad2*Δ cells is due to pleiotropic misregulation of the meiotic cell cycle. We diagram the timing of cell cycle events with respect to the stages of meiosis (as defined by spindle morphology) in Figure 5.

In the absence of Mad2, the APC/C is prematurely active in prometaphase of meiosis I

We considered that Mad2 regulates the meiotic cell cycle by modulating APC^{Cdc20} activity. During spindle checkpoint signaling, Mad2, together with other checkpoint proteins, inhibits APC^{Cdc20} activity to delay the cell cycle in metaphase I (Musacchio and Salmon, 2007) To determine whether APC^{Cdc20} is prematurely active in the absence of Mad2, we investigated the timing of the degradation of the APC^{Cdc20} substrate securin/Pds1. Using time-lapse microscopy, we monitored Pds1-GFP and mCherry-TUB1. In wild-type cells, Pds1-GFP is degraded, and the cells enter anaphase I (Figure 6A). We were surprised to find that in the 2 division *mad2*Δ cells, Pds1 is degraded on average 13 ± 12 min prior to anaphase I spindle assembly (Figure 6A and Table 1). Because prometaphase I/metaphase I is

	Average time from Pds1 degradation to anaphase I (min)	SD	Range (min)	n
Wild type	1	2	0–10	100
*mad2*Δ (2 divisions)	13	12	0–60	100
*mad2*Δ (1 division)	81	35	35–190	50
*swm1*Δ	0	0	0	100
*mad2*Δ *swm1*Δ	1	2	0–10	100
*mad3*Δ	1	2	0–10	100
*ama1*Δ	1	2	0–10	100
*mad2*Δ *ama1*Δ	11	8	0–30	100

TABLE 1: Average time from securin/Pds1 degradation to anaphase I spindle formation.



	Average time from first Rec8 cleavage to anaphase I (min ± SD)	n
Wildtype	0 ± 2	100
*mad2*Δ (2 divisions)	0 ± 2	100
*mad2*Δ (1 division)	45 ± 12	50

FIGURE 4: In the *mad2*Δ cells that undergo one meiotic division, protection of the meiotic cohesin Rec8 is lost prematurely. (A) Time lapse images of meiosis in wild-type and 1 division *mad2*Δ/*mad2*Δ cells. Cells are expressing Tub1-mCherry and Rec8-GFP. A green arrow marks the time of the first Rec8 cleavage. The time from first cleavage to anaphase I was calculated (± SD). (B) Time lapse images of meiosis in wild-type and 1 division *mad2*Δ/*mad2*Δ cells. Cells are expressing Tub1-mCherry and Sgo1-GFP. A green arrow marks the time at which Sgo1 is lost in the 1 division *mad2*Δ cells. One hundred wild-type cells and 50 *mad2*Δ cells that formed dyads were analyzed.

~18 min faster in 2 division *mad2*Δ cells than in wild-type cells (Figure 1B), Pds1 is in fact degraded ~31 min early (Figure 5). It is not clear why the cells do not enter anaphase I immediately after loss of Pds1, but there may also be a misregulation of other cell cycle events preventing cohesin cleavage. It is striking that in the 1 division *mad2*Δ cells, Pds1 is degraded even more prematurely: 81 min prior to anaphase I spindle assembly (Figure 6A and Table 1).

If prematurely active APC is indeed the cause of premature Pds1 degradation, then decreasing APC/C activity should prevent the early Pds1 degradation. We measured the timing of the loss of Pds1-GFP in *mad2*Δ cells that also have a deletion of Swm1, a nonessential component of the APC/C. Cells that lack Swm1 target substrates for ubiquitination less efficiently than wild-type cells (Hall *et al.*, 2003; Schwickart *et al.*, 2004; Oelschlaegel *et al.*, 2005), but Pds1 is degraded at anaphase I in *swm1*Δ cells (Figure 6B and Table 1). In *mad2*Δ *swm1*Δ cells, we do not see premature degradation of Pds1; the degradation of Pds1 occurs within 1 ± 2 min of anaphase I spindle formation (Figure 6B and Table 1). Using time-lapse microscopy, we find that no *mad2*Δ *swm1*Δ cells undergo only one meiotic division. Therefore, down-regulating APC/C activity prevents premature degradation of Pds1 in *mad2*Δ cells and rescues the single-division phenotype. We conclude that in *mad2*Δ cells, the APC/C is prematurely active.

The role of Mad2 in down-regulating APC/C activity in metaphase I is distinct from its role in delaying the cell cycle if a chromosome is not attached to the spindle

Because the activity of the APC/C is inhibited during spindle checkpoint signaling when a chromosome is not attached to spindle microtubules, we wanted to determine whether another protein required for the spindle checkpoint signaling in meiosis, Mad3, is also required to decrease APC/C activity during prometaphase I and metaphase I (Shonn *et al.*, 2000; Musacchio and Salmon, 2007). We monitored loss of Pds1-GFP with respect to anaphase I spindle assembly in *mad3*Δ cells. Pds1-GFP is degraded within 1 ± 2 min of anaphase I spindle formation in *mad3*Δ cells, similar to what occurs in wild-type cells (Table 1). We also do not see the formation of dyad spores or the single-division phenotype. Our results indicate that in the absence of Mad3, the APC/C is not prematurely active, suggesting that Mad2 functions independently of Mad3 to down-regulate APC/C activity during prometaphase I/ metaphase I. This is consistent with previous studies showing that *mad3*Δ cells do not have an increase in meiosis I nondisjunction (Shonn *et al.*, 2003).

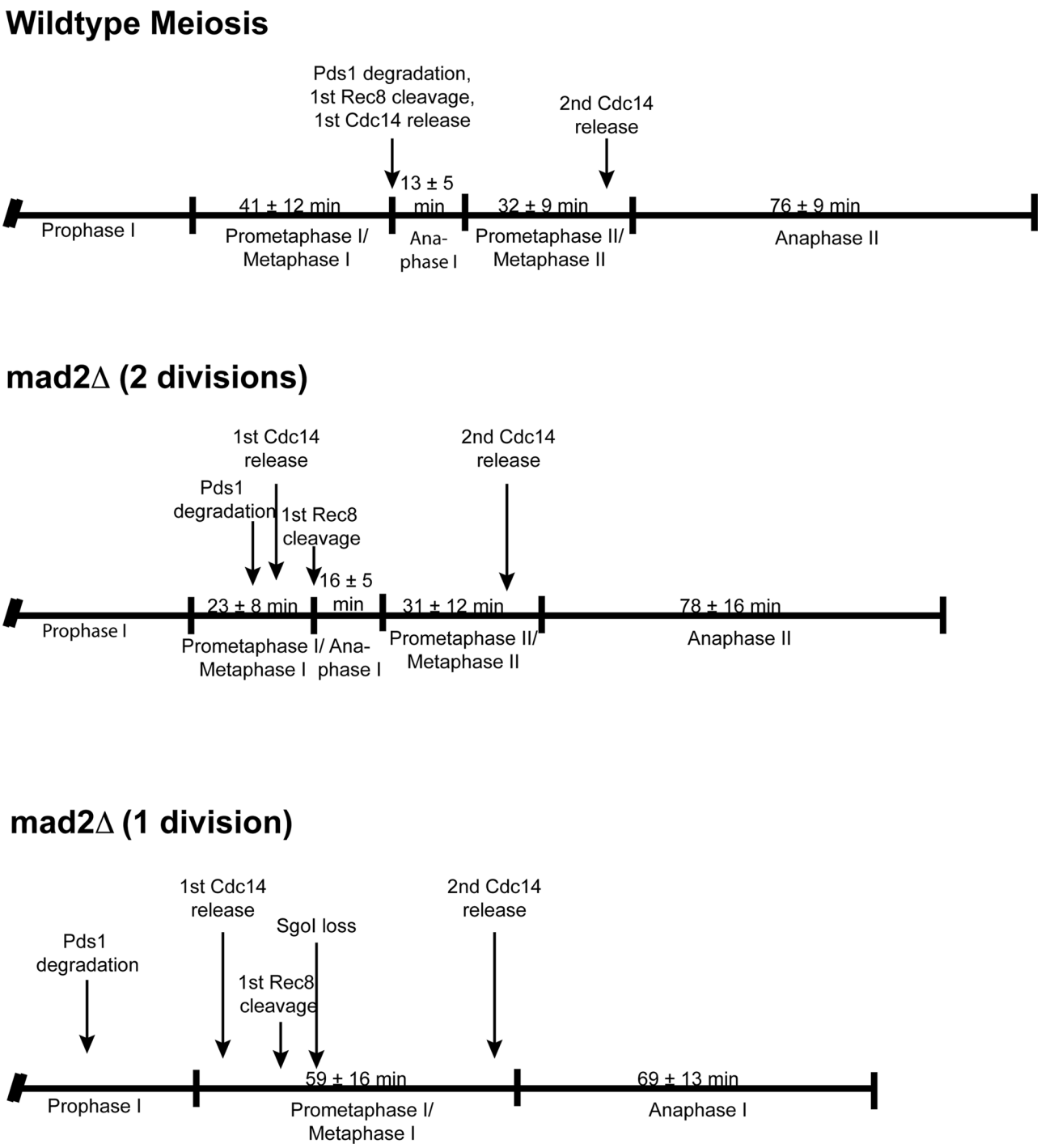


FIGURE 5: Schematic representing the timing of different cell cycle events with respect to the stage of the cell cycle in wild-type, 2 division *mad2*Δ, and 1 division *mad2*Δ cells.

Both APC^{Cdc20} and APC^{Ama1} are prematurely active in 1 division mad2Δ cells

The meiosis-specific cofactor of the APC/C, Ama1, also targets Pds1 for ubiquitination and subsequent degradation and, therefore, may be prematurely active in *mad2*Δ cells (Cooper *et al.*, 2000; Oelschlaegel *et al.*, 2005; Penkner *et al.*, 2005). Ama1 is not essential for meiosis but does function in meiosis I to promote the rapid degradation of APC/C substrates (Oelschlaegel *et al.*, 2005). To determine whether APC^{Ama1} is prematurely active in *mad2*Δ cells, we deleted Ama1 in wild-type and *mad2*Δ cells and followed the degradation of Pds1-GFP with respect to spindle assembly by live-cell imaging. In *ama1*Δ cells, Pds1 is degraded within 1 ± 2 min of anaphase I spindle assembly (Table 1). In *ama1*Δ *mad2*Δ cells, Pds1 is degraded ~11 ± 8 min prior to anaphase I spindle assembly, similar to 2 division *mad2*Δ cells. We do not see any cells that undergo a single meiotic division. We conclude that premature APC^{Ama1} activity results in the very premature Pds1 degradation and the single-division phenotype in *mad2*Δ cells.

Our results suggest that Mad2 may have a role in preventing premature APC^{Ama1} activity. However, Mad2 could directly or

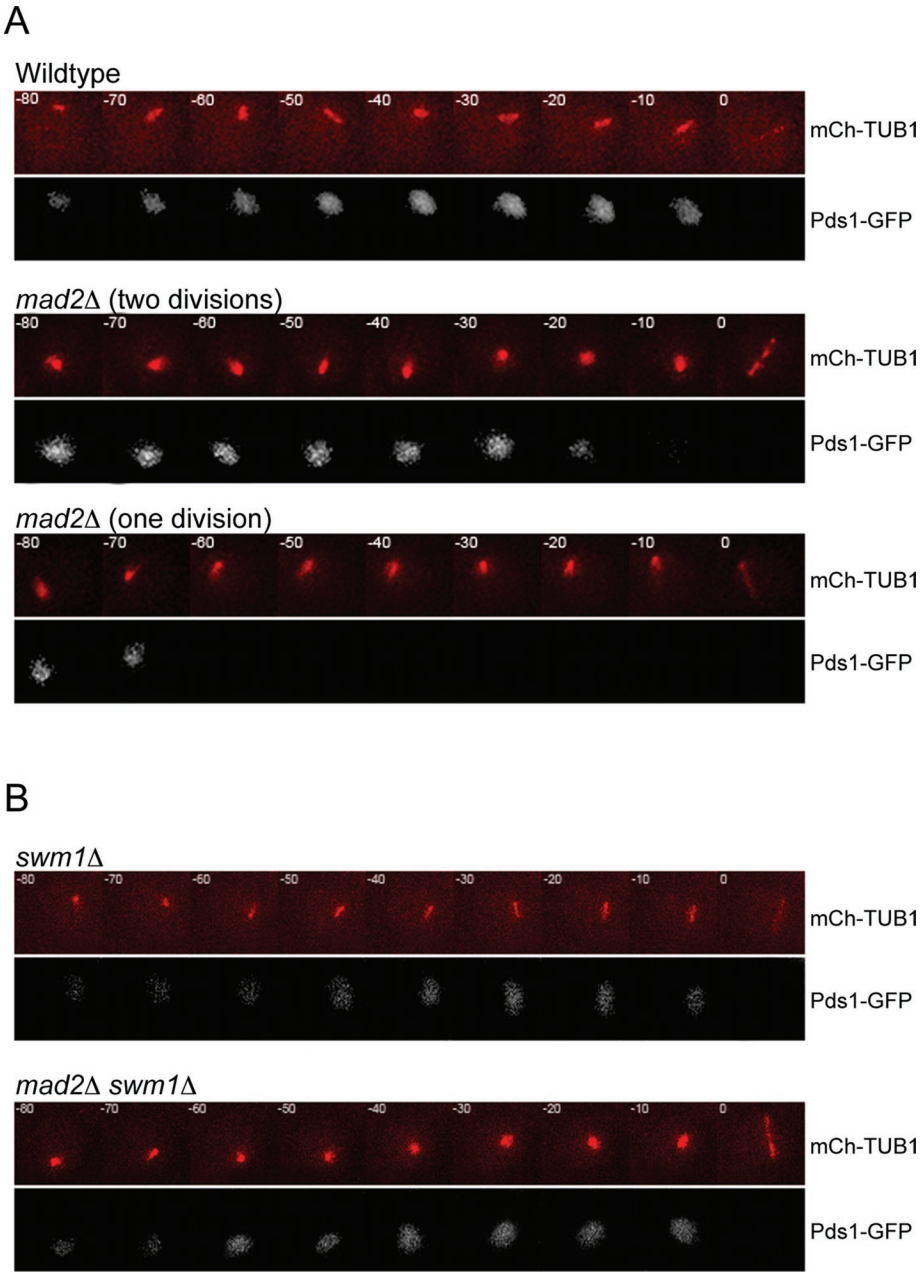


FIGURE 6: The APC is prematurely active in *mad2*Δ cells. (A) Time-lapse images of meiosis in wild-type and *mad2*Δ/*mad2*Δ cells. Both strains are expressing Pds1-GFP and Tub1-mCherry. One hundred wild-type cells, 100 *mad2*Δ cells that formed tetrads, and 50 *mad2*Δ cells that formed dyads were analyzed. (C) Time lapse images of meiosis in *swm1*Δ/*swm1*Δ and *swm1*Δ/*swm1*Δ *mad2*Δ/*mad2*Δ cells. Both strains are expressing Pds1-GFP and Tub1-mCherry. No single meiotic division in *swm1*Δ/*swm1*Δ *mad2*Δ/*mad2*Δ was observed. One hundred cells of each genotype were analyzed.

indirectly inhibit APC^{Ama1}. In wild-type cells, Cdc20 is required for Pds1 degradation, suggesting that APC^{Ama1} is only active after APC^{Cdc20} targets substrates for degradation (Salah and Nasmyth, 2000; Oelschlaegel *et al.*, 2005). We reasoned that Mad2 may not directly inhibit APC^{Ama1}, but might instead inhibit APC^{Cdc20} activity, and that this inhibition prevents APC^{Ama1} activity. To determine whether Cdc20 is required for the premature activity of APC^{Ama1}, we analyzed whether Pds1 can be degraded in the absence of Cdc20 in *mad2*Δ cells. We replaced the Cdc20 promoter with the mitosis-specific Clb2 promoter to make a Cdc20 meiotic null (*cdc20-mn*; Lee and Amon, 2003) and monitored Pds1 degradation with respect to metaphase I spindle assembly. The *cdc20-mn* and *cdc20-mn mad2*Δ cells do not degrade Pds1 prematurely. Ninety-eight percent of the *cdc20-mn* and 96% of *cdc20-mn mad2*Δ cells that enter meiosis are blocked in metaphase I with Pds1-GFP present for at least 200 min (Figure 7). Our results demonstrate that APC^{Cdc20} activity is required for the activation of APC^{Ama1} in 1 division *mad2*Δ cells. We conclude that in prometaphase I, Mad2 indirectly prevents premature APC^{Ama1} activity by inhibiting APC^{Cdc20}.

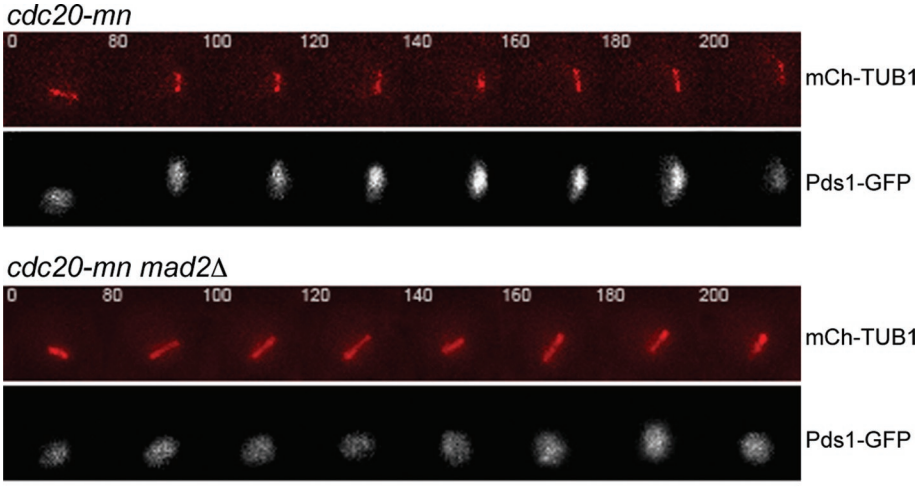
We decided to further explore the regulation of APC^{Ama1} by APC^{Cdc20}. A previous study demonstrates that APC^{Ama1} activity is inhibited during metaphase I by cyclin-dependent kinase (CDK; Oelschlaegel *et al.*, 2005). However, an allele of *AMA1*, *Ama1-m8*, with all eight putative CDK phosphorylation sites mutated to alanine does not result in premature APC^{Ama1} activity, suggesting that the inhibition of APC^{Ama1} by CDK is not direct or that there is redundancy in the regulatory pathway. To determine whether Ama1 phosphorylation regulates APC^{Ama1} activity in *mad2*Δ cells, we analyzed *mad2*Δ *Ama1-m8* cells expressing mCherry-TUB1 by time-lapse microscopy. Surprisingly, 87% of sporulated *mad2*Δ *Ama1-m8* cells undergo a single meiotic division (Table 2). The percentage sporulation of *mad2*Δ *Ama1-m8* cells was similar to that in wild-type cells. In accordance with previous observations, we did not see a phenotype of *Ama1-m8* in the wild-type background (Oelschlaegel *et al.*, 2005). Our results suggest that there is redundancy in the regulatory network to prevent premature activity of APC^{Ama1} in metaphase I. We propose that Ama1 is inhibited by phosphorylation and by another activity of CDK. In the absence of Mad2, premature APC^{Cdc20} activity could lead to less CDK activity and, in some cells, the dephosphorylation of Ama1, resulting in the one-division phenotype.

Premature APC^{Ama1} activity results in the premature release of the Cdc14 phosphatase

Our results demonstrate that in the absence of Mad2, securin/Pds1 is degraded

Genotype	Percentage sporulation	Percentage of sporulated cells that undergo one meiotic division	n
Wild-type	65	0	100
Ama1-m8	67	0	100
*mad2*Δ	65	34	100
*mad2*Δ + Ama1-m8	60	87	100

TABLE 2: A nonphosphorylatable form of Ama1 increases the percentage of *mad2*Δ cells that undergo 1 meiotic division.



	% of cells that arrest with Pds1	n
cdc20-mn	98	100
cdc20-mn mad2Δ	96	100

FIGURE 7: APC^{Cdc20} activity is required for the premature APC^{Ama1} activity in *mad2Δ* cells. (A) Time-lapse images of meiosis in *cdc20-mn/cdc20-mn* and *mad2Δ/mad2Δ cdc20-mn/cdc20-mn* cells. All strains are expressing Pds1-GFP and Tub1-mCherry. The percentage of cells that arrest with Pds1 is shown.

prematurely. The degradation of securin results in the release of the protease separase, which cleaves sister chromatid cohesion. Separase also functions in the Cdc14 early anaphase release (FEAR) network, promoting the release of the Cdc14 phosphatase from the nucleolus (Stegmeier *et al.*, 2002; Buonomo *et al.*, 2003; Marston *et al.*, 2003). Once released, Cdc14 counteracts CDK activity by dephosphorylating CDK substrates. Because the 1 division *mad2Δ* cells degrade securin/Pds1 prematurely, we analyzed whether Cdc14 is released prematurely. We monitored the timing of release and resequestration of Cdc14-GFP in wild-type and *mad2Δ* cells using time-lapse microscopy. In wild-type cells, Cdc14-GFP is released from the nucleolus 1 ± 4 min before anaphase I, is resequestered into the nucleolus in metaphase II, and then is released again 5 ± 5 min before anaphase II (Figure 8A). The 2 division *mad2Δ* cells have similar timing of Cdc14 release; Cdc14 is released 6 ± 6 min before anaphase I, resequestered, and released again 6 ± 5 min before anaphase II. Surprisingly, in 1 division *mad2Δ* cells, Cdc14 is also released twice, but both releases occur before the first division. Cdc14 is first released 54 ± 7 min before anaphase I spindle formation, resequestered, and released again 6 ± 6 min before anaphase I spindle formation (Figure 8B). These results support our conclusion that meiosis II cell cycle events are uncoupled from the chromosome segregation cycle in the 1 division *mad2Δ* cells (Figure 5).

Our results suggest that the premature degradation of Pds1 leads to separase activation of the FEAR network and Cdc14 release. Because Ama1 is required for the premature degradation of Pds1 in *mad2Δ* cells that undergo one division, Ama1 should also be required for the premature release of Cdc14. Analysis of Cdc14 release in *mad2Δ ama1Δ* cells reveals that it occurs with timing similar to that of wild-type cells during both meiotic divisions (Figure 8A). These data show that the activity of AMA1 is required for the premature release of Cdc14, most likely through targeting Pds1 for ubiquitination and subsequent degradation.

DISCUSSION

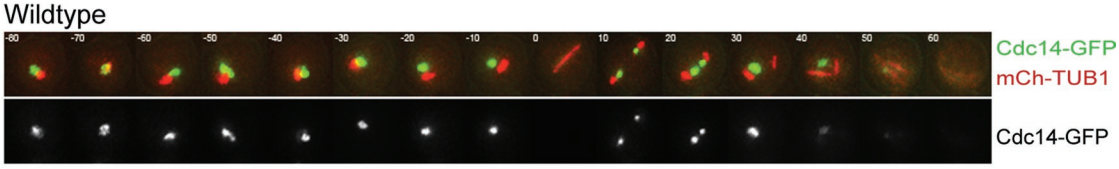
Mad2 regulates the timing of the degradation of APC/C substrates

In this study, we observed that a deletion of Mad2 can disrupt the normal timing of meiotic cell cycle events. In the absence of Mad2, cells execute one of two alternative meiotic programs: 1) 64% of cells undergo two meiotic divisions, but prometaphase I/metaphase I is shorter in duration, and 2) 34% of cells undergo a single meiotic division in which sister chromatids separate inappropriately. Both pathways are the result of the APC/C prematurely targeting substrates such as securin/Pds1 for ubiquitination. Down-regulation of APC/C activity, by deleting the APC/C component Swm1, rescues the premature securin/Pds1 degradation and the single-division phenotype. Our data indicate that premature APC^{Cdc20} activity causes the faster prometaphase I/metaphase I in the 2 division *mad2Δ* cells, and premature APC^{Cdc20} and APC^{Ama1} result in the single-division phenotypes. We propose that Mad2 restrains APC^{Cdc20} activity, setting the duration of prometaphase I and metaphase I to prevent a misregulation of cell cycle events and the missegregation of chromosomes.

In the 2 division *mad2Δ* cells, the premature APC^{Cdc20} activity is likely to lead to the increase in meiosis I nondisjunction by targeting substrates for degradation and transitioning into the next cell cycle stage too rapidly. By using time-lapse microscopy to monitor cell cycle proteins in individual cells instead of a population of cells, we find that securin/Pds1 is degraded ~30 min earlier in 2 division *mad2Δ* cells and the duration of prometaphase I/metaphase I is ~18 min shorter when compared with wild-type cells. This change in duration of prometaphase I/metaphase I in *mad2Δ* cells shortens the time allotted for chromosomes to properly attach to the bipolar spindle. Because the disruption of Mad2 in mammalian oocytes, worms, fission yeast, and budding yeast results in an increase in meiosis I nondisjunction, regulation of cell cycle timing may be a conserved role of Mad2 in meiosis (Kitagawa and Rose, 1999; Shonn *et al.*, 2000, 2003; Bernard *et al.*, 2001; Tsurumi *et al.*, 2004; Homer *et al.*, 2005a, 2005b; Stein *et al.*, 2007). Similarly, in *Drosophila* oocytes, disruption of the spindle checkpoint component Ald/Mps1 leads to an advance in anaphase I onset and an increase in meiosis I nondisjunction (Gilliland *et al.*, 2007).

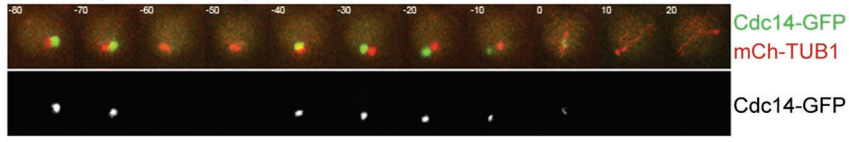
Can premature APC^{Ama1} and APC^{Cdc20} activity result in all of the phenotypes seen in 1 division *mad2Δ* cells to produce two viable spores? The premature activity of both APC^{Ama1} and APC^{Cdc20} could lead to the early degradation of substrates, such as Pds1, Sgo1, Dbf4, and Spo13 (Cooper *et al.*, 2000; Ferreira *et al.*, 2000; Salah and Nasmyth, 2000; Oelschlaegel *et al.*, 2005; Penkner *et al.*, 2005; Sullivan and Morgan, 2007). Pds1 regulates the meiotic divisions by inhibiting separase to ensure the timely segregation of chromosomes and release of Cdc14 phosphatase (Rock and Amon, 2009). Sgo1 protects centromeric cohesins (Katis *et al.*, 2004; Kitajima

A



	Average time from 1st Cdc14 release to anaphase I (min ± SD)	Average time from 2nd Cdc14 release to anaphase II (min ± SD)	n
Wildtype	1 ± 4	5 ± 5	100
*mad2*Δ (two divisions)	6 ± 6	6 ± 5	100
*mad2*Δ *ama1*Δ	5 ± 6	8 ± 10	100

B



	Average time from 1st Cdc14 release to anaphase I (min ± SD)	Average time from 2nd Cdc14 release to anaphase I (min ± SD)	n
*mad2*Δ (one division)	54 ± 7	6 ± 6	50

FIGURE 8: The phosphatase Cdc14 is released from the nucleolus prematurely in the 1 division *mad2*Δ cells, and the premature release is dependent on APC^{Ama1} activity. (A) Time-lapse images of meiosis in wild-type, *mad2*Δ/*mad2*Δ, and *mad2*Δ/*mad2*Δ *ama1*Δ/*ama1*Δ cells. All strains are expressing Cdc14-GFP and Tub1-mCherry. Average time of Cdc14 release from the nucleolus before anaphase I and anaphase II spindle formation is shown (± SD). (B) Time-lapse images of meiosis in 1 division *mad2*Δ/*mad2*Δ cells. All strains are expressing Cdc14-GFP and Tub1-mCherry. Average times of the two Cdc14 releases from the nucleolus before anaphase I are shown.

et al., 2004; Marston *et al.*, 2004). Dbf4, as a component of the DDK kinase, regulates the localization of monopolin to kinetochores (Matos *et al.*, 2008). Finally, Spo13 has a role in monopolin localization and protection of sister chromatid cohesion. The premature degradation of these substrates could result in the phenotypes of the 1 division *mad2*Δ cells: 1) Cdc14 phosphatase is prematurely released from the nucleolus; 2) cohesin does not remain protected in metaphase I; 3) kinetochores on sister chromatids are not clamped together; and 4) sister chromatids separate in a single division. Therefore, the production of viable spores after a single meiotic division in *mad2*Δ cells is likely the consequence of the combined loss of many APC/C substrates.

Our results show that APC^{Ama1} activity at the end of prophase I results in an uncoupling of cell cycle events from the chromosome segregation cycle, and meiosis II events occur in a single meiotic division. The cell has evolved many different mechanisms to prevent premature APC^{Ama1} activity. AMA1 is transcribed and spliced only in meiosis (Cooper *et al.*, 2000). An inhibitor of Ama1, Mnd2, prevents APC^{Ama1} activity in the early meiotic stages (Oelschlaegel *et al.*, 2005; Penkner *et al.*, 2005). In addition, CDK/M-phase cyclin also inhibits APC^{Ama1} activity (Oelschlaegel *et al.*, 2005). In this study, we show that the premature APC^{Ama1} activity in *mad2*Δ cells requires APC^{Cdc20} activity. Therefore, we do not believe that Mad2 directly inhibits APC^{Ama1} but is more likely to indirectly inhibit APC^{Ama1} through the regulation of APC^{Cdc20} activity. We are unsure why only 34% of *mad2*Δ cells have premature APC^{Ama1} activity and why this phenotype is uncovered in the W303, but not SK1 strain background (S. Lacefield, unpublished data). We suspect that the regulation of Ama1 activity in W303 and SK1 is slightly different. In the *mad2*Δ cells that have premature activity of APC^{Ama1}, we predict that the timing of the meiotic cell cycle was altered such that APC^{Cdc20} was activated early, leading to activation of APC^{Ama1} prior to the inhibition by CDK/M-phase cyclin activity. Indeed, in *mad2*Δ cells with a version of Ama1 with all putative CDK phosphorylation sites mutated to alanine, 87% of cells undergo a single division.

Strain name	Genotype
LY56	*MATa/α, LacO:TRP1/LacO:TRP1, P_{TUB1}-GFP-TUB1-URA3/ P_{CYC1}-GFP-LacI$_2$-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15*
LY7	*MATa/α, mad2::LEU2/mad2::LEU2, LacO:TRP1/LacO:TRP1, P_{CYC1}-GFP-LacI$_2$-URA3/ura3-1, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15*
LY9	*MATa/α, mad2::LEU2/mad2::LEU2, LacO:TRP1/trp1-1, P_{CYC1}-GFP-LacI$_2$-URA3/ura3-1, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15*
LY806	*MATa/α, mad2::LEU2/mad2::kanMX4, P_{TUB1}-GFP-TUB1-URA3/ P_{CYC1}-GFP-LacI$_2$-URA3, ZIP1-GFP-700/ZIP1, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY838	*MATa/α, P_{TUB1}-GFP-TUB1-URA3/ ura3-1, ZIP1-GFP-700/ZIP1, LacO:TRP1/trp1-1, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15,*
LY849	*MATa/α, mad2:: kanMX4 /mad2::kanMX4, P_{CYC1}-GFP-LacI$_2$-URA3/ura3-1, LacO:LEU2/leu2-3112, ade2-1/ade2-1, can1-100/can1-100, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY850	*MATa/α, mad2:: kanMX4 /mad2::kanMX4, P_{CYC1}-GFP-LacI$_2$-URA3/ura3-1, LacO:TRP1(at chromIV genomic fragment 550857)/+, leu2-3112 /leu2-3112, ade2-1/ade2-1, can1-100/can1-100, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY851	*MATa/α, mad2:: kanMX4/mad2::kanMX4, P_{CYC1}-GFP-LacI$_2$-URA3/ura3-1, LacO:TRP1(at chromIV genomic fragment 793927)/+, leu2-3112 /leu2-3112, ade2-1/ade2-1, can1-100/can1-100, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY578	*MATa/α, mad2::LEU2/mad2::LEU2, LRS4-GFP-HIS3/LRS4-GFP-HIS3, LacO:TRP1/LacO:TRP1, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15*
LY579	*MATa/α, LRS4-GFP-HIS3/LRS4-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY500	*MATa/α, mad2::kanMX4/mad2::kanMX4, REC8-GFP-kanMX4/REC8-GFP-kanMX4, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY527	*MATa/α, REC8-GFP-kanMX4/REC8-GFP-kanMX4, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY685	*MATa/α, mad2::kanMX4/mad2::kanMX4, SGO1-GFP-HIS3/SGO1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY684	*MATa/α, SGO1-GFP-HIS3/SGO1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY389	*MATa/α, PDS1-GFP-HIS3/PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY358	*MATa/α, mad2::kanMX4/mad2::kanMX4, PDS1-GFP-HIS3/PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY768	*MATa/α, mad2::LEU2/mad2::LEU2, swm1::kanMX4/ swm1::kanMX4, LacO:TRP1/LacO:TRP1, PDS1-GFP-HIS3/PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY774	*MATa/α, swm1::kanMX4/ swm1::kanMX4, PDS1-GFP-HIS3/PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY528	*MATa/α, CDC14-GFP-HIS3/CDC14-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, P_{HIS3}-mCherry-TUB1-ADE2/ P_{HIS3}-mCherry-TUB1-ADE2, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY411	*MATa/α, mad2::LEU2/mad2::LEU2, CDC14-GFP-HIS3/CDC14-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY698	*MATa/α, mad2::LEU2/mad2::LEU2, ama1::kanMX4/ ama1::kanMX4, CDC14-GFP-HIS3/CDC14-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY274	*MATa/α, PDS1-GFP-HIS3/PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, P_{CLB2}-3HA-CDC20-kanMX6 / P_{CLB2}-3HA-CDC20-kanMX6, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY720	*MATa/α, mad2::LEU2/mad2::LEU2, PDS1-GFP-HIS3/PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3 (at mad2)/ P_{HIS3}-mCherry-TUB1-URA3 (at mad2), P_{CLB2}-3HA-CDC20-kanMX6 / P_{CLB2}-3HA-CDC20-kanMX6, P_{CYC1}-GFP-LacI$_2$-URA3/, P_{CYC1}-GFP-LacI$_2$-URA3, LacO:TRP1/trp1-1 ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15*

TABLE 3: Strains used in this study.

(Continues)

Strain name	Genotype
LY752	*MATa/α, mad3::kanMX4/ mad3::kanMX4, PDS1-GFP-HIS3/PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, P_{HIS3}-mCherry-TUB1-ADE2/ P_{HIS3}-mCherry-TUB1-ADE2, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY689	*MATa/α, cdc20-127/cdc20-127, PDS1-GFP-HIS3/PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY657	*MATa/α, ama1::kanMX4/ ama1::kanMX4, PDS1-GFP-HIS3/ PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY643	*MATa/α, mad2::LEU2/mad2::LEU2, ama1::kanMX4/ ama1::kanMX4, LacO:TRP1/LacO:TRP1, PDS1-GFP-HIS3/PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY829	*MATa/α, mad2::kanMX/mad2::kanMX, PDS1-GFP-HIS3/PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, P_{Ama1}-Ama1-m8-LEU2/ P_{Ama1}-Ama1-m8-LEU2 ade2-1/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*
LY802	*MATa/α, PDS1-GFP-HIS3/PDS1-GFP-HIS3, P_{HIS3}-mCherry-TUB1-URA3/ P_{HIS3}-mCherry-TUB1-URA3, ama1::kanMX/ ama1::kanMX P_{Ama1}-Ama1-m8-LEU2/ P_{Ama1}-Ama1-m8-LEU2, P_{HIS3}-mCherry-TUB1-ADE2/ade2-1, can1-100/can1-100, leu2-3112/leu2-3112, his3-11,15/his3-11,15, trp1-1/trp1-1*

All strains are derivatives of W303 (ade2-1 his3-11,15 leu2-3112 trp1-1 ura3-1 can1-100).

TABLE 3: Strains used in this study. (Continued)

Mad2 functions as a meiotic cell cycle regulator to promote faithful chromosome segregation

The activity of Mad2 in meiosis in budding yeast may be similar to its essential activity in meiosis in mammals. As in budding yeast, depletion of Mad2 in mouse oocytes causes a shortened duration of metaphase I, premature degradation of securin, and chromosomal missegregation (Wassmann *et al.*, 2003; Tsurumi *et al.*, 2004; Homer *et al.*, 2005a, 2005b). We showed that Mad2 prevents premature APC/C substrate degradation in prometaphase I/metaphase I in budding yeast. This activity is independent of the spindle checkpoint component Mad3, although both Mad2 and Mad3 are required to delay the cell cycle at the metaphase I–to–anaphase I transition if chromosomes are not attached to microtubules. Mad3 was previously implicated in regulating the duration of prophase I, an earlier meiotic stage (Cheslock *et al.*, 2005). In mammalian oocytes, BubR1 (which shares a homology domain with Mad3) was also shown to have a role in prophase I, and depletion of BubR1 does not cause the same metaphase I phenotypes as depletion of Mad2 (Homer *et al.*, 2009). We note that the consequences of loss of checkpoint components are different in meiosis I than in mitosis in both budding yeast and mammalian cells (Shonn *et al.*, 2003; Meraldi *et al.*, 2004; Homer *et al.*, 2009). In budding yeast, the absence of Mad2 does not cause a difference in cell cycle timing in mitosis and only has a modest effect on chromosome segregation. The APC/C may be differentially regulated in meiosis I compared with mitosis to ensure that the substrates that have meiotic functions are degraded at the proper time. In the absence of Mad2, cell cycle events are uncoupled from the chromosome segregation cycle. Our results demonstrate that Mad2 is an important meiotic cell cycle regulator, preventing premature APC/C activity in prometaphase I and metaphase I and ensuring the proper orchestration of meiotic cell cycle events.

MATERIALS AND METHODS

Strains and manipulations

All strains are W303 derivatives and are described in Table 3. Deletions were made using standard methods (Longtine *et al.*, 1998). Chromosomes were tagged with GFP as described (Straight *et al.*, 1996; Shonn *et al.*, 2000). Lrs4, Rec8, Sgo1, Pds1, and Cdc14 C-terminal GFP-fusion proteins were made by targeting GFP to the endogenous locus, as described (Wach *et al.*, 1997; Sheff and Thorn, 2004). Zip1-GFP with GFP located at the end of the second coiled-coil domain at position 700 replaced Zip1 at the endogenous locus as described (Scherthan *et al.*, 2007). To visualize tubulin, constructs containing P_{TUB1}TUB1-GFP were integrated into the URA3 locus. Constructs containing P_{HIS3}mCherry-TUB1 were integrated into the URA3 or ADE2 locus. The Clb2 promoter replaced the Cdc20 promoter using a PCR-based method described in Longtine *et al.* (1998). Strains were sporulated in liquid culture by growing in peptone, yeast extract, and dextrose at 30°C to saturation, diluted into 1% yeast extract, 2% bactopeptone, and 1% potassium acetate for 12–15 h at 30°C, washed with water, and resuspended in 1% potassium acetate at 25°C.

Time-lapse microscopy

To monitor meiosis using live-cell microscopy, cells were induced to sporulate, and after 8 h in potassium acetate they were transferred on a concanavalin A–treated (1 mg/ml) cover glass bottom chamber containing 1% potassium acetate and imaged using a Nikon Ti-E inverted microscope (Melville, NY) equipped with a 60× objective (PlanApo, numerical aperture 1.4, oil), a Lambda 10-3 optical filter changer and SmartShutter (Sutter Instrument, Novato, CA), GFP and mCherry filters (Chroma Technology, Bellows Falls, VT), and a CoolSNAP HQ2 charge-coupled device camera (Photometrics, Tucson, AZ). Z-stacks of four to eight sections were acquired in 5- to 10-min intervals for 12–15 h using a 12.5% ND filter and exposure times of 50–300 ms. Z-stacks were combined into a single maximum-intensity projection with NIS-Elements software (Nikon).

Meiotic chromosome spreads

Meiosis-induced cells were cultured in 1% potassium acetate for 10–12 h at 25°C and fixed with 4% paraformaldehyde overnight at 4°C. Cells were washed twice in phosphate-buffered saline, resuspended in 1 M sorbitol, and digested in 1–1.5 mg/ml Zymolyase

(Zymo Research, Irvine, CA) buffered with 1 M sorbitol for 20–30 min at 30°C. After washing twice with 1 M sorbitol, protoplasts were resuspended in 1 M sorbitol and 50 mM EDTA, pH 8.0, and put on ice for 10 min. Protoplasts were placed on a cold, positively charged slide, 100–200 µl of cold hypotonic solution (7.5 mM KCl, 0.05% Triton X) was added, and the slides were then air dried overnight. The specimens were stained with 4′,6-diamidino-2-phenylindole (1 µg/ml) and observed under an epifluorescence microscope.

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