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Regulation of APC/C Activators in Mitosis and Meiosis

Jillian A. Pesin and Terry L. Orr-Weaver¹

Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Nine Cambridge Center, Cambridge, Massachusetts 02142, USA

Abstract

The anaphase-promoting complex/cyclosome is a multi-subunit E3 ubiquitin ligase that triggers the degradation of multiple substrates during mitosis. Cdc20/Fizzy and Cdh1/Fizzy-related activate the APC/C and confer substrate specificity through complex interactions with both the core APC/C and substrate proteins. The regulation of Cdc20 and Cdh1 is critical for proper APC/C activity and occurs in multiple ways: targeted protein degradation, phosphorylation, and direct binding of inhibitory proteins. During meiosis, the activity of the APC/C must be modified in order to achieve proper chromosome segregation during these specialized divisions. Recent studies show that one way in which APC/C activity is modified is through the use of meiosis-specific APC/C activators. Furthermore, regulation of the APC/C during meiosis is carried out by both mitotic regulators of the APC/C as well as meiosis-specific regulators. Here, we review the regulation of APC/C activators during mitosis and the role and regulation of the APC/C during female meiosis.

Keywords

Cdc20; Cdh1; Emi1; spindle assembly checkpoint; meiosis-specific activators; Emi2

INTRODUCTION

Proteolysis is a key mechanism that drives the events of mitosis. The rapid degradation of mitotic proteins provides an irreversible and directional switch to restart the cell cycle after division. The anaphase-promoting complex (APC/C) is a large multi-subunit E3 ubiquitin ligase that targets substrates for degradation through the addition of a ubiquitin chain onto target proteins, which are then recognized and degraded by the 26S proteasome (reviewed in Peters 2006).

Cdc20/Fzy and Cdh1/Fzr comprise the non-core subunits of the APC/C and activate and confer substrate specificity to the complex (Dawson et al. 1995, Schwab et al. 1997, Visintin et al. 1997, Sigrist and Lehner 1997). Cdc20 directs the ubiquitination of securin, mitotic cyclins, and other substrates for anaphase onset, while Cdh1 targets mitotic cyclins and additional substrates for degradation in mitotic exit and G1. Cdc20 and Cdh1 are members of the Cdc20 protein family whose members contain seven WD-40 repeats in their C-

¹Corresponding author: weaver@wi.mit.edu.

terminus (for a review, see Smith et al. 1999). These repeats form a seven-bladed propeller structure that mediates protein-protein interactions. The mechanism of APC/C activation and substrate specification by these activators has been the subject of much research in the past several years (reviewed in Thornton and Toczyski 2006).

One important way in which APC/C activity is governed is through the regulation of the substrate adaptors. Because the activators are essential for APC/C activity and substrate specificity, their regulation plays a crucial role in determining what target proteins are degraded and at what time. In this review we will focus on the control of Cdc20/Fzy and Cdh1/Fzr in mitosis and on the regulation of these activators as well as meiosis-specific activators in meiosis.

REGULATION OF CDC20/FZY IN THE MITOTIC CELL CYCLE

The activity of APC/C^{Cdc20} generally parallels the protein expression profile of its activating subunit. Cdc20 accumulates in S-phase, peaks in mitosis, and drops in G1. A combination of transcriptional upregulation in mitosis and protein degradation in G1 contributes to this profile (Fang et al. 1998a, Prinz et al. 1998). Control of Cdc20 transcription is best understood in *Saccharomyces cerevisiae* where Cks1, a small Cdk-associated protein, binds to the Cdc20 promoter and recruits Cdc28 and the proteasome to induce Cdc20 transcription (Morris et al. 2003). It is not known whether this mechanism controls Cdc20 transcription in higher eukaryotes, but Cdc20 transcription has been shown to be negatively regulated by p53 and p21 in mammalian cells (Kidokoro et al. 2008).

Cdc20 protein is one of several targets of APC/C^{Cdh1} when APC/C^{Cdh1} becomes active late in mitosis (Figure 1) (Prinz et al. 1998). Degradation of *S. cerevisiae* Cdc20 is dependent on the presence of a D-box, a recognition motif found in most APC/C substrates, in its N-terminus (Prinz et al. 1998). Vertebrate Cdc20 sequences do not contain a D-box, and analysis of human Cdc20 led to the identification of the KEN box, another recognition motif found mainly in APC/C^{Cdh1} substrates (Pfleger and Kirschner 2000). In mammalian oocytes and embryos, the degradation of Cdc20 is mediated through an additional motif called the CRY box (Reis et al. 2006a).

Another major player contributing to the presence of functional Cdc20 in cells is the CCT chaperonin (chaperonin-containing TCP1). In *S. cerevisiae* CCT is required for proper folding of Cdc20 in an ATP-dependent manner. CCT-dependent folding of Cdc20 is required for its associations and activity with the APC/C and for its regulation by the spindle checkpoint (Camasses et al. 2003).

In addition to regulation of levels of Cdc20 protein in the cell, the activity of APC/C^{Cdc20} is controlled by several inhibitors, which are discussed below.

Emi1

Xenopus Emi1 was isolated in a yeast two-hybrid screen for Skp1 binding proteins, the F-box protein of the E3 SCF complex (Reimann et al. 2001a). It contains an F-box and a C-terminal Zn²⁺ binding region (ZBR) (Figure 2). In cycling embryos, levels of Emi1 increase

in S-phase and decrease in mitosis, and addition of recombinant Emi1 to cycling egg extracts stabilizes APC/C substrates and prevents their ubiquitination. Emi1 associates with Cdc20 in interphase egg extracts, and inhibition of APC/C^{Cdc20}-mediated ubiquitination of mitotic substrates requires the ZBR domain in Emi1 (Reimann et al. 2001a).

The levels of Emi1 itself are regulated in a cell-cycle dependent manner by several mechanisms (Figure 2). Two mechanisms protect Emi1 from SCF-mediated degradation outside of mitosis. In S/G2 phase, the Evi5 oncogene binds directly to Emi1 and blocks phosphorylation of Emi1 by Plk1, thus preventing its recognition by SCF^{β-TrCP} (Eldridge et al. 2006). Emi1's degradation also seems to be inhibited during G2 by Pin1, a peptidyl-prolyl *cis/trans* isomerase. Emi1 associates with Pin1 in vivo during G2 and is stabilized by Pin1 in an isomerization-dependent pathway (Bernis et al. 2007).

Emi1 degradation in late prophase immediately precedes Cyclin A degradation by the APC/C, suggesting a requirement for Emi1 destruction for APC/C activation in mitosis. In addition, overexpression of non-degradable Emi1 in mammalian cells causes a prometaphase block (Margottin-Goguet et al. 2003). These results, combined with Reimann et al.'s demonstration of a direct interaction between Emi1 and Cdc20, has led to a model in which degradation of Emi1 is essential for activation of APC/C^{Cdc20} in mitosis. A recent study by Di Fiore and Pines questions this model (Di Fiore and Pines 2007). They timed degradation of fluorescently labeled Emi1 and Cyclin A in HeLa cells and found that Emi1 is degraded between 6 and 45 minutes before Cyclin A, whereas the timing observed by Margottin-Goguet et al. was just 5 to 10 minutes. Additionally, injection of RNA encoding non-degradable Emi1 into HeLa cells did not affect the timing of initiation of Cyclin A, Cyclin B, or Securin degradation (Di Fiore and Pines 2007). Technical differences in the experiments, such as levels of the non-degradable form of Emi1, may be responsible for these contradictory results. Additional experiments are needed to determine whether degradation of Emi1 is essential.

An additional function for Emi1 is as a member of a novel regulatory network END (Emi1/NuMA/dynein-dynactin) that restricts the activity of APC/C in early mitosis (Ban et al. 2007). A population of Emi1, along with the APC/C, localizes to spindle poles in early mitosis after the bulk of Emi1 has been degraded via SCF^{β-TrCP} (Hansen et al. 2004, Ban et al. 2007). This localization is dependent on binding of Emi1 and APC/C to microtubules through the action of dynein-dynactin, a minus end-directed microtubule motor. APC/C, Emi1, and NuMA, a nuclear matrix and spindle assembly protein, form a complex in mitosis that spatially restricts APC/C activity. Emi1 promotes NuMA-dependent formation of microtubule asters through its inhibition of Cyclin B degradation by the APC/C at spindle poles (Ban et al. 2007).

Emi1 was originally thought to inhibit APC/C activity simply by binding directly to Cdc20. Emi1 was shown to associate with Cdc20 through binding to a domain on Cdc20 that is distinct from the domain through which Mad2 binds and inhibits Cdc20 during spindle checkpoint arrest (Reimann et al. 2001a, Reimann et al. 2001b). However, a recent study has revealed a more complex mechanism for inhibition of the APC/C by Emi1 (Miller et al. 2006). Emi1 was found to associate with the core APC/C by co-fractionation experiments.

Assays using an Emi1 affinity column showed that Emi1 efficiently captures APC/C in the presence or absence of Cdc20 or Cdh1, suggesting that Emi1 binds to the APC/C core independently of an interaction with an activator protein. Furthermore, this direct interaction is dependent on a conserved D-box in the C-terminus of Emi1 but not on the ZBR domain. Both the D-box and the ZBR domain contribute to Emi1's ability to compete with APC/C substrates for binding to the APC/C and to inhibit APC/C's ubiquitination activity. Finally, wild-type Emi1 is a poor substrate of the APC/C itself, but mutation of the ZBR domain converts Emi1 into an efficient D-box dependent APC/C substrate. Thus, Emi1 acts as a pseudo-substrate inhibitor of the APC/C. The D-box is the domain through which Emi1 binds the D-box receptor on the core APC/C, whereas the ZBR domain seems to act to inhibit access of substrates to the complex.

Spindle Assembly Checkpoint

The spindle-assembly checkpoint (SAC) is a surveillance mechanism that prevents premature separation of sister chromatids in mitosis by monitoring the attachment of spindles to microtubules in prometaphase. SAC inhibits the ability of APC/C^{Cdc20} to target substrates for degradation, particularly Cyclin B and Securin, to prevent anaphase onset until each kinetochore is stably bi-oriented on the spindle. Inhibition of Cdc20 by SAC involves many proteins and several different mechanisms, which will not be discussed here but are well-reviewed by Musacchio and Salmon (Musacchio and Salmon 2007). Exciting recent developments in the spindle checkpoint field have revealed a role for auto-ubiquitination of Cdc20 in inactivation of the checkpoint (Reddy et al. 2007, Stegmeier et al. 2007) and important structural data for Mad2, a key spindle checkpoint protein (Mapelli et al. 2007, Yang et al. 2007, Yang et al. 2008).

DNA damage and PKA pathway

One output of the DNA damage checkpoint is arrest of the cell cycle in the G2-M transition and the metaphase to anaphase transition. In *S. cerevisiae* the PKA pathway inhibits the metaphase to anaphase transition in response to DNA damage (Searle et al. 2004). An accumulation of single-stranded DNA at telomeres causes a PKA-dependent stabilization of Clb2 and Pds1 and phosphorylation of Cdc20. Cdc20 contains 2 consensus sites for PKA phosphorylation that are required for this DNA damage induced modification. In the presence of DNA damage, Cdc20 does not interact with Clb2, causing a metaphase arrest. When the phosphorylation sites of Cdc20 are mutated, Cdc20 does interact with Clb2 upon DNA damage, and Pds1 and Clb2 are degraded with faster kinetics. These results suggest that DNA damage induces PKA phosphorylation of Cdc20 to inhibit the targeting of APC/C substrates in mitosis. It is not known whether Cdc20 is inhibited by DNA damage in other organisms.

REGULATION OF CDH1/FZR IN THE MITOTIC CELL CYCLE

Cdh1 RNA levels are constant throughout the cell cycle, and Cdh1 protein levels are high in mitosis, but lowered in late G1 and S-phases (Prinz et al. 1998, Kramer et al. 2000, Hsu et al. 2002). The decrease in Cdh1 levels in late G1 and S-phase are thought to be due to E3 ligase-mediated protein degradation (Figure 1). Cdh1 contains 2 putative D-boxes that are

required for its degradation in G1, suggesting that Cdh1 targets its own degradation. Furthermore, Cdh1 is ubiquitinated in vitro and degraded in an APC/C^{Cdh1}-dependent manner (Listovsky et al. 2004). In S-phase Cdh1 levels remain low, but APC/C^{Cdh1} cannot be responsible for maintaining low levels of Cdh1 at this stage because it is inactivated by inhibitory phosphorylation. Benmaamar and Pagano investigated a possible role for SCF in degradation of Cdh1 at this cell cycle stage (Benmaamar and Pagano 2005). They found that inactivation of SCF by expression of a dominant-negative Cul1 or by RNA interference against Cul1 results in an accumulation of Cdh1, suggesting that SCF activity is required either directly or indirectly for the degradation of Cdh1.

Phosphorylation inhibits the association of Cdh1 with the APC/C. Cdh1 is phosphorylated by cyclin-dependent kinases during S, G2, and M-phase, and only upon dephosphorylation in G1 is it able to bind and activate APC/C (Figure 1) (Zachariae et al. 1998, Jaspersen et al. 1999, Lukas et al. 1999, Blanco et al. 2000, Kramer et al. 2000; Yamaguchi et al. 2000, Huang et al. 2001, Sorensen et al. 2001, Keck et al. 2007).

Like Cdc20, Cdh1 is also subject to regulation by a several inhibitors, but unlike Cdc20, it seems to be crucial for the cell to modulate APC/C^{Cdh1} activity throughout the cell cycle, even at times when Cdh1 is already subject to inhibitory phosphorylation.

Rca1/ Emi1

The first inhibitor of Cdh1, *regulator of cyclin A (rca1)*, was discovered in *Drosophila* in a screen for dominant suppressors of *roughex* mutants (Dong et al. 1997). *rca1* mutants, like *cyclin A* mutants, arrest in G2 of embryonic cell cycle 16, and ectopic expression of *rca1* drives cells into S-phase and causes an increase in Cyclin A protein levels. A subsequent study demonstrated that Rca1 acts to inhibit APC/C^{Fzr/Cdh1} in G2 (Grosskortenhaus and Sprenger 2002). Although Emi1, the vertebrate ortholog of Rca1, is thought to inhibit both Cdc20 and Cdh1, Rca1 only seems to affect Cdh1. Grosskortenhaus and Sprenger failed to detect an association between FZY/ Cdc20 and Rca1, and genetic studies confirmed that the effect of Rca1 on cyclin levels is specific to Fzr (Grosskortenhaus and Sprenger 2002).

In vertebrates, Emi1 is important for inhibiting APC/C^{Cdh1} at the G1 to S-phase transition (Figure 1). Emi1 binds to Cdh1 and inhibits APC/C^{Cdh1} in vitro (Reimann et al. 2001b, Miller et al. 2006). An in vivo role for Emi1 has been investigated in HeLa cells (Hsu et al. 2002). Similar to Cyclin A, Emi1 transcription is activated by E2F at the G1 to S-phase transition. Cells that are depleted of Emi1 by RNA interference fail to accumulate Cyclin A and do not enter S-phase. Overexpression of Cdh1 causes a G1 arrest, and this arrest can be overcome by overexpression of Emi1, suggesting that Emi1 regulates S-phase entry via inhibition of APC/C^{Cdh1} (Hsu et al. 2002). It is not clear whether Rca1 also regulates S-phase entry in *Drosophila* embryos in addition to its role in G2 or whether Emi1 acts to inhibit Cdh1 in G2 in vertebrate cells.

RASSF1A

RASSF1A is a tumor suppressor gene that is silenced by promoter methylation in lung cancer patients. Overexpression of RASSF1A induces mitotic arrest and the accumulation of

Cyclins A and B in HeLa cells, whereas depletion of RASSF1A by RNA interference accelerates cyclin degradation and mitotic progression (Song et al. 2004). RASSF1A was once thought to be an inhibitor of APC/C^{Cdc20}, but a recent report suggests that it restricts APC/C^{Cdh1} activity at the G1-S transition through regulation of Emi1 levels via SCF^{βTRCP} (Whitehurst et al. 2008).

Rae1-Nup98

Inhibitory phosphorylation of Cdh1 was thought to fully inactivate APC/C^{Cdh1} during mitosis, but an investigation of the roles of Rae1 and Nup98 revealed an additional layer of regulation of APC/C^{Cdh1} in mitosis. Rae1 is an mRNA export factor that acts by anchoring Nup98, a nucleoporin, to the nuclear pore complex (Pritchard et al. 1999). Splenocytes from Rae1^{+/-}Nup98^{+/-} mice exhibit premature separation of sister chromatids, and Rae1^{+/-}Nup98^{+/-} murine embryonic fibroblasts contain decreased levels of securin, which suggested a role for Rae1 and Nup98 in mitotic control (Jeganathan et al. 2005). In mitotic HeLa cell extracts, Cdc27, Cdc16, and Cdh1 co-immunoprecipitate with Rae1 and Nup98, whereas Cdc20 does not associate. Furthermore, the association of Cdh1 with Rae1 and Nup98 is specific to mitosis. Rae1-Nup98 was found to inhibit ubiquitination of Securin but not Cyclin B by APC/C^{Cdh1}. Interestingly, Rae1-Nup98 dissociates from APC/C^{Cdh1} at the same time that BubR1 dissociates from APC/C^{Cdc0} upon release of the spindle checkpoint-mediated metaphase arrest (Jeganathan et al. 2005). These results suggest that, contrary to what was previously thought, APC/C^{Cdh1} may indeed play a role in targeting securin for degradation upon anaphase onset. The mechanism of Rae1-Nup98-mediated inhibition of APC/C^{Cdh1} remains to be elucidated as well as an understanding of how Rae1-Nup98 is targeted to the APC/C and triggered to dissociate from the APC/C.

Finally, in addition to the Cdc20 and Cdh1 inhibitors discussed above, two transcription factors, CBP and p300, are thought to be positive regulators of the APC/C in mitosis (Turnell et al. 2005). In mammalian cells CBP and p300 are associated with an active APC/C complex in vivo, and they co-immunoprecipitate with core APC/C subunits as well as both Cdc20 and Cdh1. Knockdown of CBP by RNA interference leads to an increase of Cyclin B and Plk1 levels and an accumulation of cells in mitosis. APC/C precipitated from these CBP-depleted cells has reduced ubiquitination activity (Turnell et al. 2005). The mechanism by which CBP-p300 positively regulates mitotic APC/C is not yet understood.

It is becoming increasingly clear that regulation of APC/C activators in mitosis as well as G1, S, and G2 occurs in a complex network and at multiple levels: transcriptional control, protein stability, phosphorylation, and direct binding of inhibitors.

ROLE OF THE APC/C IN FEMALE MEIOSIS

Meiosis is a modified cell division in which one parent cell generates four haploid cells, in contrast to the two identical diploid cells generated in a canonical mitotic cell division. Understanding the function and regulation of the APC/C in meiosis presents an interesting and complex problem given the differences of both chromosome segregation dynamics and developmental context in meiosis compared to mitosis.

A reductional division in meiosis I and an equational division in meiosis II without an intervening S phase generate haploid gametes in a meiotic cell division. Control of the meiotic divisions is coordinated by both general cell-cycle regulators as well as meiosis-specific proteins (Reviewed in Marston and Amon 2004). Meiosis I is unique because homologous chromosome pairs, as opposed to sister chromatids, must be segregated from each other. Homologous chromosomes are held together by chiasmata and arm cohesion. Segregation is achieved by loss of cohesion along chromosome arms but not in the centromeric regions. Subsequently, in meiosis II, sister chromatids are segregated through loss of cohesion at the centromeres in a process very similar to that in mitosis. Regulation of APC/C activity is likely to be important in this process as separase must be activated twice, through APC/C-mediated degradation of securin, in one full meiotic cell division.

Furthermore, the absence of an S-phase in between meiosis I and meiosis II also likely requires specialized regulation of the APC/C. In *Xenopus* oocytes it has been shown that mitotic Cdk activity is not abolished, but rather maintained at a low level in between the two meiotic divisions (Figure 3) (Furuno et al. 1994, Iwabuchi et al. 2000). Mitotic Cdk activity must be kept at an intermediate level to satisfy the unique requirements of meiosis: Cdk activity must be low enough to allow for disassembly of the meiosis I spindle, but high enough to repress initiation of DNA replication. Presumably, APC/C-mediated degradation of mitotic and meiotic cyclins must be regulated to contribute to this modulation of Cdk activity.

Regulation of APC/C activity in meiosis is particularly crucial during oogenesis of multicellular organisms. In most animals meiosis is arrested twice to coordinate development of the oocyte with the events of meiosis (Figure 3) (Reviewed in Kishimoto 2003, Tunquist and Maller 2003). During both meiotic arrests, APC/C activity must be suppressed. In prophase I, unscheduled activity of the APC/C could prevent proper maintenance of chromosome cohesion. In the secondary metaphase arrests, inhibition of the APC/C is crucial for preventing premature anaphase onset. Thus, understanding the function and regulation of the APC/C during meiosis is critical for understanding control of chromosome segregation and cell cycle progression and coordination of this control with developmental signals during meiosis.

Requirement for APC/C in Meiosis

A requirement for APC/C activity during the meiotic divisions has been shown in all organisms tested with the exception of *Xenopus* oocytes. In yeast, Cdc20 is required for the degradation of Pds1/Securin and resulting activation of separase in both meiotic divisions (Salah and Nasmyth 2000). Separase activity is required for the degradation of Rec8, the meiosis-specific cohesin subunit, along chromosome arms to allow for homolog disjunction in meiosis I (Buonomo et al. 2000, Kitajima et al. 2003).

It has been more difficult to demonstrate a requirement for APC/C in meiosis in multicellular organisms. In *C. elegans*, mutations in or RNA interference against several subunits of the APC/C cause a meiotic metaphase I arrest (Furuta et al. 2000, Golden et al. 2000, Davis et al. 2002). Female-sterile mutations in *fzy* cause both meiosis I and meiosis II arrests in *Drosophila* eggs (Swan and Schupbach 2007). Finally, several studies in mouse

oocytes have demonstrated a requirement for APC/C-mediated degradation of Securin and activation of separase for Rec8 removal from chromosome arms and homolog disjunction in meiosis I (Herbert et al. 2003, Terret et al. 2003, Kudo et al. 2006).

Two studies in *Xenopus* oocytes have suggested that APC/C is not required for meiosis I in this organism. Microinjection of oocytes with antibodies against Fzy or Cdc27 does not disrupt progression through meiosis I but only causes an arrest in meiosis II (Peter et al. 2001). Injection of antisense oligonucleotides against Fzy also does not block progression through meiosis I (Taieb et al. 2001). A more recent study in *Xenopus* demonstrates that Securin and Cyclin B are degraded at anaphase I, reaccumulate before metaphase II, and are degraded again at anaphase II (Fan et al. 2006). These data are suggestive of an active APC/C in both meiosis I and meiosis II, although they do not demonstrate a requirement for this activity for homolog disjunction and progression through meiosis I. Additionally, misexpression of Emi2, an APC/C inhibitor that is active later in meiosis, induces a metaphase I arrest in *Xenopus* oocytes, again suggesting that APC/C is required for the metaphase I to anaphase I transition (Ohe et al. 2007, Tung et al. 2007). Given the conservation of APC/C function in meiosis in other eukaryotes, it is possible that the antibody blocking and antisense oligonucleotide experiments failed to eliminate APC/C activity.

Meiosis-specific APC/C activators

An intriguing development in our knowledge of meiotic cell cycle control has been the identification of meiosis-specific APC/C activators in yeast and flies. The existence of these activators suggests a unique role and set of substrates for the APC/C in meiosis that are outside of the functions of Cdc20 or Cdh1.

In yeast, meiosis-specific activators are expressed exclusively during meiosis and are required for proper spore formation (Cooper et al. 2000, Asakawa et al. 2001, Blanco et al. 2001). Aml1 in *S. cerevisiae* associates with the core APC/C throughout meiosis, becomes active after meiosis I, and is required for Clb1 and Pds1 degradation. Furthermore, it can drive the ubiquitination of Pds1 in vitro (Cooper et al. 2000, Oelschlaegel et al. 2005, Penkner et al. 2005). In *Saccharomyces pombe* mfr1/fzr1 also associates with the core APC/C during meiosis and is required for the degradation of M-phase cyclin Cdc13. Degradation of Cdc13 in meiosis II is required for proper spore formation at the end of meiosis (Blanco et al. 2001).

In *Drosophila*, *cortex* (*cort*) encodes a distant member of the Cdc20/FZY protein family that is transcribed only during oogenesis, and null mutants are viable but female sterile, indicative of a role solely in meiosis (Chu et al. 2001). *cort* mutant females lay eggs that never complete meiosis and arrest terminally in metaphase II (Lieberfarb et al. 1996, Page and Orr-Weaver 1996). *cort* seems to be required as soon as the metaphase I to anaphase I transition occurs, as meiosis II spindles often exhibit unequal numbers of chromosomes indicating aberrant chromosome segregation in meiosis I (Page and Orr-Weaver 1996). Consistent with *cort* functioning as an APC/C activator, mitotic cyclin levels are elevated in *cort* eggs, and misexpression of *cort* triggers degradation of these cyclins in wing imaginal discs (Swan and Schupbach 2007, Pesin and Orr-Weaver 2007).

The *Drosophila* genome also contains a fourth Cdc20-related gene, *fizzy-related 2* (*fzr2*), that is expressed exclusively in male meiosis. When misexpressed, *fzr2* can rescue *fzr* function by triggering degradation of Cyclin B, which suggests a true function for *fzr2* as an APC/C activator (Jacobs et al. 2002). The use of a female and a male meiosis-specific APC/C activator in *Drosophila* provides an interesting system in which to delineate the meiosis-specific roles of APC/C in the two different developmental contexts of oogenesis and spermatogenesis.

Meiosis-specific activators are not the only APC/C activators present during meiosis. In *S. cerevisiae* both Cdc20 and Ama1 are thought to contribute to APC/C function in meiosis. Ama1 protein is present beginning in pre-meiotic S-phase, although it does not become essential until late in meiosis for spore formation (Cooper et al. 2000, Oelschlaegel et al. 2005, Penkner et al. 2005). This disparity in timing of Ama1 presence and function is explained by the action of an APC/C^{Ama1} inhibitor that will be discussed below. Cdc20, in contrast, is required in meiosis I, as Cdc20 meiotic mutants arrest in metaphase I with high levels of Pds1 (Salah and Nasmyth 2000). However, both Ama1 and Cdc20 are likely to be involved in both meiotic divisions. Ama1 mutants do not arrest in meiosis I but do display a delay in spindle elongation and an increase in Pds1 and Clb5 protein levels (Oelschlaegel et al. 2005). Additionally, Cdc20 levels peak in both meiosis I and meiosis II, suggesting that it is important for both divisions (Salah and Nasmyth 2000).

Similarly, in *Drosophila*, both *fzy* and *cort* are required for female meiotic divisions. Swan and Schupbach used double mutant genetic analysis to demonstrate that *fzy* and *cort* have redundant roles in meiosis I but non-redundant roles in meiosis II (Swan and Schupbach 2007). In *fzy* single mutants, a small percentage of eggs arrest with one spindle in meiosis I, but the majority of eggs arrest with 2 spindles in anaphase II. In *cort* single mutants, a similar percentage of eggs arrest in meiosis I, but the rest arrest with 2 spindles in metaphase II. Double mutants display an increased number of eggs arresting in meiosis I, consistent with a redundant role for these two genes in the first division. However, the remaining double mutant eggs arrest in metaphase II, suggesting that *cort* is epistatic to *fzy* in meiosis II. The effects of these mutations on localized Cyclin B degradation also support the idea that *cort* and *fzy* have non-redundant and different temporal roles in meiosis II. *cort* is required for the degradation of Cyclin B specifically at the spindle midzone, while *fzy* seems to target Cyclin B after *cort* and along the entire spindle in meiosis II.

In addition to *cort* and *fzy* playing some non-redundant roles in meiosis, their protein expression patterns during meiosis are quite different. FZY is expressed at a constant level throughout oogenesis and embryogenesis, while CORT is specifically expressed in a narrow window during the female meiotic divisions (Pesin and Orr-Weaver 2007). The unique expression pattern of CORT is caused by both post-transcriptional and post-translational control. Polyadenylation of *cort* mRNA at oocyte maturation correlates with appearance of CORT protein as the oocyte enters the meiotic divisions (Pesin and Orr-Weaver 2007). At the completion of meiosis, APC/C-dependent degradation of CORT ensures that the protein is no longer present in the subsequent rapid embryonic mitotic divisions. In this situation, the organism is utilizing existing developmental control mechanisms to restrict activity of a meiosis-specific form of the APC/C.

The role of Cdh1 in meiosis is not entirely clear. It appears to be important for prophase I and prometaphase I. In *S. cerevisiae*, *cdh1* mutant cells show incomplete synapsis of bivalents in prophase I (Penkner et al. 2005). This result could be due to a role for Cdh1 in meiosis, or Cdh1 may be required in the previous mitotic cell cycle and its failure affect the subsequent meiotic prophase. In mouse oocytes, APC/C^{Cdh1} mediated degradation of substrates appears to be required for maintaining prophase I arrest and preventing entry into meiotic divisions (Reis et al. 2006b, Marangos et al. 2007). In contrast, injection of Cdh1 antisense oligonucleotides into *Xenopus* oocytes inhibits meiotic entry (Papin et al. 2004).

A role for Cdh1 after germinal vesicle breakdown has been shown in mouse oocytes (Reis et al. 2007). Injection of oocytes with *cdh1* antisense morpholino accelerates progression through meiosis I resulting in premature anaphase and non-disjunction of homologs. These defects are specific to inhibition of *cdh1* after germinal vesicle breakdown. Furthermore, Reis et al. demonstrated that Cdc20 is degraded in these oocytes shortly after GVBD in a Cdh1-dependent manner. Cdc20 must be resynthesized for proper degradation of Cyclin B1 and Securin in metaphase I and polar body extrusion. These results suggest a new paradigm for use of APC/C activators during meiosis. In this new model, APC/C^{Cdh1} prolongs prometaphase I by causing degradation of Cdc20. This delay could serve to allow more time for homologs to properly congress at the metaphase I plate.

REGULATION OF THE APC/C IN MEIOSIS

Proper regulation of the APC/C in meiosis is crucial to prevent unscheduled APC/C activity and premature activation of Separase through Securin degradation. At both metaphase I and metaphase II, premature chromosome or sister chromatid segregation could lead to aneuploid gametes and offspring, which is a leading cause of spontaneous abortion and mental retardation (Hassold and Hunt 2001). Additionally, a unique feature of female meiosis is a prolonged prophase I arrest, up to several decades in length in humans, in which sister chromatid cohesion must be maintained for proper chromosome segregation in meiosis I.

Mnd2

As mentioned above, Aml1, the meiosis-specific APC/C activator in *S. cerevisiae*, is present beginning in premeiotic S-phase, yet is not required until late meiosis, suggesting that Aml1 may be inhibited in early meiosis. Two studies in *S. cerevisiae* revealed a crucial inhibitor of APC/C^{Aml1} in prophase I (Figure 4). Mnd2 was originally identified as being associated with the APC/C core (Hall et al. 2003, Passmore et al. 2003). Subsequently, it was found to inhibit APC/C^{Aml1} through genetic suppression and in vitro ubiquitination assays (Oelschlaegel et al. 2005, Penkner et al. 2005). The association of Mnd2 with APC/C from meiotic cells can inhibit the ubiquitination activity of APC/C^{Aml1} but not APC/C^{Cdc20} or APC/C^{Cdh1} (Oelschlaegel et al. 2005).

The mechanism of Mnd2 inhibition of APC/C^{Aml1} is unclear. In vivo, the absence of Mnd2 causes an increased association of Aml1 with the APC/C core, but in vitro, Aml1 binds with equal frequency to APC/C that is associated with Mnd2 or not (Oelschlaegel et al.

2005). The inhibitory actions of Mnd2 may occur after both Mnd2 and Ama1 are bound to APC/C.

The mutant phenotype of *mnd2* reveals the disastrous consequences of unrestrained APC/C^{Ama1} activity in meiotic prophase. *mnd2* cells fail to accumulate Pds1 in S and prophase and arrest in a prophase-like state (Oelschlaegel et al. 2005, Penkner et al. 2005). Furthermore, these cells show premature sister chromatid separation as well as a failure to fully synapse homologs. Both groups demonstrated that these defects are the result of premature activation of separase and cleavage of Rec8 in prophase and are dependent on APC/C^{Ama1}-mediated degradation of Pds1.

Mnd2 inhibition of APC/C^{Ama1} must be relieved in late meiosis when Ama1 becomes required for spore formation. Mnd2 protein disappears from meiotic cells during anaphase II, and this is thought to be one way in which APC/C^{Ama1} becomes active at this time (Oelschlaegel et al. 2005, Penkner et al. 2005). Additionally, Ama1 may be inhibited by the activity of Cdk1 kinases. Inhibition of Cdk1 in *cdc20* mutant metaphase I arrested cells triggers spindle disassembly and Ama1-dependent degradation of Pds1 (Oelschlaegel et al. 2005). This result suggests that APC/C^{Cdc20}-mediated degradation of cyclins in meiosis I is required for the activation of APC/C^{Ama1} in meiosis II.

Emi1

Based on Emi1's role in APC/C inhibition in mitosis, it initially seemed to be a likely candidate for an APC/C regulator in meiosis. However, after numerous studies the role of Emi1 in meiosis is controversial. It was long thought to be a key component of cytostatic factor arrest (CSF) in metaphase II (discussed below), but this role for Emi1 has recently been called into question. Investigations of a role for Emi1 in earlier stages of meiosis suggest that it may function at an earlier time point.

Tung and Jackson found evidence suggestive of Emi1 acting in prophase I and during the meiosis I – meiosis II transition in *Xenopus* oocytes. Injection of an Emi-neutralizing antibody in prophase I prior to maturation inhibited germinal vesicle breakdown (GVBD) upon progesterone stimulation and entry into the meiotic divisions (Tung and Jackson 2005). Injection of the same antibody at the onset of GVBD induced chromosome decondensation in between meiosis I and meiosis II, DNA replication, and failure to reaccumulate Cyclin B and Cdc2 activity before meiosis II. These defects were rescued by co-injection of Cdc20 oligonucleotides, suggesting they were the result of unrestrained APC/C^{Cdc20} activity. The confounding factor in these experiments pointed out by the authors is that their anti-Emi1 antibody cross reacts with Emi2/Erp1 (Emi-related protein 1), and thus, these results may be due to inhibition of Emi2 instead (discussed below). Exogenous Emi1 has been shown to be rapidly degraded during oocyte maturation in *Xenopus* oocytes (Ohsumi et al. 2004). Therefore, it may be more likely that Emi1 has a role in prophase and not at the meiosis I to meiosis II transition in *Xenopus* (Figure 4).

In mouse a similar story is unfolding. Emi1 protein is present in mouse oocytes but is degraded at GVBD in a SCF^{β-TrCP} dependent manner, mirroring the timing of Emi1 destruction in mitotic cells in late prophase (Marangos et al. 2007). As in *Xenopus*,

inhibition of Emi1 function in prophase I delays entry into the first meiotic division by preventing the accumulation of Cyclin B necessary for MPF activation and progression through meiosis I. These effects of inhibiting Emi1 function in mouse oocytes are mediated through APC/C^{Cdh1} activity (Marangos et al. 2007). Thus, a conserved role exists for Emi1 during prophase I arrest in vertebrate oocytes.

Mes1

The transition between meiosis I and meiosis II requires a balance between lowering Cyclin B-Cdk activity sufficiently to exit meiosis I but maintaining levels high enough to suppress DNA replication and promote entry into meiosis II. In *S. pombe*, Mes1 may be the solution to this problem (Figure 4). *mes1* is transcribed in a narrow window between late meiosis I and late meiosis II (Mata et al. 2002). *mes1* mutants arrest before meiosis II and completely degrade M-phase cyclin Cdc13 prematurely in anaphase I, instead of in anaphase II (Shimoda et al. 1985, Izawa et al. 2005). The *mes1* mutant phenotype is suppressed by mutation of *slp1*, the Cdc20 homolog in *S. pombe*, and addition of Mes1 to a *Xenopus* egg extract inhibits APC/C activity, both consistent with a role for Mes1 as an APC/C inhibitor (Izawa et al. 2005). Mes1 may inhibit APC/C^{Mfr1} in addition to APC/C^{Slp1}, but this remains to be shown conclusively.

Mes1 appears to be a true substrate of the APC/C. It contains a KEN box and a D-box, and these motifs are required for its degradation by APC/C in an in vitro destruction assay performed in *Xenopus* egg extracts (Kimata et al. 2008). Furthermore, Mes1 is ubiquitinated, unlike other APC/C inhibitors, and this ubiquitination is required for partial degradation of Cyclin B in meiosis I and onset of anaphase I (Kimata et al. 2008). Non-ubiquitable Mes1 cannot dissociate from a ternary APC/C-Cdc20-Mes1 complex, and this binding probably prevents APC/C from targeting other substrates. This study leads to several interesting questions about the mechanism of Mes1 inhibition of the APC/C. Mes1 behaves as a normal APC/C substrate, yet how is it able to compete for binding to the APC/C much more efficiently than other substrates, and how does it stay bound to the complex? Further investigation should provide more insight into the regulation of Mes1 ubiquitination by the APC/C.

Emi2/Erp1

Just as Mes1 is required in *S. pombe* to inhibit APC/C activity in between meiosis I and meiosis II, Emi2/Erp1, a homolog of Emi1, is required in this role in *Xenopus* and mouse oocytes (Figure 4). A role for Emi2 in *Xenopus* at this time was first suggested by Tung and Jackson when they used a neutralizing Emi1 antibody that cross-reacted with Emi2 (discussed above, Tung and Jackson 2005). Two groups later found that Emi2 is not expressed until after GVBD, and its expression is coincident with polyadenylation of *emi2* mRNA (Ohe et al. 2007, Tung et al. 2007). Inhibition of Emi2 by injection of morpholinos or antisense oligonucleotides reduces Cyclin B2 reaccumulation after meiosis I, prevents entry into meiosis II, and, in the case of morpholino injection, induces DNA replication (Ohe et al. 2007, Tung et al. 2007). Injection of Emi2 morpholinos into mouse oocytes generates a very similar phenotype, suggesting that Emi2/Erp1 inhibits APC/C-mediated

degradation of Cyclin B after meiosis I to prevent DNA replication and allow entry into meiosis II (Madgwick et al. 2006).

A critical role for Emi2 in meiosis is inhibition of APC/C during cytostatic factor (CSF) arrest (Figure 4). Vertebrate eggs are arrested in metaphase II while they await fertilization. This arrest functions to prevent premature egg activation or completion of meiosis before fertilization occurs. Many years of research determined that establishment of CSF arrest involves the Mos/MAPK/p90^{RSK} signaling pathway and Cyclin E/ Cdk2 activity through inhibition of APC/C^{Cdc20} (for review, see Tunkquist and Maller 2003). Release of CSF arrest is triggered by a transient increase in free cytosolic Ca²⁺ levels induced by fertilization. An additional component contributing to CSF arrest was thought to be Emi1, although this idea has recently been questioned, as some groups report that Emi1 is not present and unstable in mature eggs, whereas others show that it is stabilized in mature eggs at low levels (Ohsumi et al. 2004, Tung et al. 2005, Shoji et al. 2006). Cross-reactivity of antibodies to both proteins has been the source of some of this confusion.

Nevertheless, Emi2/Erp1 has emerged as a crucial mediator of CSF arrest. Emi2 is present in *Xenopus* CSF extracts and degraded upon addition of calcium, consistent with a role as a component of CSF (Schmidt et al. 2005, Tung et al. 2005). Furthermore, depletion of Emi2 causes premature release from CSF arrest, independent of calcium addition (Schmidt et al. 2005). Conversely, addition of exogenous or non-degradable Emi2 prevents calcium-induced CSF release. In mouse oocytes, Emi2 appears to play the same role in CSF arrest. These effects are likely to occur through Emi2 inhibition of APC/C^{Cdc20}, as Emi2 can inhibit ubiquitination of substrates by APC/C^{Cdc20} in vitro (Schmidt et al. 2005, Tung et al. 2005). In addition, mouse Emi2 was shown to bind to Cdc20 in vitro (Shoji et al. 2006).

Degradation of Emi2 upon Ca²⁺-induced CSF release appears to be the critical event that relieves inhibition of APC/C^{Cdc20} and allows passage from metaphase II to anaphase II. Ca²⁺ plays a critical role in controlling Emi2 degradation by indirectly affecting phosphorylation of Emi2 by Plx2 through its effect on calmodulin kinase II (CaMKII) (Lorca et al. 1993, Hansen et al. 2006, Schmidt et al. 2005, Tung et al. 2005). Plx1 kinase phosphorylation of Emi2 promotes its degradation by SCF^{βTrCP}. A relationship between CaMKII and Plx1 was shown by the observation that CSF release triggered by a constitutively-active CaMKII (CamCat) requires Plx1, and Plx1-induced CSF release requires active CaMKII (Liu and Maller 2005). The polo-box domain (PBD) of Plx1 is thought to bind a phosphopeptide motif in target proteins generated by phosphorylation of the target by a “priming kinase.” Once Plx1 docks onto the target through its PBD, its phosphorylation of the target protein is enhanced. Phosphorylation of Emi2 by CaMKII enhances Plx1 binding to and phosphorylation of Emi2 in vitro, and degradation of Emi2 in anaphase extracts is dependent on phosphorylation by CaMKII, strongly suggesting that CaMKII is the priming kinase for Plx1 upon CSF release (Liu and Maller 2005, Rauh et al. 2005, Hansen et al. 2006). These studies explain the link between calcium elevation and release of APC/C^{Cdc20} inhibition at fertilization.

Finally, recent studies in *Xenopus* have found additional sites of phosphorylation on Emi2, some of which directly link Emi2 to the Mos/MAPK/p90^{RSK} pathway in CSF arrest (Figure

2). The ability of Emi2 to inhibit APC/C after meiosis I and in CSF arrest is dependent on the MAPK pathway (Inoue et al. 2007). p90^{RSK} phosphorylates Emi2 in vitro, and these sites of phosphorylation are required in vivo for Emi2 stability during oocyte maturation and its CSF activity (Inoue et al. 2007, Nishiyama et al. 2007a). This work strongly suggests that, at least in amphibian oocytes, Emi2 is the effector protein of Mos/MAPK/p90^{RSK} signaling in CSF arrest.

Nishiyama et al. shed light on the mechanism of Emi2 inhibition of APC/C. Phosphorylation of Emi2 by p90^{RSK} enhances association of Emi2 with the APC/C, consistent with its role in activating Emi2 inhibitory activity (Nishiyama et al. 2007a). Like Emi1, Emi2 contains a D-box that also enhances association of Emi2 with APC/C. This association is inhibited in vitro by D-box peptides, suggesting that Emi2 blocks substrate access to the APC/C. In addition, phosphorylation of Emi2 by Cyclin B/Cdc2 (cdk1) at sites distinct from those targeted by CaMKII and p90^{RSK} may disrupt the interaction between Emi2 and APC/C (Figure 2) (Wu et al. 2007, Hansen et al. 2007). This phosphorylation is antagonized by protein phosphatase 2A and may represent a feedback loop that serves to maintain proper levels of Cyclin B and Cdc2/cdk1 kinase activity during CSF arrest.

An additional Ca²⁺-induced pathway was found to be involved in exit from CSF arrest in *Xenopus* egg extracts. Upon its activation at fertilization, calcineurin, a Ca²⁺-dependent phosphatase, triggers several downstream events, including the dephosphorylation of Apc3, a core subunit of the APC/C, and of Cdc20/Fzy (Mochida and Hunt 2007). Addition of a calcineurin inhibitor prevents this dephosphorylation, delays degradation of CyclinB2, and prevents exit from meiosis II after the addition of Ca²⁺ (Mochida and Hunt 2007, Nishiyama et al. 2007b). Mochida and Hunt report that co-immunoprecipitation experiments provide some evidence of a physical association between Fzy/Cdc20 and calcineurin, and incubation of purified APC/C from CSF egg extracts with calcineurin causes a mobility shift of Apc3 and Fzy/Cdc20 protein. It has been shown that phosphorylation of Cdc20/Fzy inhibits its ability to activate APC/C during spindle checkpoint arrest (Yudkovsky et al. 2000, Chung and Chen 2003, Tang et al. 2004). Thus, dephosphorylation of Cdc20/Fzy by calcineurin upon fertilization may play an important role in calcineurin's contribution to release of the metaphase II arrest in CSF eggs (Figure 4). Consistent with the *Xenopus* results, calcineurin signaling also has been shown to be involved in meiosis in *Drosophila* because mutations in *sarah*, a gene encoding an inhibitor of calcineurin, disrupt the meiotic divisions in females (Horner et al. 2006).

Spindle Assembly Checkpoint

In a normal mitosis in the absence of spindle damage, the spindle checkpoint genes are dispensable, with the exception of *mps1*, in *S. cerevisiae*, but are essential in metazoans (Hoyt et al. 1991, Li et al. 1991, Kitagawa et al. 1999, Dobles et al. 2000, Kalitsis et al. 2000). However, a recent study suggests that *mad2* is not an essential gene in *Drosophila* (Buffin et al. 2007). In meiosis, these genes appear to be required not only upon spindle damage but also in a normal undisturbed meiotic division (Figure 4). In *S. cerevisiae*, mutations in *mad1* or *mad2* cause increased nondisjunction of homologous chromosomes in meiosis I (Shonn et al. 2000). Levels of nondisjunction are restored if anaphase is artificially

delayed, suggesting that Mad1 and Mad2 are important for inducing a metaphase I delay in a normal meiosis. Loss of recombination in a *spo11* mutant, which causes a lack of tension on kinetochores, induces a Mad2-dependent suppression of APC/C activity, suggesting that the checkpoint also responds to spindle defects in meiosis I. Furthermore, Mad2 may have an additional role in promoting the biorientation of homologs perhaps through a mechanism other than inhibition of APC/C (Shonn et al. 2003). The requirement for spindle checkpoint function in a normal meiosis I division reflects the increased complexity of bi-orienting homologous chromosome pairs on the spindle compared with bi-orienting sister chromatid kinetochores in mitosis.

Higher organisms also display a requirement for spindle checkpoint genes in an undisturbed meiosis I. Mutations in spindle checkpoint genes in *C. elegans* suppress a metaphase I arrest in a *cdc23* mutant, indicating a likely role for SAC in inhibiting APC/C during meiosis I (Stein et al. 2007). In mouse oocytes from females heterozygous for Mad2, meiosis I is shortened and anaphase I onset accelerated (Niault et al. 2007). These oocytes display a large increase in aneuploidy in metaphase II, which is likely the result of the premature anaphase I onset. Similar to in *S. cerevisiae*, meiosis I must be of a sufficient length to allow enough time for homologous chromosomes to properly orient on the spindle and form stable connections with microtubules. A spindle checkpoint in mouse oocytes also responds to spindle damage (Wassman et al. 2003, Niault et al. 2007). A wild-type oocyte responds to nocodazole by arresting in metaphase I and is subsequently able to properly segregate chromosomes in anaphase I. In the oocytes from Mad2 heterozygotes, however, oocytes missegregate chromosomes at a high rate after a nocodazole-induced arrest (Niault et al. 2007). Furthermore, *bubR1* mutant female mice contain oocytes with chromosome segregation defects (Baker et al. 2004). Finally, microinjection of Bub1 antibodies into mouse oocytes causes chromosome misalignment on the meiosis I spindle that is not corrected by delaying anaphase onset, suggesting that, like Mad2 in yeast, Bub1 has a specific role in chromosome alignment during metaphase I (Yin et al. 2006).

Control of chromosome segregation in meiosis I in *Drosophila* females is an interesting problem because in *Drosophila* the secondary meiotic arrest occurs in metaphase I, unlike CSF arrest in metaphase II. Oocytes mutant for *mps1* enter anaphase I prematurely, suggesting a role for the spindle checkpoint in mediating this arrest (Gilliland et al. 2007). Reduction of *mps1* function in these oocytes causes non-disjunction of both exchange and non-exchange chromosomes, which is likely due, in part, to defects in bi-orientation of homologous chromosomes in meiosis I (Gilliland et al. 2005, Gilliland et al. 2007).

Additionally, in female meiosis of *bubR1* mutants in *Drosophila*, non-disjunction of sister chromatids is elevated (Malmanche et al. 2007). Cohesin is lost from chromosomes in prophase I of these oocytes, and it is not clear whether this effect occurs through loss of an inhibitory effect on APC/C activity in the *bubR1* mutant. In *S. cerevisiae* Mad3 mediates a prophase I delay that becomes essential for chromosome segregation when chromosomes do not recombine (Cheslock et al. 2005). It is also unclear whether this defect is due to uninhibited APC/C.

A role for the spindle checkpoint in CSF arrest in meiosis II has not been shown conclusively. The spindle checkpoint was hypothesized to be a component of CSF arrest because Bub1 is phosphorylated by p90^{RSK} during oocyte maturation in *Xenopus* oocytes, placing it downstream of the Mos/ MAPK pathway that is required for establishment of CSF arrest (Schwab et al. 2001). Immunodepletion of Bub1, Mad1, or Mad2 all prevent establishment of CSF arrest in *Xenopus* oocytes, and this effect is thought to occur downstream of Mos (Tunquist et al. 2002, Tunquist et al. 2003). In contrast, inhibition of Mps1, another kinase required for spindle checkpoint-mediated arrest in mitosis, has no effect on CSF arrest (Grimison et al. 2006). Furthermore, although Bub1 and Mad2 are required for establishment of CSF arrest, they are not required for maintenance of the arrest in CSF extracts (Tunquist et al. 2002, Tunquist et al. 2003). In mouse oocytes, expression of dominant negative forms of Bub1, Mad2, or BubR1 do not affect CSF arrest, further bringing into question a role for the spindle checkpoint in inhibiting APC/C during CSF arrest (Tsurumi et al. 2004). CSF arrest is quite different from a mitotic spindle checkpoint-mediated arrest because kinetochores are already under tension and attached correctly to the spindle. If there is a role for spindle checkpoint proteins in establishment of CSF arrest, they may act while kinetochores achieve bipolar attachments, but not after, or they may have a different role or target during CSF establishment.

CONCLUSIONS

Regulation of Cdc20/Fzy and Cdh1/Fzr is crucial for proper activity of the APC/C during the mitotic cell cycle and during meiosis. The balance of APC/C^{Cdc20} and APC/C^{Cdh1} activity is achieved with multiple mechanisms: transcriptional control, targeted degradation, phosphorylation, and the direct binding of inhibitors. During meiosis in some organisms, special forms of the APC/C exist that are activated by meiosis-specific activators. An attractive hypothesis that remains to be tested is that these activators target the degradation of a unique, meiosis-specific set of APC/C substrates. Regulation of the APC/C during meiosis utilizes both mitotic APC/C regulators in addition to meiosis-specific regulators, demonstrating a need for increased control of APC/C activity during the more complex meiotic cell cycle.

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LITERATURE CITED

- Asakawa H, Kitamura K, Shimoda C. A novel Cdc20-related WD-repeat protein, Fzr1, is required for spore formation in *Schizosaccharomyces pombe*. *Mol Genet Genomics*. 2001; 265:424–435. [PubMed: 11405625]
- Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja S, et al. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat Genet*. 2004; 36:744–749. [PubMed: 15208629]
- Ban KH, Torres JZ, Miller JJ, Mikhailov A, Nachury MV, et al. The END network couples spindle pole assembly to inhibition of the anaphase-promoting complex/cyclosome in early mitosis. *Dev Cell*. 2007; 13:29–42. [PubMed: 17609108]

- Benmaamar R, Pagano M. Involvement of the SCF complex in the control of Cdh1 degradation in S-phase. *Cell Cycle*. 2005; 4:1230–1232. [PubMed: 16123585]
- Bernis C, Vigneron S, Burgess A, Labbe JC, Fesquet D, et al. Pin1 stabilizes Emi1 during G2 phase by preventing its association with SCF(betatrpc). *EMBO Rep*. 2007; 8:91–98. [PubMed: 17159919]
- Blanco MA, Pelloquin L, Moreno S. Fission yeast mfr1 activates APC and coordinates meiotic nuclear division with sporulation. *J Cell Sci*. 2001; 114:2135–2143. [PubMed: 11493649]
- Blanco MA, Sanchez-Diaz A, de Prada JM, Moreno S. APC(ste9/srw1) promotes degradation of mitotic cyclins in G(1) and is inhibited by cdc2 phosphorylation. *Embo J*. 2000; 19:3945–3955. [PubMed: 10921876]
- Buffin E, Emre D, Karess RE. Flies without a spindle checkpoint. *Nat Cell Biol*. 2007; 9:565–572. [PubMed: 17417628]
- Buonomo SB, Clyne RK, Fuchs J, Loidl J, Uhlmann F, Nasmyth K. Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. *Cell*. 2000; 103:387–398. [PubMed: 11081626]
- Camasses A, Bogdanova A, Shevchenko A, Zachariae W. The CCT chaperonin promotes activation of the anaphase-promoting complex through the generation of functional Cdc20. *Mol Cell*. 2003; 12:87–100. [PubMed: 12887895]
- Cheslock PS, Kemp BJ, Boumil RM, Dawson DS. The roles of MAD1, MAD2 and MAD3 in meiotic progression and the segregation of nonexchange chromosomes. *Nat Genet*. 2005; 37:756–760. [PubMed: 15951820]
- Chu T, Henrion G, Haegeli V, Strickland S. *Cortex* a Drosophila gene required to complete oocyte meiosis, is a member of the Cdc20/fizzy protein family. *Genesis*. 2001; 29:141–152. [PubMed: 11252055]
- Chung E, Chen RH. Phosphorylation of Cdc20 is required for its inhibition by the spindle checkpoint. *Nat Cell Biol*. 2003; 5:748–753. [PubMed: 12855955]
- Cooper KF, Mallory MJ, Egeland DB, Jarnik M, Strich R. Ama1p is a meiosis-specific regulator of the anaphase promoting complex/cyclosome in yeast. *Proc Natl Acad Sci U S A*. 2000; 97:14548–14553. [PubMed: 11114178]
- Davis ES, Wille L, Chestnut BA, Sadler PL, Shakes DC, Golden A. Multiple subunits of the *Caenorhabditis elegans* anaphase-promoting complex are required for chromosome segregation during meiosis I. *Genetics*. 2002; 160:805–813. [PubMed: 11861581]
- Dawson IA, Roth S, Artavanis-Tsakonas S. The Drosophila cell cycle gene *fizzy* is required for normal degradation of cyclins A and B during mitosis and has homology to the CDC20 gene of *Saccharomyces cerevisiae*. *J Cell Biol*. 1995; 129:725–737. [PubMed: 7730407]
- Di Fiore B, Pines J. Emi1 is needed to couple DNA replication with mitosis but does not regulate activation of the mitotic APC/C. *J Cell Biol*. 2007; 177:425–437. [PubMed: 17485488]
- Dobles M, Liberal V, Scott ML, Benezra R, Sorger PK. Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2. *Cell*. 2000; 101:635–645. [PubMed: 10892650]
- Dong X, Zavitz KH, Thomas BJ, Lin M, Campbell S, Zipursky SL. Control of G1 in the developing Drosophila eye: *rcal* regulates Cyclin A. *Genes Dev*. 1997; 11:94–105. [PubMed: 9000053]
- Eldridge AG, Loktev AV, Hansen DV, Verschuren EW, Reimann JD, Jackson PK. The *evi5* oncogene regulates cyclin accumulation by stabilizing the anaphase-promoting complex inhibitor emi1. *Cell*. 2006; 124:367–380. [PubMed: 16439210]
- Fan HY, Sun QY, Zou H. Regulation of Separase in meiosis: Separase is activated at the metaphase I-II transition in *Xenopus* oocytes during meiosis. *Cell Cycle*. 2006; 5:198–204. [PubMed: 16357532]
- Fang G, Yu H, Kirschner MW. Direct binding of CDC20 protein family members activates the anaphase-promoting complex in mitosis and G1. *Mol Cell*. 1998; 2:163–171. [PubMed: 9734353]
- Furuno N, Nishizawa M, Okazaki K, Tanaka H, Iwashita J, et al. Suppression of DNA replication via Mos function during meiotic divisions in *Xenopus* oocytes. *Embo J*. 1994; 13:2399–2410. [PubMed: 8194530]
- Furuta T, Tuck S, Kirchner J, Koch B, Auty R, et al. EMB-30: an APC4 homologue required for metaphase-to-anaphase transitions during meiosis and mitosis in *Caenorhabditis elegans*. *Mol Biol Cell*. 2000; 11:1401–1419. [PubMed: 10749938]

- Gilliland WD, Hughes SE, Cotitta JL, Takeo S, Xiang Y, Hawley RS. The Multiple Roles of Mps1 in *Drosophila* Female Meiosis. *PLoS Genet.* 2007; 3:e113. [PubMed: 17630834]
- Gilliland WD, Wayson SM, Hawley RS. The meiotic defects of mutants in the *Drosophila* mps1 gene reveal a critical role of Mps1 in the segregation of achiasmate homologs. *Curr Biol.* 2005; 15:672–677. [PubMed: 15823541]
- Golden A, Sadler PL, Wallenfang MR, Schumacher JM, Hamill DR, et al. Metaphase to anaphase (mat) transition-defective mutants in *Caenorhabditis elegans*. *J Cell Biol.* 2000; 151:1469–1482. [PubMed: 11134076]
- Grimison B, Liu J, Lewellyn AL, Maller JL. Metaphase arrest by cyclin E-Cdk2 requires the spindle-checkpoint kinase Mps1. *Curr Biol.* 2006; 16:1968–1973. [PubMed: 17027495]
- Grosskortenhaus R, Sprenger F. Rca1 inhibits APC-Cdh1(Fzr) and is required to prevent cyclin degradation in G2. *Dev Cell.* 2002; 2:29–40. [PubMed: 11782312]
- Guardavaccaro D, Kudo Y, Boulaire J, Barchi M, Busino L, et al. Control of meiotic and mitotic progression by the F box protein beta-Trcp1 in vivo. *Dev Cell.* 2003; 4:799–812. [PubMed: 12791266]
- Hall MC, Torres MP, Schroeder GK, Borchers CH. Mnd2 and Swm1 are core subunits of the *Saccharomyces cerevisiae* anaphase-promoting complex. *J Biol Chem.* 2003; 278:16698–16705. [PubMed: 12609981]
- Hansen DV, Loktev AV, Ban KH, Jackson PK. Plk1 regulates activation of the anaphase promoting complex by phosphorylating and triggering SCFbetaTrCP-dependent destruction of the APC Inhibitor Emi1. *Mol Biol Cell.* 2004; 15:5623–5634. [PubMed: 15469984]
- Hansen DV, Pomerening JR, Summers MK, Miller JJ, Ferrell JE Jr, Jackson PK. Emi2 at the crossroads: where CSF meets MPF. *Cell Cycle.* 2007; 6:732–738. [PubMed: 17361104]
- Hansen DV, Tung JJ, Jackson PK. CaMKII and polo-like kinase 1 sequentially phosphorylate the cytostatic factor Emi2/XErp1 to trigger its destruction and meiotic exit. *Proc Natl Acad Sci U S A.* 2006; 103:608–613. [PubMed: 16407128]
- Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet.* 2001; 2:280–291. [PubMed: 11283700]
- Herbert M, Levasseur M, Homer H, Yallop K, Murdoch A, McDougall A. Homologue disjunction in mouse oocytes requires proteolysis of securin and cyclin B1. *Nat Cell Biol.* 2003; 5:1023–1025. [PubMed: 14593421]
- Horner VL, Czank A, Jang JK, Singh N, Williams BC, et al. The *Drosophila* calpippresin *sarah* is required for several aspects of egg activation. *Curr Biol.* 2006; 16:1441–1446. [PubMed: 16860744]
- Hoyt MA, Totis L, Roberts BT. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell.* 1991; 66:507–517. [PubMed: 1651171]
- Hsu JY, Reimann JD, Sorensen CS, Lukas J, Jackson PK. E2F-dependent accumulation of hEmi1 regulates S phase entry by inhibiting APC(Cdh1). *Nat Cell Biol.* 2002; 4:358–366. [PubMed: 11988738]
- Huang JN, Park I, Ellingson E, Littlepage LE, Pellman D. Activity of the APC(Cdh1) form of the anaphase-promoting complex persists until S phase and prevents the premature expression of Cdc20p. *J Cell Biol.* 2001; 154:85–94. [PubMed: 11448992]
- Inoue D, Ohe M, Kanemori Y, Nobui T, Sagata N. A direct link of the Mos-MAPK pathway to Erp1/Emi2 in meiotic arrest of *Xenopus laevis* eggs. *Nature.* 2007; 446:1100–1104. [PubMed: 17410130]
- Iwabuchi M, Ohsumi K, Yamamoto TM, Sawada W, Kishimoto T. Residual Cdc2 activity remaining at meiosis I exit is essential for meiotic M-M transition in *Xenopus* oocyte extracts. *Embo J.* 2000; 19:4513–4523. [PubMed: 10970845]
- Izawa D, Goto M, Yamashita A, Yamano H, Yamamoto M. Fission yeast Mes1p ensures the onset of meiosis II by blocking degradation of cyclin Cdc13p. *Nature.* 2005; 434:529–533. [PubMed: 15791259]
- Jacobs H, Richter D, Venkatesh T, Lehner C. Completion of mitosis requires neither *fzr/rap* nor *fzr2* a male germline-specific *Drosophila* Cdh1 homolog. *Curr Biol.* 2002; 12:1435–1441. [PubMed: 12194827]

- Jaspersen SL, Charles JF, Morgan DO. Inhibitory phosphorylation of the APC regulator Hct1 is controlled by the kinase Cdc28 and the phosphatase Cdc14. *Curr Biol.* 1999; 9:227–236. [PubMed: 10074450]
- Jeganathan KB, Malureanu L, van Deursen JM. The Rael-Nup98 complex prevents aneuploidy by inhibiting securin degradation. *Nature.* 2005; 438:1036–1039. [PubMed: 16355229]
- Kalitsis P, Earle E, Fowler KJ, Choo KH. Bub3 gene disruption in mice reveals essential mitotic spindle checkpoint function during early embryogenesis. *Genes Dev.* 2000; 14:2277–2282. [PubMed: 10995385]
- Keck JM, Summers MK, Tedesco D, Ekholm-Reed S, Chuang LC, et al. Cyclin E overexpression impairs progression through mitosis by inhibiting APCCdh1. *J Cell Biol.* 2007; 178:371–385. [PubMed: 17664332]
- Kidokoro T, Tanikawa C, Furukawa Y, Katagiri T, Nakamura Y, Matsuda K. CDC20, a potential cancer therapeutic target, is negatively regulated by p53. *Oncogene.* 2008; 27:1562–1571. [PubMed: 17873905]
- Kimata Y, Trickey M, Izawa D, Gannon J, Yamamoto M, Yamano H. A Mutual Inhibition between APC/C and Its Substrate Mes1 Required for Meiotic Progression in Fission Yeast. *Dev Cell.* 2008; 14:446–454. [PubMed: 18331722]
- Kishimoto T. Cell-cycle control during meiotic maturation. *Curr Opin Cell Biol.* 2003; 15:654–663. [PubMed: 14644189]
- Kitagawa R, Rose AM. Components of the spindle-assembly checkpoint are essential in *Caenorhabditis elegans*. *Nat Cell Biol.* 1999; 1:514–521. [PubMed: 10587648]
- Kitajima TS, Miyazaki Y, Yamamoto M, Watanabe Y. Rec8 cleavage by separase is required for meiotic nuclear divisions in fission yeast. *Embo J.* 2003; 22:5643–5653. [PubMed: 14532136]
- Kramer ER, Scheuringer N, Podtelejnikov AV, Mann M, Peters JM. Mitotic regulation of the APC activator proteins CDC20 and CDH1. *Mol Biol Cell.* 2000; 11:1555–1569. [PubMed: 10793135]
- Kudo NR, Wassmann K, Anger M, Schuh M, Wirth KG, et al. Resolution of chiasmata in oocytes requires separase-mediated proteolysis. *Cell.* 2006; 126:135–146. [PubMed: 16839882]
- Li R, Murray AW. Feedback control of mitosis in budding yeast. *Cell.* 1991; 66:519–531. [PubMed: 1651172]
- Lieberfarb ME, Chu T, Wreden C, Theurkauf W, Gergen JP, Strickland S. Mutations that perturb poly(A)-dependent maternal mRNA activation block the initiation of development. *Development.* 1996; 122:579–588. [PubMed: 8625809]
- Liu J, Maller JL. Calcium elevation at fertilization coordinates phosphorylation of XErp1/Emi2 by Plx1 and CaMK II to release metaphase arrest by cytotstatic factor. *Curr Biol.* 2005; 15:1458–1468. [PubMed: 16040245]
- Lorca T, Cruzalegui FH, Fesquet D, Cavadore JC, Mery J, et al. Calmodulin-dependent protein kinase II mediates inactivation of MPF and CSF upon fertilization of *Xenopus* eggs. *Nature.* 1993; 366:270–273. [PubMed: 8232587]
- Lukas C, Sorensen CS, Kramer E, Santoni-Rugiu E, Lindene C, et al. Accumulation of cyclin B1 requires E2F and cyclin-A-dependent rearrangement of the anaphase-promoting complex. *Nature.* 1999; 401:815–818. [PubMed: 10548110]
- Madgwick S, Hansen DV, Levasseur M, Jackson PK, Jones KT. Mouse Emi2 is required to enter meiosis II by reestablishing cyclin B1 during interkinesis. *J Cell Biol.* 2006; 174:791–801. [PubMed: 16966421]
- Malmanche N, Owen S, Gegick S, Steffensen S, Tomkiel JE, Sunkel CE. *Drosophila* BubR1 Is Essential for Meiotic Sister-Chromatid Cohesion and Maintenance of Synaptonemal Complex. *Curr Biol.* 2007; 17:1489–1497. [PubMed: 17702574]
- Mapelli M, Massimiliano L, Santaguida S, Musacchio A. The Mad2 conformational dimer: structure and implications for the spindle assembly checkpoint. *Cell.* 2007; 131:730–743. [PubMed: 18022367]
- Marangos P, Verschuren EW, Chen R, Jackson PK, Carroll J. Prophase I arrest and progression to metaphase I in mouse oocytes are controlled by Emi1-dependent regulation of APC(Cdh1). *J Cell Biol.* 2007; 176:65–75. [PubMed: 17190794]

- Margottin-Goguet F, Hsu JY, Loktev A, Hsieh HM, Reimann JD, Jackson PK. Prophase destruction of Emi1 by the SCF(betaTrCP/Slimb) ubiquitin ligase activates the anaphase promoting complex to allow progression beyond prometaphase. *Dev Cell*. 2003; 4:813–826. [PubMed: 12791267]
- Marston AL, Amon A. Meiosis: cell-cycle controls shuffle and deal. *Nat Rev Mol Cell Biol*. 2004; 5:983–997. [PubMed: 15573136]
- Mata J, Lyne R, Burns G, Bahler J. The transcriptional program of meiosis and sporulation in fission yeast. *Nat Genet*. 2002; 32:143–147. [PubMed: 12161753]
- Miller JJ, Summers MK, Hansen DV, Nachury MV, Lehman NL, et al. Emi1 stably binds and inhibits the anaphase-promoting complex/cyclosome as a pseudosubstrate inhibitor. *Genes Dev*. 2006; 20:2410–2420. [PubMed: 16921029]
- Mochida S, Hunt T. Calcineurin is required to release *Xenopus* egg extracts from meiotic M phase. *Nature*. 2007; 449:336–340. [PubMed: 17882219]
- Moshe Y, Boulaire J, Pagano M, Hershko A. Role of Polo-like kinase in the degradation of early mitotic inhibitor 1, a regulator of the anaphase promoting complex/cyclosome. *Proc Natl Acad Sci U S A*. 2004; 101:7937–7942. [PubMed: 15148369]
- Musacchio A, Salmon ED. The spindle-assembly checkpoint in space and time. *Nat Rev Mol Cell Biol*. 2007; 8:379–393. [PubMed: 17426725]
- Niault T, Hached K, Sotillo R, Sorger PK, Maro B, et al. Changing mad2 levels affects chromosome segregation and spindle assembly checkpoint control in female mouse meiosis I. *PLoS ONE*. 2007a; 2:e1165. [PubMed: 18043727]
- Nishiyama T, Ohsumi K, Kishimoto T. Phosphorylation of Erp1 by p90rsk is required for cytostatic factor arrest in *Xenopus laevis* eggs. *Nature*. 2007b; 446:1096–1099. [PubMed: 17410129]
- Nishiyama T, Yoshizaki N, Kishimoto T, Ohsumi K. Transient activation of calcineurin is essential to initiate embryonic development in *Xenopus laevis*. *Nature*. 2007; 449:341–345. [PubMed: 17882220]
- Oelschlaegel T, Schwickart M, Matos J, Bogdanova A, Camasses A, et al. The yeast APC/C subunit Mnd2 prevents premature sister chromatid separation triggered by the meiosis-specific APC/C-Ama1. *Cell*. 2005; 120:773–788. [PubMed: 15797379]
- Ohe M, Inoue D, Kanemori Y, Sagata N. Erp1/Emi2 is essential for the meiosis I to meiosis II transition in *Xenopus* oocytes. *Dev Biol*. 2007; 303:157–164. [PubMed: 17141208]
- Ohsumi K, Koyanagi A, Yamamoto TM, Gotoh T, Kishimoto T. Emi1-mediated M-phase arrest in *Xenopus* eggs is distinct from cytostatic factor arrest. *Proc Natl Acad Sci U S A*. 2004; 101:12531–12536. [PubMed: 15314241]
- Page AW, Orr-Weaver TL. The *Drosophila* genes *grauzone* and *cortex* are necessary for proper female meiosis. *J Cell Sci*. 1996; 109(Pt 7):1707–1715. [PubMed: 8832393]
- Papin C, Rouget C, Lorca T, Castro A, Mandart E. XCdh1 is involved in progesterone-induced oocyte maturation. *Dev Biol*. 2004; 272:66–75. [PubMed: 15242791]
- Passmore LA, McCormack EA, Au SW, Paul A, Willison KR, et al. Doc1 mediates the activity of the anaphase-promoting complex by contributing to substrate recognition. *Embo J*. 2003; 22:786–796. [PubMed: 12574115]
- Penkner AM, Prinz S, Ferscha S, Klein F. Mnd2, an essential antagonist of the anaphase-promoting complex during meiotic prophase. *Cell*. 2005; 120:789–801. [PubMed: 15797380]
- Pesin JA, Orr-Weaver TL. Developmental Role and Regulation of *cortex* a Meiosis-Specific Anaphase-Promoting Complex/Cyclosome Activator. *PLoS Genet*. 2007; 3:e202. [PubMed: 18020708]
- Peter M, Castro A, Lorca T, Le Peuch C, Magnaghi-Jaulin L, et al. The APC is dispensable for first meiotic anaphase in *Xenopus* oocytes. *Nat Cell Biol*. 2001; 3:83–87. [PubMed: 11146630]
- Peters JM. The anaphase promoting complex/cyclosome: a machine designed to destroy. *Nat Rev Mol Cell Biol*. 2006; 7:644–656. [PubMed: 16896351]
- Pfleger CM, Kirschner MW. The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1. *Genes Dev*. 2000; 14:655–665. [PubMed: 10733526]
- Prinz S, Hwang ES, Visintin R, Amon A. The regulation of Cdc20 proteolysis reveals a role for APC components Cdc23 and Cdc27 during S phase and early mitosis. *Curr Biol*. 1998; 8:750–760. [PubMed: 9651679]

- Pritchard CE, Fornerod M, Kasper LH, van Deursen JM. RAE1 is a shuttling mRNA export factor that binds to a GLEBS-like NUP98 motif at the nuclear pore complex through multiple domains. *J Cell Biol.* 1999; 145:237–254. [PubMed: 10209021]
- Rauh NR, Schmidt A, Bormann J, Nigg EA, Mayer TU. Calcium triggers exit from meiosis II by targeting the APC/C inhibitor XErp1 for degradation. *Nature.* 2005; 437:1048–1052. [PubMed: 16127448]
- Reddy SK, Rape M, Margansky WA, Kirschner MW. Ubiquitination by the anaphase-promoting complex drives spindle checkpoint inactivation. *Nature.* 2007; 446:921–925. [PubMed: 17443186]
- Reimann JD, Freed E, Hsu JY, Kramer ER, Peters JM, Jackson PK. Emi1 is a mitotic regulator that interacts with Cdc20 and inhibits the anaphase promoting complex. *Cell.* 2001; 105:645–655. [PubMed: 11389834]
- Reimann JD, Gardner BE, Margottin-Goguet F, Jackson PK. Emi1 regulates the anaphase-promoting complex by a different mechanism than Mad2 proteins. *Genes Dev.* 2001; 15:3278–3285. [PubMed: 11751633]
- Reis A, Chang HY, Levasseur M, Jones KT. APCcdh1 activity in mouse oocytes prevents entry into the first meiotic division. *Nat Cell Biol.* 2006b; 8:539–540. [PubMed: 16715549]
- Reis A, Levasseur M, Chang HY, Elliott DJ, Jones KT. The CRY box: a second APCcdh1-dependent degron in mammalian cdc20. *EMBO Rep.* 2006a; 7:1040–1045. [PubMed: 16878123]
- Reis A, Madgwick S, Chang HY, Nabti I, Levasseur M, Jones KT. Prometaphase APCcdh1 activity prevents non-disjunction in mammalian oocytes. *Nat Cell Biol.* 2007; 9:1192–1198. [PubMed: 17891138]
- Salah SM, Nasmyth K. Destruction of the securin Pds1p occurs at the onset of anaphase during both meiotic divisions in yeast. *Chromosoma.* 2000; 109:27–34. [PubMed: 10855492]
- Schmidt A, Duncan PI, Rauh NR, Sauer G, Fry AM, et al. Xenopus polo-like kinase Plx1 regulates XErp1, a novel inhibitor of APC/C activity. *Genes Dev.* 2005; 19:502–513. [PubMed: 15713843]
- Schwab M, Lutum AS, Seufert W. Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. *Cell.* 1997; 90:683–693. [PubMed: 9288748]
- Schwab MS, Roberts BT, Gross SD, Tunquist BJ, Taieb FE, et al. Bub1 is activated by the protein kinase p90(Rsk) during Xenopus oocyte maturation. *Curr Biol.* 2001; 11:141–150. [PubMed: 11231148]
- Searle JS, Schollaert KL, Wilkins BJ, Sanchez Y. The DNA damage checkpoint and PKA pathways converge on APC substrates and Cdc20 to regulate mitotic progression. *Nat Cell Biol.* 2004; 6:138–145. [PubMed: 14743219]
- Shimoda C, Hirata A, Kishida M, Hashida T, Tanaka K. Characterization of meiosis-deficient mutants by electron microscopy and mapping of four essential genes in the fission yeast *Schizosaccharomyces pombe*. *Mol Gen Genet.* 1985; 200:252–257. [PubMed: 3861929]
- Shoji S, Yoshida N, Amanai M, Ohgishi M, Fukui T, et al. Mammalian Emi2 mediates cytostatic arrest and transduces the signal for meiotic exit via Cdc20. *Embo J.* 2006; 25:834–845. [PubMed: 16456547]
- Shonn MA, McCarroll R, Murray AW. Requirement of the spindle checkpoint for proper chromosome segregation in budding yeast meiosis. *Science.* 2000; 289:300–303. [PubMed: 10894778]
- Shonn MA, Murray AL, Murray AW. Spindle checkpoint component Mad2 contributes to biorientation of homologous chromosomes. *Curr Biol.* 2003; 13:1979–1984. [PubMed: 14614824]
- Sigrist SJ, Lehner CF. *Drosophila* *fizzy-related* down-regulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles. *Cell.* 1997; 90:671–681. [PubMed: 9288747]
- Smith TF, Gaitatzes C, Saxena K, Neer EJ. The WD repeat: a common architecture for diverse functions. *Trends Biochem Sci.* 1999; 24:181–185. [PubMed: 10322433]
- Song MS, Song SJ, Ayad NG, Chang JS, Lee JH, et al. The tumour suppressor RASSF1A regulates mitosis by inhibiting the APC-Cdc20 complex. *Nat Cell Biol.* 2004; 6:129–137. [PubMed: 14743218]
- Sorensen CS, Lukas C, Kramer ER, Peters JM, Bartek J, Lukas J. A conserved cyclin-binding domain determines functional interplay between anaphase-promoting complex-Cdh1 and cyclin A-Cdk2 during cell cycle progression. *Mol Cell Biol.* 2001; 21:3692–3703. [PubMed: 11340163]

- Stegmeier F, Rape M, Draviam VM, Nalepa G, Sowa ME, et al. Anaphase initiation is regulated by antagonistic ubiquitination and deubiquitination activities. *Nature*. 2007; 446:876–881. [PubMed: 17443180]
- Stein KK, Davis ES, Hays T, Golden A. Components of the spindle assembly checkpoint regulate the anaphase-promoting complex during meiosis in *Caenorhabditis elegans*. *Genetics*. 2007; 175:107–123. [PubMed: 17057243]
- Swan A, Schupbach T. The Cdc20 (Fzy)/Cdh1-related protein, Cort, cooperates with Fzy in cyclin destruction and anaphase progression in meiosis I and II in *Drosophila*. *Development*. 2007; 134:891–899. [PubMed: 17251266]
- Taieb FE, Gross SD, Lewellyn AL, Maller JL. Activation of the anaphase-promoting complex and degradation of cyclin B is not required for progression from Meiosis I to II in *Xenopus* oocytes. *Curr Biol*. 2001; 11:508–513. [PubMed: 11413001]
- Tang Z, Shu H, Oncel D, Chen S, Yu H. Phosphorylation of Cdc20 by Bub1 provides a catalytic mechanism for APC/C inhibition by the spindle checkpoint. *Mol Cell*. 2004; 16:387–397. [PubMed: 15525512]
- Terret ME, Wassmann K, Waizenegger I, Maro B, Peters JM, Verlhac MH. The meiosis I-to-meiosis II transition in mouse oocytes requires separase activity. *Curr Biol*. 2003; 13:1797–1802. [PubMed: 14561405]
- Thornton BR, Toczyski DP. Precise destruction: an emerging picture of the APC. *Genes Dev*. 2006; 20:3069–3078. [PubMed: 17114580]
- Tsurumi C, Hoffmann S, Geley S, Graeser R, Polanski Z. The spindle assembly checkpoint is not essential for CSF arrest of mouse oocytes. *J Cell Biol*. 2004; 167:1037–1050. [PubMed: 15611331]
- Tung JJ, Jackson PK. Emi1 class of proteins regulate entry into meiosis and the meiosis I to meiosis II transition in *Xenopus* oocytes. *Cell Cycle*. 2005; 4:478–482. [PubMed: 15701974]
- Tung JJ, Padmanabhan K, Hansen DV, Richter JD, Jackson PK. Translational unmasking of Emi2 directs cytostatic factor arrest in meiosis II. *Cell Cycle*. 2007; 6:725–731. [PubMed: 17361107]
- Tunquist BJ, Maller JL. Under arrest: cytostatic factor (CSF)-mediated metaphase arrest in vertebrate eggs. *Genes Dev*. 2003; 17:683–710. [PubMed: 12651887]
- Tunquist BJ, Schwab MS, Chen LG, Maller JL. The spindle checkpoint kinase bub1 and cyclin e/cdk2 both contribute to the establishment of meiotic metaphase arrest by cytostatic factor. *Curr Biol*. 2002; 12:1027–1033. [PubMed: 12123578]
- Turnell AS, Stewart GS, Grand RJ, Rookes SM, Martin A, et al. The APC/C and CBP/p300 cooperate to regulate transcription and cell-cycle progression. *Nature*. 2005; 438:690–695. [PubMed: 16319895]
- Visintin R, Prinz S, Amon A. CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. *Science*. 1997; 278:460–463. [PubMed: 9334304]
- Wassmann K, Niault T, Maro B. Metaphase I arrest upon activation of the Mad2-dependent spindle checkpoint in mouse oocytes. *Curr Biol*. 2003; 13:1596–1608. [PubMed: 13678590]
- Whitehurst AW, Ram R, Shivakumar L, Gao B, Minna JD, White MA. The RASSF1A Tumor Suppressor Restrains APC/C Activity During the G1/S Phase Transition to Promote Cell Cycle Progression in Human Epithelial Cells. *Mol Cell Biol*. 2008
- Wu Q, Guo Y, Yamada A, Perry JA, Wang MZ, et al. A role for Cdc2- and PP2A-mediated regulation of Emi2 in the maintenance of CSF arrest. *Curr Biol*. 2007; 17:213–224. [PubMed: 17276914]
- Yamaguchi S, Okayama H, Nurse P. Fission yeast Fizzy-related protein *srw1p* is a G(1)-specific promoter of mitotic cyclin B degradation. *Embo J*. 2000; 19:3968–3977. [PubMed: 10921878]
- Yang M, Li B, Liu CJ, Tomchick DR, Machius M, et al. Insights into Mad2 Regulation in the Spindle Checkpoint Revealed by the Crystal Structure of the Symmetric Mad2 Dimer. *PLoS Biol*. 2008; 6:e50. [PubMed: 18318601]
- Yang M, Li B, Tomchick DR, Machius M, Rizo J, et al. p31^{comet} blocks Mad2 activation through structural mimicry. *Cell*. 2007; 131:744–755. [PubMed: 18022368]
- Yin S, Wang Q, Liu JH, Ai JS, Liang CG, et al. Bub1 prevents chromosome misalignment and precocious anaphase during mouse oocyte meiosis. *Cell Cycle*. 2006; 5:2130–2137. [PubMed: 16969117]

- Yudkovsky Y, Shteinberg M, Listovsky T, Brandeis M, Hershko A. Phosphorylation of Cdc20/fizzy negatively regulates the mammalian cyclosome/APC in the mitotic checkpoint. *Biochem Biophys Res Commun.* 2000; 271:299–304. [PubMed: 10799291]
- Zachariae W, Schwab M, Nasmyth K, Seufert W. Control of cyclin ubiquitination by CDK-regulated binding of Hct1 to the anaphase promoting complex. *Science.* 1998; 282:1721–1724. [PubMed: 9831566]

Key Terms/ Definitions Abbreviations and Acronyms

E3 ubiquitin ligase	protein or protein complex that covalently attaches ubiquitin to a lysine residue on a target protein
spindle assembly checkpoint	ensures that chromosomes do not segregate until they are properly attached to the spindle
sister chromatids	replicated copies of a single chromosome
meiosis	two sequential cell divisions that generate 4 haploid cells from one diploid cell
homologous chromosomes	chromosomes from different parent cells containing the same genetic loci that pair and then segregate during meiosis I
securin	a protein that inhibits the catalytic activity of separase
separase	a cysteine protease that triggers anaphase by cleaving cohesin complex subunit to release sister chromatid cohesion
sister chromatid cohesion	physical attachment of sister chromatids mediated by the cohesin complex
germinal vesicle breakdown	disintegration of the large meiotic nuclear envelope at the end of meiotic prophase I
APC/C	anaphase-promoting complex
Fzy	Fizzy
Fzr	Fizzy-related
SCF complex	Skp, Cullin, F-box containing complex
SAC	spindle assembly checkpoint
GVBD	germinal vesicle breakdown
MPF	maturation-promoting factor
CSF	cytostatic factor
CaMKII	calmodulin kinase II

Summary Points

1. Cdc20/Fzy is transcribed in S-phase, and the protein is rapidly degraded in late mitosis and G1 through targeting by APC/C^{Cdh1}.
2. APC/C^{Cdc20} activity is inhibited by Emi1 in prophase and by spindle assembly checkpoint proteins in prometaphase and metaphase.
3. Cdh1/Fzr is uniformly transcribed during the mitotic cell cycle but inhibited by phosphorylation in S, G2, and M phases and E3 ubiquitin ligase-mediated degradation in late G1 and S.
4. APC/C^{Cdh1} activity is inhibited by Emi1 at the G1-S transition and by Rae1-Nup98 in prometaphase.
5. In addition to Cdc20 and Cdh1, yeast and *Drosophila* have been shown to use meiosis-specific APC/C activators: Ama1 in *S. cerevisiae*, mfr1/fzr1 in *S. pombe*, and CORTEX in *Drosophila*.
6. During metaphase I and metaphase II, APC/C activity must be suppressed, but in addition it needs to remain inactive in female meiosis during the long prophase arrest to prevent premature loss of cohesion and in between the two meiotic divisions to prevent DNA replication.
7. In vertebrates, Emi2/Erp1, a homolog of Emi1, is an important regulator of APC/C^{Cdc20} activity between meiosis I and meiosis II and during cytostatic factor arrest.

Future Issues

1. What is the mechanism of Rae1-Nup98 mediated inhibition of APC/C^{Cdh1}?
2. Do meiosis-specific APC/C activators target a unique set of substrates, and how is this specificity achieved?
3. What are the substrates of APC/C^{Cdh1} in prometaphase of mouse meiosis I?
4. What is the mechanism of Mes1-mediated inhibition of the APC/C?
5. Is dephosphorylation of Cdc20/Fzy the key downstream target of calcineurin required for release of CSF arrest?
6. Are there meiosis-specific APC/C activators in vertebrates?

Figure 1. Regulation of Cdc20 and Cdh1 during the Cell Cycle

(top) During S and G2 phases, APC/C^{Cdc20} is thought to be bound and inhibited by Emi1. Emi1 is degraded in prometaphase, and APC/C^{Cdc20} becomes active against early mitotic APC/C substrates like Cyclin A and Nek2A. In prometaphase APC/C^{Cdc20} remains inhibited from targeting Cyclin B, Securin, and other substrates by the spindle assembly checkpoint through direct binding of Mad2, Bub3, and BubR1. Upon release of the spindle assembly checkpoint, APC/C^{Cdc20} becomes fully active. In late anaphase and G1, Cdc20 is targeted by APC/C^{Cdh1} for degradation by the proteasome. It will not be expressed again until S phase.

(bottom) Cdh1 is inhibited from associating with the core APC/C in S, G2, and M phases by phosphorylation by cyclin-dependent kinases. In *Drosophila*, Rca1 is thought to inhibit Cdh1 in G2, and in vertebrates Emi1 is thought to inhibit APC/C^{Cdh1} at the G1-S transition. Recent findings suggest that APC/C^{Cdh1} must be inhibited from targeting Securin from degradation by Rae1-Nup98 in metaphase. Dephosphorylation of Cdh1 by Cdc14 phosphatase as well as a decrease in mitotic Cdk-cyclin activity leads to loss of inhibitory phosphorylation on Cdh1 in late mitosis and allows for full activation of APC/C^{Cdh1} in late mitosis and G1. In addition, Cdh1 may be subject to ubiquitin-mediated degradation in late G1 by APC/C^{Cdh1} (indicated by dashed arrow) and in S phase by SCF ubiquitin ligase. See text for references.

a **b** **Figure 2. Structure and regulation of Emi1 and Emi2/Erp1**

(A) The Emi1 protein contains an F-box domain, a C-terminal Zn^{2+} binding region (ZBR), and a D-box. The role of the F-box is unclear, but the D-box is the domain through which Emi1 binds the D-box receptor on the core APC/C, whereas the ZBR domain seems to act to inhibit access of substrates to the complex. See text for details and references. Emi1 is a substrate of the E3 ubiquitin ligase $\text{SCF}^{\beta\text{-TrCP}}$ (Guardavaccaro et al. 2003, Margottin-Goguet et al. 2003). Phosphorylation by both Cdk and Plk1 contribute to recognition and ubiquitination of Emi1 by $\text{SCF}^{\beta\text{-TrCP}}$ in late prophase (Margottin-Goguet et al. 2003, Moshe et al. 2004, Hansen et al. 2004).

(B) The Emi2/Erp1 protein also contains an F-box domain, a C-terminal Zn^{2+} binding region (ZBR), and a D-box. Upon fertilization, activated calmodulin kinase II (CaMKII) acts as a priming kinase for Plx1 on Emi2. Phosphorylation by Plx1 targets Emi2 for degradation targeted by $\text{SCF}^{\beta\text{-TrCP}}$. Phosphorylation of Emi2 by p90^{RSK} , part of the pathway that establishes CSF arrest, is required for Emi2 stability during oocyte maturation. Phosphorylation of Emi2 by Cdc2 (Cdk1)/CycB may disrupt the interaction between Emi2 and APC/C. See text for details and references.

Figure 3. Meiotic Progression in Females

Oocytes are arrested in prophase I to allow for oocyte growth and differentiation before initiation of the meiotic divisions. Then, oocytes arrest again at different times to await egg activation or fertilization. This secondary arrest ensures that the completion of meiosis is properly coordinated with fertilization of the oocyte. In *Drosophila*, the secondary meiotic arrest takes place in metaphase I. Egg activation releases this arrest. In most vertebrates the secondary meiotic arrest takes place in metaphase II and is called cytotstatic factor (CSF) arrest. CSF is released by fertilization. MPF (CycB/Cdc2 (Cdk1)) activity levels rise upon entry into the meiotic divisions. They must drop in between meiosis I and meiosis II to exit meiosis I, but some activity must be maintained to suppress DNA replication in between the two divisions. MPF levels drop again at exit from meiosis II. See text for details and references.

Figure 4. Meiotic APC/C Regulators

(A) In *S. cerevisiae* Mnd2 inhibits APC/C^{Ama1}, a meiosis-specific form of the APC/C, beginning in prophase. Inhibition is not released until late meiosis when Mnd2 protein disappears from meiotic cells. Full activation of APC/C^{Ama1} may also require APC/C^{Cdc20}-mediated degradation of cyclins in meiosis I.

(B) In *S. pombe* Mes1 is an inhibitor of APC/C^{Slp1/Cdc20} in between the meiotic divisions. Mes1 itself is an APC/C substrate but is able to compete for binding to the APC/C more efficiently than other substrates.

(C) In vertebrates regulation of the APC/C during meiosis has been well-studied. In prophase, prior to germinal vesicle breakdown (GVBD), inhibition of APC/C^{Cdh1} by Emi1 allows for an accumulation of MPF that is required for entry into the first meiotic division. Emi1 is targeted for degradation by SCF ^{β -TrCP} at GVBD. APC/C^{Cdc20} activity is thought to be regulated by the spindle assembly checkpoint in meiosis I for proper homolog alignment and disjunction. Similar to the role of Mes1, Emi2/Erp1 helps maintain low APC/C^{Cdc20} activity levels in between the two meiotic divisions. Emi2/Erp1 is also a main component of CSF arrest and prevents APC/C^{Cdc20} from being active until fertilization. The spindle assembly checkpoint may play a role in establishing but not in maintaining CSF arrest. At fertilization, a transient increase in free cytosolic Ca²⁺ activates calmodulin kinase II and the phosphatase calcineurin. Calmodulin kinase II phosphorylates Emi2, which ultimately leads to its degradation by SCF ^{β -TrCP}. Calcineurin dephosphorylates Apc3, a core APC/C subunit, and Cdc20/Fzy, both of which may contribute to activation of APC/C^{Cdc20} upon fertilization. See text for references and details.

TABLE 1

Orthologs of APC/C activators, inhibitors, and other APC/C pathway components^a

	<i>Saccharomyces cerevisiae</i>	<i>Schizosaccharomyces pombe</i>	<i>Drosophila melanogaster</i>	<i>Xenopus laevis</i>	Mouse
APC/C activators	Cdc20	Slp1	Fzy	Fizzy	Cdc20
	Cdh1/Hct1	Ste9	Fzr	Fizzy-related	Cdh1
	Amal	^a <i>b</i>	?	n/i ^c	n/i
Meiosis-specific APC/C activators	?	?	Cort	n/i	n/i
	?	Mfr1/Fzr1	?	n/i	n/i
	?	?	Fzr2	n/i	n/i
	n/i	n/i	Rcal	Emi1	Emi1
APC/C inhibitors	n/i	n/i	n/i	Erp1	Emi2
	Mnd2	n/i	n/i	n/i	n/i
	n/i	Mes1	n/i	n/i	n/i
	Acm1	n/i	n/i	n/i	n/i
	Esp1	Cut1	Sse + Thr	Separase	Separase
	Pds1	Cut2	Pim	Securin	Securin
	Cdc5	Plx1	Polo	Plx1	Plk
Additional regulators of the meta-ana transition					

^a Orthologous genes from each species are contained in one row.
^b It is unknown what the exact homologous relationship is between the meiosis-specific activators that have been identified in yeast and *Drosophila*.
^c n/i : not identified