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Regulation of APC/C Activators in Mitosis and Meiosis

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Key Words

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

Abstract

The anaphase-promoting complex/cyclosome (APC/C) is a multisubunit 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 the specialized divisions of meiosis, the activity of the APC/C must be modified to achieve proper chromosome segregation. 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.

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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/cyclosome (APC/C) is a large multisubunit E3 ubiquitin ligase that targets substrates for degradation through the addition of a polyubiquitin chain onto target proteins, which are then recognized and degraded by the 26S proteasome (reviewed in Peters 2006).

Cdc20/Fzy (Fizzy) and Cdh1/Fzr (Fizzy-related) compose the noncore subunits of the APC/C and activate and confer substrate speci-

ficity to the complex (**Table 1**) (Dawson et al. 1995, Schwab et al. 1997, Sigrist & Lehner 1997, Visintin et al. 1997). Cdc20 directs the ubiquitination of Securin, mitotic cyclins, and other substrates for anaphase onset. Cdc20 is responsible for the separation of sister chromatids because the degradation of Securin leads to the activation of the Separase protease, which cleaves the cohesin complex ring responsible for physically attaching the sister chromatids. Cdh1 targets mitotic cyclins and additional substrates for degradation in mitotic exit and G1. The form of the APC/C with a specific activator is designated by a superscript, for example, APC/C^{Cdc20}. Cdc20 and Cdh1 are members of the Cdc20 protein family, whose members contain seven WD-40 repeats in their C 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 & Toczyski 2006).

One important way in which APC/C activity is governed is through the regulation of these activators that act as substrate adaptors. Because the activators are essential for APC/C activity and substrate specificity, their regulation plays a crucial role in determining which target proteins are degraded as well as the timing of their degradation. In this review we 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. (Although the nomenclature differs between experimental organisms, for consistency in this review we refer to proteins by capitalizing the first letter of the name, and genes are in lowercase italic font.)

REGULATION OF Cdc20/Fzy IN THE MITOTIC CELL CYCLE

One mechanism of regulation of APC/C^{Cdc20} is via the control of protein levels of Cdc20. In this section we discuss this control first and then

APC/C: anaphase-promoting complex/cyclosome

E3 ubiquitin ligase: a protein or protein complex that covalently attaches ubiquitin to a lysine residue on a target protein

Fzy: Fizzy

Fzr: Fizzy-related

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
Meiosis-specific APC/C activators	Ama1	?	?	n/i	n/i
	?	?	Cort	n/i	n/i
	?	Mfr1/Fzr1	?	n/i	n/i
	?	?	Fzr2	n/i	n/i
APC/C inhibitors	n/i	n/i	Rca1	Emi1	Emi1
	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
Additional regulators of the metaphase-anaphase transition	Esp1	Cut1	Sse + Thr	Separase	Separase
	Pds1	Cut2	Pim	Securin	Securin
	Cdc5	Plo1	Polo	Plx1	Plk

^aOrthologous genes from each species are contained in one row.

^bIt is unknown what the exact homologous relationship is between the meiosis-specific activators that have been identified in yeast and *Drosophila*.

^cn/i, not identified.

review additional inhibitory mechanisms affecting APC/C^{Cdc20} in mitosis (**Figure 1**). Cdc20 protein 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. 1998, Prinz et al. 1998). Control of Cdc20 transcription is best understood in *Saccharomyces cerevisiae*, in which its cell cycle regulation is dependent on Cks1, a small Cdk-associated protein that binds to the Cdc20 promoter. It appears that Cks1 can displace Cdc28 (Cdk1) and promote association of the proteasome, which enhances Cdc20 transcription (Morris et al. 2003). It is not known whether this mechanism controls Cdc20 transcription in higher eukaryotes, but Cdc20 transcription is negatively regulated by p53 and p21 in mammalian cells (Kidokoro et al. 2008).

Cdc20 protein is subjected to APC/C-triggered degradation because it 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 its D-box, a

recognition motif found in most APC/C substrates, in Cdc20's 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 & Kirschner 2000). In mammalian oocytes and embryos, the degradation of Cdc20 is mediated through an additional motif called the CRY box (Reis et al. 2006b).

Another major player contributing to the presence of functional Cdc20 in cells is the CCT (chaperonin-containing TCP1) chaperonin. 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 control of the activity of APC/C^{Cdc20} by regulation of levels of Cdc20 protein in the cell, APC/C^{Cdc20} can be inhibited by the Emi1 (Early mitotic inhibitor 1) protein, by the spindle assembly checkpoint, and by PKA signaling, as detailed below.

Securin: a protein that inhibits the catalytic activity of Separase. It is also called Pds1 in budding yeast, Cut2 in fission yeast, and Pim in *Drosophila* (**Table 1**)

Separase: a cysteine protease that triggers anaphase by cleaving a cohesin complex subunit to release sister chromatid cohesion

Sister chromatids: replicated copies of a single chromosome

Sister chromatid cohesion: the physical attachment of sister chromatids mediated by the cohesin complex

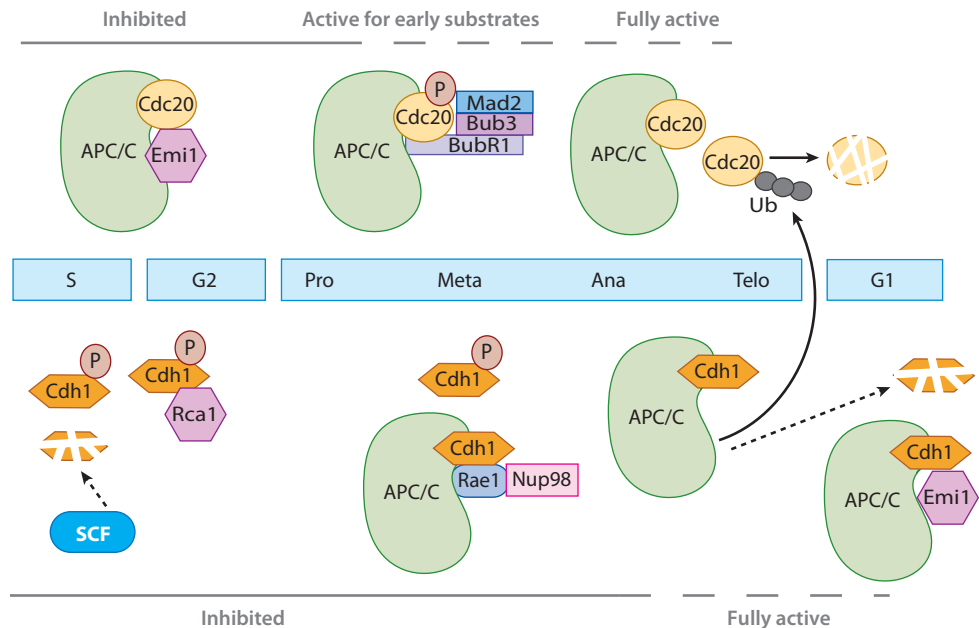


Figure 1

Regulation of Cdc20 and Cdh1 during the cell cycle. (*Top*) During S and G2 phases, Emi1 is thought to bind and inhibit APC/C^{Cdc20}. 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. Cdc20 will not be expressed again until S phase. (*Bottom*) Phosphorylation by cyclin-dependent kinases inhibits Cdh1 from associating with the core APC/C in S, G2, and M phases. 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. In budding yeast, the inhibitor Acm1 binds to Cdh1 (not shown) until it is degraded by APC/C^{Cdc20}, providing an additional mechanism to restrict the timing of APC/C^{Cdh1} activation. Dephosphorylation of Cdh1 by Cdc14 phosphatase as well as a decrease in mitotic Cdk-Cyclin activity lead to loss of inhibitory phosphorylation on Cdh1 in late mitosis and allow for full activation of APC/C^{Cdh1} in late mitosis and G1. In addition, Cdh1 may be subject to ubiquitin (Ub)-mediated degradation in late G1 by APC/C^{Cdh1} (indicated by *dashed arrow*) and in S phase by SCF ubiquitin ligase (SCF). See text for references. Pro, prophase; Meta, metaphase; Ana, anaphase; Telo, telophase.

Emi1

The Emi1 protein inhibits APC/C^{Cdc20} activity by its ability to bind to Cdc20 and the core APC/C complex. As discussed in the next section, Emi1 also inhibits APC/C^{Cdh1}. Emi1 contains three motifs important for its function: an F-box protein-protein interaction domain, a C-terminal Zn²⁺-binding region (ZBR), and a D-box (**Figure 2**). Emi1 was initially identified in *Xenopus*. In cycling embryos, levels of Emi1 increase in S phase and decrease in mi-

toosis, and the addition of recombinant Emi1 to cycling egg extracts stabilizes APC/C substrates and prevents their ubiquitination. One critical cell cycle function of Emi1 is to permit the accumulation of mitotic cyclin proteins during G2 to promote entry into mitosis and to block re-replication of DNA (Di Fiore & Pines 2007).

Emi1 inhibits APC/C activity in part by binding directly to Cdc20, but it also binds the APC/C core (Reimann et al. 2001a,b; Miller et al. 2006). Assays using an Emi1-affinity

Meiosis: two sequential cell divisions that generate four haploid cells from one diploid cell

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 (Miller et al. 2006). This direct interaction is dependent on the D-box in the C terminus of Emi1, but not on the ZBR domain. Both the D-box and the ZBR domain, however, 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, but mutation of the ZBR domain converts Emi1 into an efficient D-box-dependent APC/C substrate. Thus, Emi1 acts as a pseudosubstrate 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 inhibit access of substrates to the complex.

Several mechanisms regulate levels of Emi1 in a cell cycle-dependent manner. Transcription of the Emi1 gene is activated at the G1-S transition by the E2F transcription factor. Emi1 itself is targeted for degradation by another E3 ubiquitin ligase, the SCF ^{β -TrCP} complex (Figure 2). Emi1 is protected from SCF-mediated degradation outside of mitosis in at least two ways. Recognition of Emi1 by the SCF requires phosphorylation by the Plk1 kinase (Figure 2a). In S/G2 phases, the Evi5 oncogene binds directly to Emi1 and blocks phosphorylation of Emi1 by Plk1, thus preventing recognition of Emi1 by SCF ^{β -TrCP} (Eldridge et al. 2006). Pin1, a peptidyl-prolyl *cis/trans* isomerase, seems to inhibit the degradation of Emi1 during G2. Emi1 associates with Pin1 *in vivo* during G2 and is stabilized by Pin1 in an isomerization-dependent pathway that prevents an association between Emi1 and SCF ^{β -TrCP} (Bernis et al. 2007).

Emi1 degradation in late prophase immediately precedes Cyclin A degradation by the APC/C, and overexpression of nondegradable Emi1 in mammalian cells causes a prometaphase block (Margottin-Goguet et al.

2003). It is not clear, however, that Emi1 degradation causes or even is essential for activation of APC/C^{Cdc20} (Di Fiore & Pines 2007).

Emi1 additionally functions as a member of a novel regulatory network END (Emi1/NuMA/dynein-dynactin), which 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 the 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).

Spindle Assembly Checkpoint

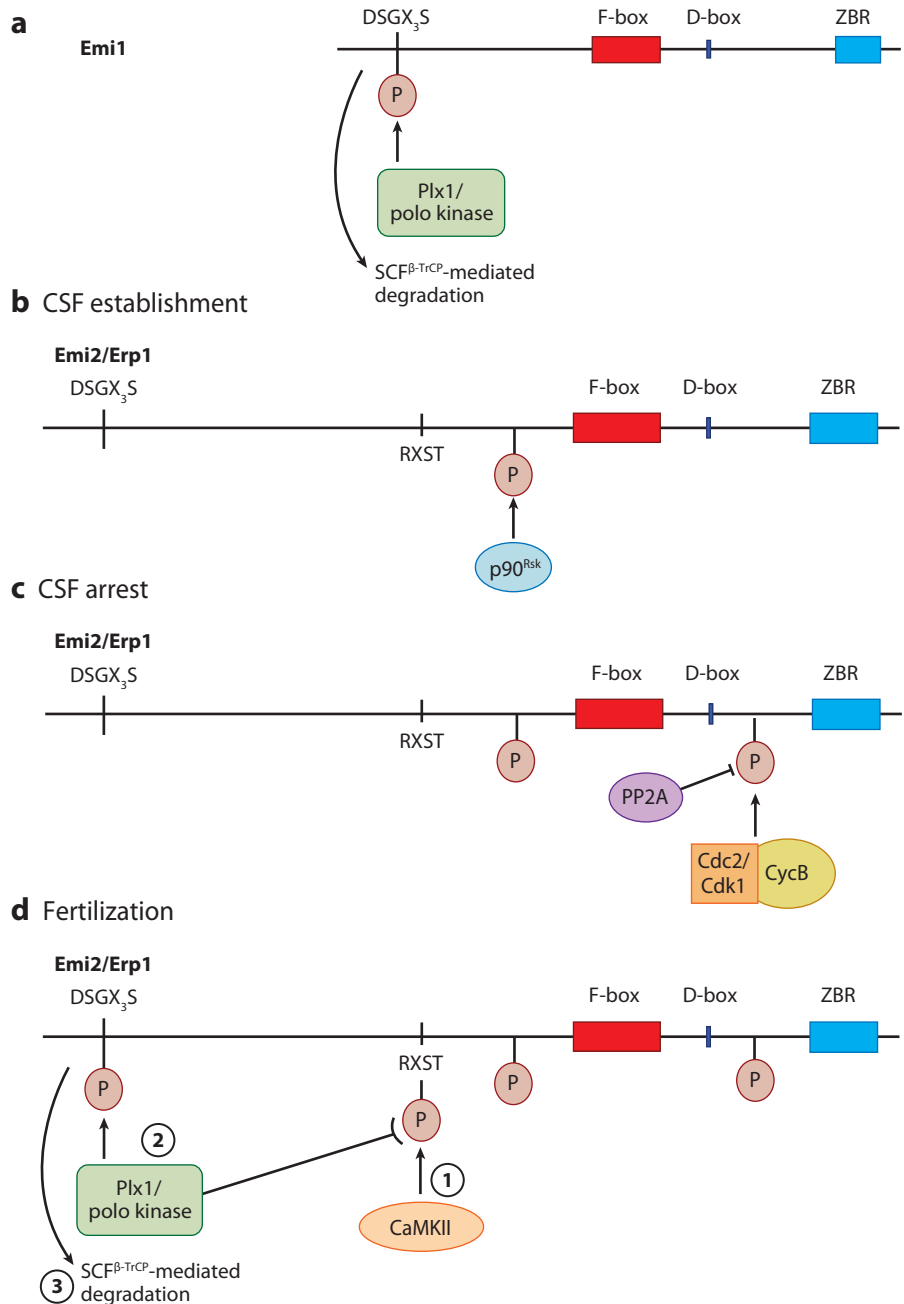
The spindle assembly checkpoint (SAC) is a surveillance mechanism that prevents premature separation of sister chromatids in mitosis by monitoring the attachment of spindles to microtubules in prometaphase. SAC inhibits the ability of APC/C^{Cdc20} to target substrates for degradation, particularly Cyclin B and Securin, to prevent anaphase onset until each kinetochore is stably bioriented on the spindle. Inhibition of Cdc20 by SAC involves many proteins and several different mechanisms, which are not discussed here but are well-reviewed by Musacchio & 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). Important structural data for Mad2, a key spindle checkpoint protein, have provided insights into conformational changes in the protein associated with binding to Cdc20 and inhibition

SCF complex: Skp-, Cullin-, F-box-containing complex

of APC/C^{Cdc20}. These changes, mediated through dimerization, may serve to propagate a signal from improperly attached kinetochores (Mapelli et al. 2007; Yang et al. 2007, 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 two 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

APC/C^{Cdh1} activity is regulated by protein levels as well as phosphorylation of the Cdh1 activator. In addition, APC/C^{Cdh1} activity is controlled by Emi1 and by several other proteins detailed below. 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 phases is thought to be due to E3 ubiquitin ligase-mediated protein degradation, both via auto-activity of APC/C^{Cdh1} and the SCF (**Figure 1**). Cdh1 contains two putative D-boxes that are required for its degradation in G1, consistent with Cdh1 targeting 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 & Pagano (2005) investigated a possible role for SCF in the degradation of Cdh1 at this cell cycle stage. They found that inactivation of SCF by expression of a dominant-negative Cul1, a subunit of SCF, 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 APC/C. Cdh1 is phosphorylated by cyclin-dependent kinases during S, G2, and M phases, and only upon dephosphorylation in late mitosis and G1 can it bind and activate APC/C (**Figure 1**) (Zachariae et al. 1998, Jaspersen et al. 1999, Lukas et al. 1999, Blanco

Spindle assembly checkpoint (SAC): ensures that chromosomes do not segregate until they are properly attached to the spindle

Figure 2

Structure and regulation of Emi1 and Emi2/Erp1 (Early mitotic inhibitor 1 and 2 and Emi-related protein 1, respectively). (a) The Emi1 protein contains an F-box domain, a C-terminal Zn²⁺-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 inhibit access of substrates to the complex. See text for further details and references. Emi1 is a substrate of the E3 ubiquitin ligase SCF^{β-TrCP} (Guardavaccaro et al. 2003, Margottin-Goguet et al. 2003). Phosphorylation by both Cdk and Plk1 contributes to recognition and ubiquitination of Emi1 by SCF^{β-TrCP} in late prophase (Margottin-Goguet et al. 2003, Hansen et al. 2004, Moshe et al. 2004). (b) The Emi2/Erp1 protein also contains an F-box domain, a C-terminal ZBR, and a D-box. Phosphorylation of Emi2/Erp1 by p90^{Rsk}, part of the pathway that establishes cytotostatic factor (CSF) arrest, is required for Emi2/Erp1 stability during oocyte maturation. (c) Phosphorylation of Emi2/Erp1 by Cdc2 (Cdk1)/CycB (Cyclin B) may disrupt the interaction between Emi2/Erp1 and APC/C. The action of the PP2A phosphatase counteracts this phosphorylation so that Emi2/Erp1 inhibits the APC/C during CSF arrest. Additional sites of Cdc2 (Cdk1)/CycB phosphorylation counteracted by PP2A have been recently mapped N-terminal to the p90^{Rsk} phosphorylation site (J.Q. Wu et al. 2007). (d) Upon fertilization, activated calmodulin kinase II (CaMKII) (I) acts as a priming kinase for Plx1 on Emi2/Erp1. Phosphorylation by Plx1 (2) targets Emi2/Erp1 for degradation targeted by SCF^{β-TrCP} (3). See text for further details and references.

et al. 2000, Kramer et al. 2000, Yamaguchi et al. 2000, Huang et al. 2001, Sørensen et al. 2001, Keck et al. 2007).

Like Cdc20, Cdh1 is also subject to regulation by several inhibitors. However, 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.

Emi1/Rca1

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). Similar to Cyclin A, Emi1 transcription is activated by E2F at the G1-to-S-phase transition. HeLa cells depleted of Emi1 by RNA interference fail to accumulate Cyclin A and do not enter S phase. Overexpression of Cdh1 causes a G1 arrest. 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).

The *Drosophila* ortholog of Emi1, *regulator of cyclin A1 (rca1)*, when mutated, causes an embryonic cell cycle arrest identical to *cyclin A* mutants. Ectopic expression of *rca1* drives cells into S phase and causes an increase in Cyclin A protein levels (Dong et al. 1997). A subsequent study demonstrated that Rca1 acts to inhibit APC/C^{Fzr/Cdh1} in G2 (Grosskortenhaus & Sprenger 2002). Although vertebrate Emi1 is thought to inhibit both Cdc20 and Cdh1, Rca1 seems to affect only Cdh1. Grosskortenhaus & Sprenger (2002) failed to detect an association between Fzr/Cdc20 and Rca1 and confirmed through genetic studies that the effect of Rca1 on cyclin levels is specific to Fzr. It is not clear whether, in addition to its role in G2, Rca1 also regulates S phase entry in *Drosophila* embryos 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 inhibit APC/C^{Cdc20}, but a recent report suggests that it restricts APC/C^{Cdh1} activity at the G1-S transition by inhibiting SCF ^{β -TrCP} and thereby permitting accumulation of Emi1 (Whitehurst et al. 2008).

Acm1

The APC/C^{Cdh1} modulator 1 (Acm1) protein was identified in *S. cerevisiae* by its ability to bind to Cdh1 together with a 14-3-3 protein (Martinez et al. 2006, Dial et al. 2007). Acm1 inhibits Cdh1 activity as a pseudosubstrate, and it provides an additional mechanism to restrict Cdh1 activity to the proper cell cycle phase. The inhibitory effect of Acm1 is released by its ubiquitination by APC/C^{Cdc20} after the metaphase-to-anaphase transition, when it also becomes subject to ubiquitination by activated APC/C^{Cdh1} (Enquist-Newman et al. 2008). Although the protein is conserved among budding yeast species, orthologs have not been identified in other organisms (Martinez et al. 2006).

Rae1-Nup98

In mammalian cells, as in yeast, inhibitory phosphorylation of Cdh1 was concluded to fully inactivate APC/C^{Cdh1} during mitosis. An investigation of the roles of Rae1 and Nup98, however, revealed an additional layer of regulation of APC/C^{Cdh1} in mammalian mitosis as well. This work suggests that, at least in mammalian cells, Cdh1 is bound to APC/C early in mitosis and can participate in controlling the metaphase-to-anaphase transition. 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 (Jeganathan et al. 2005). In mitotic HeLa cell extracts, Cdc27, Cdc16, and Cdh1 coimmunoprecipitate with Rae1 and Nup98, but Cdh1 rather than Cdc20 is associated. Rae1-Nup98 inhibits ubiquitination of Securin but not that of 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 the previous model, APC/C^{Cdh1} may play a role in targeting Securin for degradation upon anaphase onset. How Rae1-Nup98 mediates inhibition of APC/C^{Cdh1}, as well as how Rae1-Nup98 is targeted to the APC/C and triggered to dissociate from the APC/C, remain to be elucidated.

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 coimmunoprecipitate with core APC/C subunits as well as with both Cdc20 and Cdh1. Knockdown of CBP by RNA interference leads to increases in 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 in 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 gener-

ated 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 the developmental context in meiosis compared with mitosis.

In meiosis, two rounds of chromosome segregation without an intervening S phase generate haploid gametes. Both general cell cycle regulators as well as meiosis-specific proteins coordinate control of the meiotic divisions (reviewed in Marston & 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 between the sister chromatids. 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 because Separase must be activated twice, in both meiosis I and meiosis II, through APC/C-mediated degradation of Securin.

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, 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). Cdk1/Cyclin B 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 the initiation of DNA replication. Presumably, APC/C-mediated degradation of 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**)

Homologous chromosomes: chromosomes from different parent cells containing the same genetic loci that pair and then segregate during meiosis I

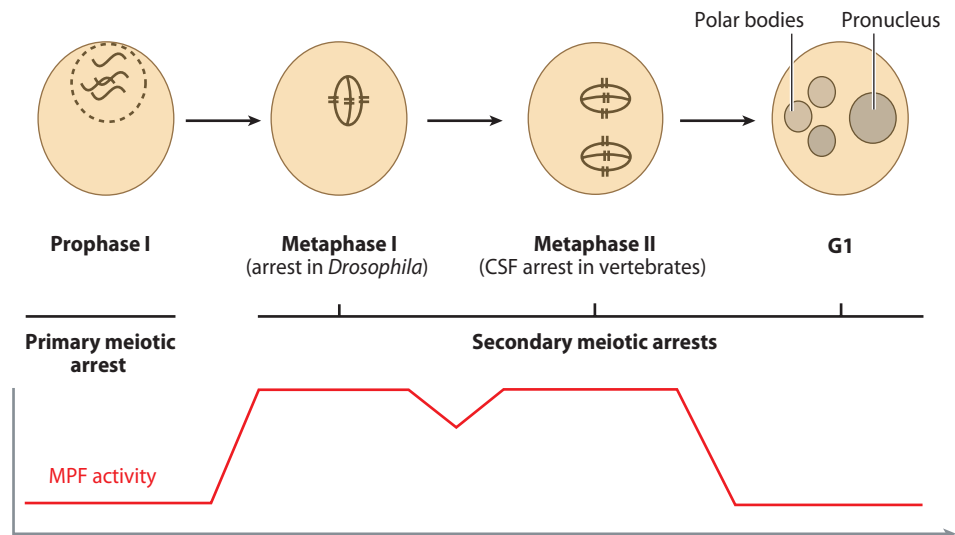


Figure 3

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. Fertilization releases the CSF arrest to permit the completion of meiosis II. MPF [CycB/Cdc2 (Cdk1)] activity levels rise upon entry into the meiotic divisions. They must drop in between meiosis I and meiosis II for exit from 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 further details and references.

(reviewed in Kishimoto 2003, Tunquist & Maller 2003). During both meiotic arrests, APC/C activity must be suppressed. In prophase I, unscheduled activity of the APC/C may 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 the control of chromosome segregation and cell cycle progression and the 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 or-

ganisms tested, with the exception of *Xenopus* oocytes. In yeast, Cdc20 is required for the degradation of Pds1/Securin and the resulting activation of Separase in both meiotic divisions (Salah & 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).

The requirement for APC/C in meiosis is conserved in multicellular organisms. In *Caenorhabditis elegans*, mutations in or RNA interference against several subunits of the APC/C cause a meiotic metaphase I arrest, as would be expected if Separase cleavage of cohesin were needed for release of arm cohesion and separation of homologs (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 & 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). Although microinjection of *Xenopus* oocytes with antibodies against Fzy or Cdc27 or antisense oligonucleotides against Fzy does not disrupt progression through meiosis I but only causes an arrest in meiosis II (Peter et al. 2001, Taieb et al. 2001), it is possible that these experiments failed to eliminate APC/C activity. Securin and Cyclin B are degraded at anaphase I, reaccumulate before metaphase II, and are degraded again at anaphase II (Fan et al. 2006). Additionally, misexpression of Emi2/Erp1, 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 transition from metaphase I to anaphase I (Ohe et al. 2007, Tung et al. 2007).

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 (**Table 1**). 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). Ama1 in *S. cerevisiae* associates with the core APC/C throughout meiosis, although it does not become fully active until after meiosis I, when it 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 *Schizosaccharomyces pombe* Mfr1/Fzr1 also associates with the core APC/C during meiosis

and is required for the degradation of the 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 & Orr-Weaver 1996). *cort* seems to be required as soon as the transition from metaphase I to anaphase I occurs; meiosis II spindles often exhibit unequal numbers of chromosomes, indicating aberrant chromosome segregation in meiosis I (Page & Orr-Weaver 1996). Consistent with *cort* functioning as an APC/C activator, Cyclin A, B, and B3 levels are elevated in *cort* eggs, and misexpression of *cort* triggers degradation of these cyclins in wing imaginal discs (Pesin & Orr-Weaver 2007, Swan & Schupbach 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 female meiosis-specific and male meiosis-specific APC/C activators 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. The Ama1 protein is present beginning in premeiotic S phase, although it does not become essential for spore formation until late in meiosis (Cooper et al. 2000, Oelschlaegel et al. 2005, Penkner et al. 2005). This disparity in the timing of Ama1 presence and function is explained by the action of an APC/C^{Ama1} inhibitor that is

Germinal vesicle breakdown (GVBD): disintegration of the large meiotic nuclear envelope at the end of meiotic prophase I

discussed below. Cdc20, in contrast, is required in meiosis I; Cdc20 meiotic mutants arrest in metaphase I with high levels of Pds1 (Salah & 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 increases in Pds1 and Clb5 protein levels (Oelschlaegel et al. 2005). Cdc20 levels peak in both meiosis I and meiosis II, suggesting that it too is important for both divisions (Salah & Nasmyth 2000).

Similarly, in *Drosophila*, both *fzy* and *cort* are required for female meiotic divisions. Swan & Schupbach (2007) used double-mutant genetic analysis to demonstrate that *fzy* and *cort* have redundant roles in meiosis I but nonredundant roles in meiosis II. In *fzy* single mutants, a small percentage of eggs arrest with one spindle indicative of meiosis I, but the majority of eggs arrest in anaphase II. In *cort* single mutants, a similar percentage of eggs arrest in meiosis I, but the rest arrest 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 exhibit the *cort* mutant phenotype of 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 nonredundant and different temporal roles in meiosis II: *cort* is required for the degradation of Cyclin B specifically at the spindle midzone, whereas *fzy* seems to target Cyclin B after *cort* and along the entire spindle.

In addition to Cort and Fzy playing some nonredundant roles in meiosis, their protein expression patterns during meiosis are quite different. Fzy is expressed at a constant level throughout oogenesis and embryogenesis, whereas Cort is specifically expressed in a narrow window during the female meiotic divisions (Pesin & Orr-Weaver 2007). Both posttranscriptional control and posttranslational control contribute to the unique expression pattern of Cort. Polyadenylation of *cort* mRNA

at oocyte maturation is controlled by the Gld2 poly(A) polymerase and is required for the appearance of the Cort protein as the oocyte enters the meiotic divisions (Pesin & Orr-Weaver 2007, Benoit et al. 2008). 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 may be due to a role for Cdh1 in meiosis, or Cdh1 may be required in the previous mitotic cell cycle, and its failure may 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. 2006a, Marangos et al. 2007). In contrast, injection of Cdh1 antisense oligonucleotides into *Xenopus* oocytes inhibits oocyte maturation and release of the prophase I arrest (Papin et al. 2004).

Reis et al. (2007) showed a role for Cdh1 after germinal vesicle breakdown (GVBD) in mouse oocytes. Injection of oocytes with *cdh1* antisense morpholinos accelerates progression through meiosis I, resulting in premature anaphase and nondisjunction of homologs. These defects are specific to inhibition of *cdh1* after GVBD. Furthermore, 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 the use of APC/C activators during meiosis. In this model, APC/C^{Cdh1} prolongs prometaphase I by causing degradation of Cdc20. This delay may serve to allow more time for homologs to congress properly 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 may lead to aneuploid gametes and offspring, which is a leading cause of spontaneous abortion and mental retardation (Hassold & 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, Ama1, 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 Ama1 may be inhibited in early meiosis. Two studies in *S. cerevisiae* revealed a crucial inhibitor of APC/C^{Ama1} 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, genetic suppression and in vitro ubiquitination assays revealed that Mnd2 inhibits APC/C^{Ama1} (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^{Ama1} but not that of APC/C^{Cdc20} or APC/C^{Cdh1} (Oelschlaegel et al. 2005). The mechanism of Mnd2 inhibition of APC/C^{Ama1} is unclear. In vivo, the absence of Mnd2 causes an increased association of Ama1 with the APC/C core, but upon addition in vitro, Ama1 equally coimmunoprecipitates with 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 *mnd2Δ* reveals the disastrous consequences of unrestrained

APC/C^{Ama1} activity in meiotic prophase I. *mnd2Δ* cells fail to accumulate Pds1 in S phase and prophase I 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 Oelschlaegel et al. (2005) and Penkner et al. (2005) demonstrated that these defects are the result of premature activation of Separase and cleavage of Rec8 in prophase I 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 phenomenon 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, the activity of Cdk1 kinases may inhibit Ama1. Inhibition of Cdk1 in *cde20* 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

In vertebrates Emi1 may function to limit APC/C activity during the prophase I arrest (Figure 4). Emi1 protein is present in mouse oocytes but is degraded at GVBD in a SCF^{β-TriCP}-dependent manner, mirroring the timing of Emi1 destruction in mitotic cells in late prophase (Marangos et al. 2007). Reduction of mouse Emi1 function by injection of morpholinos in prophase I delays entry into the first meiotic division by preventing the accumulation of Cyclin B necessary for maturation-promoting factor (MPF) activation and progression through meiosis I. APC/C^{Cdh1} activity mediates these effects of inhibiting Emi1 function in mouse oocytes (Marangos et al. 2007).

MPF: maturation-promoting factor

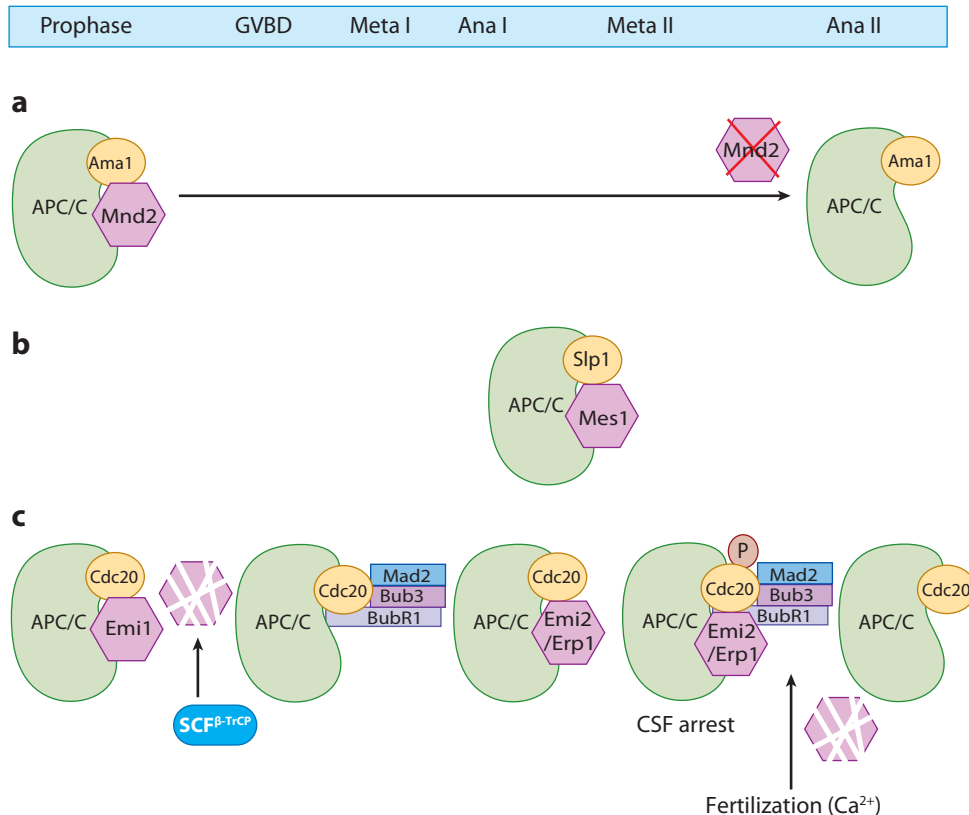


Figure 4

Meiotic APC/C regulators. (a) In *Saccharomyces cerevisiae* Mnd2 inhibits APC/C^{Ama1}, a meiosis-specific form of the APC/C, beginning in prophase I. Inhibition is not released until late meiosis, when the 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 *Schizosaccharomyces pombe* Mes1 is an inhibitor of APC/C^{Slp1/Cdc20} in between the meiotic divisions. Mes1 is an APC/C substrate but is able to compete for binding to the APC/C more efficiently than can other substrates. (c) In vertebrate prophase I, prior to germinal vesicle breakdown (GVBD), inhibition of APC/C^{Cdh1} by Emi1 allows for an accumulation of MPF, which is required for entry into the first meiotic division. Emi1 is targeted for degradation by SCF^{β-TrCP} at GVBD. The spindle assembly checkpoint is thought to regulate APC/C^{Cdc20} activity in meiosis I for proper homolog alignment and disjunction. Similar to the role of Mes1, Emi2/Erp1 (Emi-related protein 1) helps maintain low APC/C^{Cdc20} activity levels in between the two meiotic divisions. Emi2/Erp1 is also a main component of cytotstatic factor (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/Erp1, which ultimately leads to the degradation of Emi2/Erp1 by SCF^{β-TrCP} (see **Figure 2**). 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 further references and details. Meta I and II denote metaphase I and II, respectively; Ana I and II denote anaphase I and II, respectively.

Exogenous Emi1 is rapidly degraded during oocyte maturation in *Xenopus* oocytes (Ohsumi et al. 2004). Emi1 may contribute to prophase I arrest in *Xenopus* as well as mouse, but problems

with antibodies that cross react with both Emi1 and Emi2/Erp1 (see below) has led to confusion about its function in *Xenopus* meiosis (Tung & Jackson 2005).

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 the addition of Mes1 to a *Xenopus* egg extract inhibits APC/C activity; both of these observations are 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} (**Table 1**), 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-ubiquitlatable 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 do 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**). Emi2/Erp1 is not expressed until after GVBD, and its expression is coincident with polyadenylation of *Emi2/Erp1* mRNA (Ohe et al. 2007, Tung et al. 2007). Inhibition of Emi2/Erp1 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/Erp1 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 to allow entry into meiosis II (Madgwick et al. 2006).

A second critical role for Emi2/Erp1 in meiosis is the 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 the completion of meiosis before fertilization occurs. Many years of research determined that the establishment of CSF arrest involves the Mos/MAPK/p90^{Rsk} signaling pathway and Cyclin E/Cdk2 activity through the inhibition of APC/C^{Cdc20} (for a review, see Tunquist & Maller 2003). Release of CSF arrest is triggered by a transient increase in free cytosolic Ca²⁺ levels induced by fertilization.

Emi2/Erp1 is present in extracts prepared from CSF-arrested *Xenopus* eggs and degraded upon the addition of Ca²⁺, a treatment known to release CSF arrest in these extracts (Schmidt et al. 2005). Furthermore, depletion of Emi2/Erp1 causes premature release from CSF arrest, independent of Ca²⁺ addition (Schmidt et al. 2005). Conversely, the addition of exogenous or nondegradable Emi2/Erp1 prevents Ca²⁺-induced CSF release. Complementary

CSF: cytostatic factor

results were observed in intact *Xenopus* eggs (Tung et al. 2005). In mouse oocytes, Emi2/Erp1 appears to play the same role in CSF arrest. These effects are likely to occur through Emi2/Erp1 inhibition of APC/C^{Cdc20} because Emi2/Erp1 can inhibit ubiquitination of substrates by APC/C^{Cdc20} in vitro (Schmidt et al. 2005, Tung et al. 2005). In addition, mouse Emi2/Erp1 binds to Cdc20 in vitro (Shoji et al. 2006).

Recent studies in *Xenopus* have found additional sites of phosphorylation on Emi2/Erp1, some of which directly link Emi2/Erp1 to the Mos/MAPK/p90^{Rsk} pathway in CSF arrest (**Figure 2**). The ability of Emi2/Erp1 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/Erp1 in vitro, and these sites of phosphorylation are required in vivo for Emi2/Erp1 stability during oocyte maturation and for the CSF activity of Emi2/Erp1 (Inoue et al. 2007, Nishiyama et al. 2007a). This work strongly suggests that, at least in amphibian oocytes, Emi2/Erp1 is the effector protein of Mos/MAPK/p90^{Rsk} signaling in CSF arrest.

Nishiyama et al. (2007a) shed light on the mechanism of Emi2/Erp1 inhibition of APC/C. Phosphorylation of Emi2/Erp1 by p90^{Rsk} enhances the association of Emi2/Erp1 with the APC/C, consistent with the role of p90^{Rsk} in activating Emi2/Erp1 inhibitory activity. Like Emi1, Emi2/Erp1 contains a D-box that also enhances the association of Emi2/Erp1 with APC/C. This association is inhibited in vitro by D-box peptides, suggesting that Emi2/Erp1 blocks substrate access to the APC/C. In addition, phosphorylation of Emi2/Erp1 by Cyclin B/Cdc2 (Cdk1) at sites distinct from those targeted by CaMKII and p90^{Rsk} may disrupt the interaction between Emi2/Erp1 and APC/C (**Figure 2**) (Hansen et al. 2007, J.Q. Wu et al. 2007, Q. Wu et al. 2007). This phosphorylation is antagonized by protein phosphatase 2A (PP2A). PP2A is recruited to Emi2/Erp1 by p90^{Rsk} phosphorylation and may provide a feedback loop that serves to maintain proper levels of Cyclin B and Cdc2/Cdk1 kinase

activity during CSF arrest (J.Q. Wu et al. 2007).

Degradation of Emi2/Erp1 upon Ca²⁺-induced CSF release appears to be the critical event that relieves the inhibition of APC/C^{Cdc20} and allows passage from metaphase II to anaphase II. Ca²⁺ plays a crucial role in controlling Emi2/Erp1 degradation by indirectly affecting phosphorylation of Emi2/Erp1 by Plx1 through its effect on calmodulin kinase II (CaMKII) (Lorca et al. 1993, Schmidt et al. 2005, Tung et al. 2005, Hansen et al. 2006). Plx1 kinase phosphorylation of Emi2/Erp1 promotes Emi2/Erp1 degradation by SCF ^{β -TrCP} (**Figure 2**). A relationship between CaMKII and Plx1 was shown by the observations that CSF release triggered by a constitutively active CaMKII (CamCat) requires Plx1 and Plx1-induced CSF release requires active CaMKII (Liu & 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/Erp1 by CaMKII enhances Plx1 binding to and phosphorylation of Emi2/Erp1 in vitro, and degradation of Emi2/Erp1 in anaphase extracts is dependent on phosphorylation by CaMKII, strongly suggesting that CaMKII is the priming kinase for Plx1 upon CSF release (Liu & Maller 2005, Rauh et al. 2005, Hansen et al. 2006).

An additional Ca²⁺-induced pathway is involved in the 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 that of Cdc20/Fzy (Mochida & Hunt 2007). The addition of a calcineurin inhibitor prevents this dephosphorylation, delays degradation of CyclinB2, and prevents exit from meiosis II after the addition of Ca²⁺ (Mochida & Hunt 2007, Nishiyama et al. 2007b). Mochida & Hunt (2007) report that coimmunoprecipitation 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 apparent dephosphorylation of Apc3 and Fzy/Cdc20 protein. When phosphorylated, Cdc20/Fzy is inhibited from activating APC/C during spindle checkpoint arrest (Yudkovsky et al. 2000, Chung & 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 the release of the metaphase II arrest in CSF eggs (**Figure 4**). Consistent with the *Xenopus* results, calcineurin signaling is also involved in meiosis in *Drosophila* because mutations in *sarab*, a gene encoding an inhibitor of calcineurin, disrupt the meiotic divisions in females (Horner et al. 2006).

In summary, Emi2/Erp1 plays a critical role in CSF arrest by inhibition of APC/C^{Cdc20}. Mos, through p90^{Rsk}, contributes to this inhibition because p90^{Rsk} phosphorylation of Emi2/Erp1 recruits PP2A. PP2A dephosphorylates several sites of phosphorylation by Cdc2/Cyclin B on Emi2/Erp1 that would disrupt its interaction with and inhibition of APC/C^{Cdc20}. The Ca²⁺ increase at fertilization causes degradation of Emi2/Erp1 by CaMKII activation of Plx1, whose phosphorylation of Emi2/Erp1 targets it for destruction by SCF ^{β -TrCP}. The Ca²⁺ increase additionally inactivates APC/C^{Cdc20} by dephosphorylation of Apc3 and Cdc20 by the calcineurin phosphatase.

Spindle Assembly Checkpoint

In mitosis the SAC inhibits APC/C^{Cdc20} in the presence of improper kinetochore microtubule attachments. Some components of the SAC are dispensable, whereas others, particularly those in metazoans, are essential. This is likely because they carry out essential mitotic functions in addition to surveillance mechanisms (Hoyt et al. 1991, Li & Murray 1991, Kitagawa & Rose 1999, Dobles et al. 2000, Kalitsis et al. 2000, Buffin et al. 2007).

In meiosis, SAC proteins appear to be required not only upon spindle damage but also

for the normal mechanism of meiosis I. It remains to be determined whether this role of the SAC is mediated by inhibition of the APC/C. 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 the inhibition of APC/C (Shonn et al. 2003). The requirement for spindle checkpoint function in a normal meiosis I division may be the consequence of the increased complexity of biorienting homologous chromosome pairs on the spindle compared with biorienting sister chromatid kinetochores in mitosis.

Higher organisms also display a requirement for spindle checkpoint genes in an undisturbed meiosis I. 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 in mouse oocytes must be of a sufficient length to allow enough time for homologous chromosomes to orient properly on the spindle and to 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, not metaphase II, as occurs in CSF arrest in vertebrates. 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 nondisjunction of both exchange and nonexchange chromosomes, which is likely due, in part, to defects in biorientation of homologous chromosomes in meiosis I (Gilliland et al. 2005, 2007). Additionally, in female meiosis of *bubR1* mutants in *Drosophila*, nondisjunction of sister chromatids is elevated (Malmanche et al. 2007).

There is little evidence to date as to whether the SAC functions in meiosis by inhibition of the APC/C. In the *Drosophila bubR1* mutants cohesin is lost from chromosomes in prophase I of the oocytes, but it has not been determined whether this effect occurs through loss of an inhibitory effect on APC/C activity in the *bubR1* mutant. In *S. cerevisiae* Mad3 (BubR1) 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 an uninhibited APC/C. The sole example of a link between the SAC in meiosis and the APC/C comes from studies in *C. elegans*, in which mutations in spindle checkpoint genes suppress a metaphase I arrest caused by leaky alleles of the *cdc23* gene, which encodes the APC8 subunit (Stein et al. 2007).

A role for the spindle checkpoint in CSF arrest in meiosis II has not been shown conclusively. The spindle checkpoint was hypothe-

sized to be a component of CSF arrest because Bub1 is phosphorylated by p90^{Rsk} during oocyte maturation in *Xenopus* oocytes, placing the SAC proteins downstream of the Mos/MAPK pathway that is required for the establishment of CSF arrest (Schwab et al. 2001). Immunodepletion of Bub1, Mad1, or Mad2 prevents the establishment of CSF arrest in *Xenopus* oocytes, and this effect is thought to occur downstream of Mos (Tunquist et al. 2002, Tunquist & Maller 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 the establishment of CSF arrest, they are not required for maintenance of the arrest in CSF extracts (Tunquist et al. 2002, Tunquist & Maller 2003). In mouse oocytes, expression of dominant-negative forms of Bub1, Mad2, or BubR1 that compete with their endogenous counterparts 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 the 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, there are special forms of the APC/C 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.

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, in yeast by Acm1, and in mammals by Rae1-Nup98 in prometaphase.
5. In addition to Cdc20 and Cdh1, yeast and *Drosophila* use meiosis-specific APC/C activators: Ama1 in *S. cerevisiae*, Mfr1/Fzr1 in *S. pombe*, and Cort in *Drosophila*.
6. During metaphase I and metaphase II, APC/C activity must be suppressed. In addition, APC/C needs to remain inactive in female meiosis (*a*) during the long prophase arrest to prevent premature loss of cohesion and (*b*) 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. What are the relative contributions of inactivation/degradation of Emi2/Erp1 versus dephosphorylation of Apc3 and Cdc20/Fzy by calcineurin in the release of CSF arrest?
6. Are there meiosis-specific APC/C activators in vertebrates?

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Errata

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