Taming the Beast: Control of APC/C^{Cdc20}-Dependent Destruction

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The anaphase-promoting complex/cyclosome (APC/C) is a large multisubunit ubiquitin ligase that triggers the metaphase-to-anaphase transition in the cell cycle by targeting the substrates cyclin B and securin for destruction. APC/C activity toward these two key substrates requires the coactivator Cdc20. To ensure that cells enter mitosis and partition their duplicated genome with high accuracy, APC/C^{Cdc20} activity must be tightly controlled. Here, we discuss the mechanisms that regulate APC/C^{Cdc20} activity both before and during mitosis. We focus our discussion primarily on the chromosomal pathways that both accelerate and delay APC/C activation by targeting Cdc20 to opposing fates. The findings discussed provide an overview of how cells control the activation of this major cell cycle regulator to ensure both accurate and timely cell division.

During cell division, genome stability depends on tight regulation of anaphase, the mitotic stage in which sister chromatids are separated. Anaphase should only occur after sister chromatids of all replicated chromosomes have correctly attached to opposite poles of the mitotic spindle (Fig. 1A). Progression into anaphase before achieving this fully attached state can lead to errors in chromosome segregation and aneuploidy, a hallmark of birth defects and cancer (Holland and Cleveland 2012; Santaguida and Amon 2015; Funk et al. 2016).

In eukaryotes, anaphase onset is triggered by the anaphase-promoting complex/cyclosome (APC/C), a large E3 ubiquitin ligase (Fig. 1A,B; Peters 2006; Pines 2011; Primorac and Musacchio 2013; Barford 2015). When the APC/C is active, it promotes the polyubiquitination of its substrates, which leads to their proteasome-mediated degradation. The essential APC/C substrates for anaphase onset are securin and cyclin B. Securin is the inhibitor of separase, the cysteine protease that cleaves a subunit of the cohesin complex that holds sister chromatids together. Cyclin B is the activator of Cdk1, the essential kinase that drives mitotic entry. Therefore, degradation of securin and cyclin B simultaneously results in chromosome segregation and exit from mitosis.

APC/C activity requires binding to a class of proteins known as coactivators that all harbor a carboxy-terminal WD40 domain (Fig. 1C). Although there are species-specific APC/C coactivators that participate in meiosis, such as Ama1 in *Saccharomyces cerevisiae* (Cooper et al. 2000) and Cortex in *Drosophila melanogaster* (Chu et al. 2001), the two widely conserved APC/C coactivators are Cdc20 and Cdh1. Cdc20 is essential for mitotic pro-

gression and Cdc20 depletion or mutation results in highly penetrant metaphase arrest and lethality (Dawson et al. 1995; Sigrist et al. 1995; Lim et al. 1998; Kitagawa et al. 2002; Li et al. 2007). In contrast, depletion or mutation of Cdh1 results in milder cell cycle defects (Schwab et al. 1997; Sigrist and Lehner 1997; Fay et al. 2002; Garcia-Higuera et al. 2008). The current view in the field is that Cdh1 has important roles in postmitotic contexts, including during cell differentiation and in the formation of the nervous system (Eguren et al. 2011). In this perspective, we discuss Cdc20 and the control of Cdc20-activated APC/C during the cell cycle, with a focus on new findings on the control of this key activity by chromosomes during mitosis.

APC/C^{Cdc20} activity is linked to cell cycle progression (Fig. 2). During the majority of the cell cycle, APC/C^{Cdc20} activity is inhibited (Peters 2006; Pines 2011). At mitotic entry, APC/C^{Cdc20} is activated by cyclin B-Cdk1, the essential mitotic kinase complex (see below). This activation itself could explain the cell cycle oscillator: the increase in Cdk1-cyclin B gradually activates APC/C^{Cdc20} and, once its activity reached a critical threshold, APC/C^{Cdc20} degrades cyclin B to inactivate Cdk1 leading to mitotic exit and reverting APC/C^{Cdc20} back to its inhibited state. However, this view is too simplistic, as APC/C^{Cdc20} activity is tightly regulated, most importantly by the chromosomal cargo of cell division. Here, we briefly review the structure and activity of the APC/C and then discuss mechanisms that control APC/C^{Cdc20}, with a focus on the chromosomal mechanisms that balance the need for accurate segregation with timely mitotic progression.

Figure 1. Anaphase-promoting complex/cyclosome (APC/C) structure and mechanism of substrate recognition. (A) Cartoon illustrating the metaphase-to-anaphase transition, which is promoted by APC/C^{Cdc20} activity. Microtubules are in yellow, chromosomes in blue, and kinetochores in gray. (B) Structure of APC/C^{Cdc20} bound to a D-box-containing substrate, Hsl1 (Zhang et al. 2016). The substrate binds to the interphase between Cdc20 and the APC/C subunit Apc10 (adapted from Corbett 2017). (C) Schematic illustrating the domains in human Cdc20. The C-box, KILR, and IR-tail motifs contribute to APC/C binding, whereas the WD40 domain is involved in substrate recognition. Inhibitory Cdk1 phosphorylation sites are shown in red, whereas S92, which is phosphorylated by Plk1, is in orange. Note that the KILR motif is also the Mad2 interacting motif. (D) Structure of the WD40 domain of *Saccharomyces cerevisiae* Cdh1 bound to an inhibitor, Acm1 (He et al. 2013). The structure shows the interaction sites for the three APC/C degrons: D-box, KEN box, and ABBA motif. (Adapted from Corbett 2017.)

A BRIEF OVERVIEW OF APC/C STRUCTURE AND MECHANISM OF PROTEIN UBIQUITINATION

The APC/C is a large complex composed of 14–16 subunits, depending on the species (Peters 2006; Pines 2011). Coactivator binding is essential for APC/C activity and for the recruitment of substrates. All APC/C coactivators possess a carboxy-terminal WD40 domain that is required for substrate recognition. In addition, the C-box and IR-tail motifs participate in APC/C binding (Schwab et al. 2001; Passmore et al. 2003; Vodermaier et al. 2003). Other coactivator-specific motifs correspond to the KLLR motif in Cdh1 (Chang et al. 2015) and KILR motif in Cdc20 (Izawa and Pines 2012), which also contribute to APC/C binding (Fig. 1C). Understanding of APC/C regulation has been greatly advanced in recent years by high-resolution cryo-EM studies that have revealed how each

subunit is assembled into the complex, how coactivators promote APC/C activity, and how different regulators control APC/C activity (Fig. 1B; Buschhorn et al. 2011; da Fonseca et al. 2011; Frye et al. 2013; Barford 2015; Chang et al. 2015; Alfieri et al. 2016; Yamaguchi et al. 2016; Zhang et al. 2016).

The APC/C recognizes substrates that possess degrons (Fig. 1D) known as the D-box [RXXL] and KEN box (Glutzer et al. 1991; Pfleger and Kirschner 2000). Other substrate-specific degrons such as the A-box in Aurora A (Littlepage and Ruderman 2002) and the O-box in Orc1 (Araki et al. 2005) have been described, although the latter was subsequently found to function as a D-box (He et al. 2013). In addition, Cdc20 and yeast Cdh1 interact with proteins containing a motif known as the Phe box or ABBA motif (for Acm1, Bub1, BubR1, and cyclin A) (Lu et al. 2014; Di Fiore et al. 2015; Diaz-Martinez et al. 2015) that can serve as a degron in some cases,

Figure 2. Regulation of APC/C activity during the cell cycle. Schematic illustrates the current model for the temporal regulation of the activities of Cdk1–Cyclin B (red), APC/C^{Cdh1} (orange), and APC/C^{Cdc20} toward cyclin A (blue) or cyclin B (green).

such as for cyclin A (Di Fiore et al. 2015). When bound by coactivators, the APC/C forms a bipartite receptor for D-box substrates that comprises the side of the WD40 barrel and the subunit Apc10/Doc1 (Fig. 1B; Passmore et al. 2003; Carroll et al. 2005; Kraft et al. 2005; Matyskiela and Morgan 2009; Buschhorn et al. 2011; da Fonseca et al. 2011). In addition, the WD40 barrel serves as the receptor for KEN box and ABBA substrates (Fig. 1D; Chao et al. 2012; He et al. 2013). In vertebrates, the APC/C functions with two E2 ubiquitin conjugating enzymes: UbcH10/Ube2C, and UbcH5/Ube2D (King et al. 1995; Aristarkhov et al. 1996); in addition, Ube2S participates in ubiquitin chain extension (Garnett et al. 2009; Williamson et al. 2009; Wu et al. 2010). On the other hand, budding yeast uses Ubc4 for mono-ubiquitination and Ubc1 for ubiquitin chain extension (Rodrigo-Brenni and Morgan 2007). Inhibitors of the APC/C, such as Emi1 or the mitotic checkpoint complex (discussed below), are known to regulate multiple aspects of APC/C function, including coactivator binding, substrate recognition, and activity/binding of the E2 enzymes that act in conjunction with the APC/C to catalyze substrate ubiquitination.

APC/C^{Cdc20} INHIBITION DURING G₂

A major target of the APC/C activated by Cdc20 is cyclin B, the activator of the essential mitosis-promoting kinase Cdk1 (Peters 2006; Pines 2011). Thus, APC/C^{Cdc20} activity must be kept in check during interphase to allow sufficient accumulation of cyclin B for mitotic entry.

Cdc20 itself is only synthesized in late S phase and its levels reach a maximum in mitosis (Weinstein 1997; Prinz et al. 1998; Shirayama et al. 1998), which may partially contribute to limiting APC/C^{Cdc20} activity in interphase. In addition, interphase APC/C is precluded from binding to Cdc20 by an auto-inhibitory mechanism that is released upon mitotic phosphorylation (see below). However in vitro, Cdc20 can efficiently activate interphase, non-phosphorylated APC/C (Fang et al. 1998), suggesting that other mechanisms also contribute to inhibiting Cdc20 before mitotic entry.

An initial candidate was the APC/C inhibitor Emi1 (Rca1 in *Drosophila*). (Dong et al. 1997; Reimann et al. 2001a, b; Grosskortenhaus and Sprenger 2002). However, although in vitro Emi1 can inhibit both APC/C^{Cdc20} and APC/C^{Cdh1}, its physiological target appears to be Cdh1 (Di Fiore and Pines 2007; Machida and Dutta 2007). An ortholog of Emi, called Emi2 or XErp1, inhibits APC/C^{Cdc20} to maintain the metaphase II arrest of mature *Xenopus* eggs (Schmidt et al. 2005; Tung et al. 2005). A role for Emi2 beyond meiotic arrest has been reported in developing *Xenopus* embryos, where it inhibits APC/C^{Cdc20} to promote cyclin B accumulation (Tischer et al. 2012). However, a mouse knockout of Emi2 is sterile and shows defects in meiotic progression but develops normally (Gopinathan et al. 2017), suggesting that it does not make a major contribution to somatic divisions in other systems. Thus, at present, Cdc20 synthesis and auto-inhibition of the APC/C that is relieved by mitotic phosphorylation are the major mechanisms implicated in

keeping APC/C^{Cdc20} in check to allow sufficient building of cyclin B and mitotic entry.

CYTOPLASMIC AND CHROMOSOMAL REGULATION OF APC/C^{Cdc20} ACTIVITY IN MITOSIS

Upon nuclear envelope breakdown, the APC/C binds to Cdc20 and immediately becomes active toward substrates such as cyclin A and Nek2A (van Zon and Wolhuis 2010). However, cyclin B and securin are only degraded once all chromosomes have attached to spindle microtubules via their kinetochores, the protein assemblies build on their centromere regions to connect to spindle microtubules (Cheeseman 2014; Musacchio and Desai 2017). In addition to forming a dynamic microtubule interface, kinetochores function as signaling hubs where kinase and phosphatase activities are integrated to correct attachment errors and both promote as well as inhibit APC/C^{Cdc20} activation. A tight connection exists between microtubule attachment at kinetochores and APC/C^{Cdc20}-mediated degradation of securin and cyclin B, which ensures coordinated segregation of all chromosomes and prevents chromosome loss. Below we discuss both cytoplasmic and kinetochore-based mechanisms that control APC/C^{Cdc20} activity.

Cytosolic APC/C^{Cdc20} Activation by Phosphorylation

Studies in the late 1990s and early 2000s showed that APC/C phosphorylation during mitosis was a prerequisite for its activation by Cdc20 (Lahav-Baratz et al. 1995; Peters et al. 1996; Patra and Dunphy 1998; Shteinberg et al. 1999; Golan et al. 2002; Kraft et al. 2003). The mitotic kinases Cdk1 and Plk1 phosphorylate multiple APC/C subunits and this phosphorylation increases binding affinity for Cdc20 (Kraft et al. 2003). However, the biochemical and structural mechanism of this phospho-dependent regulation has only recently been elucidated (Fig. 3; Fujimitsu et al. 2016; Qiao et al. 2016; Zhang et al. 2016). In brief, the APC/C subunit Apc1 possesses an internal loop that blocks the binding of the C-box of Cdc20 to the APC/C subunit Apc8. Thus, apo-APC/C is normally in an auto-inhibited state (Fig. 3A). Phosphorylation of the Apc1 loop by Cdk1 and Plk1 releases it from Apc8 and thereby promotes Cdc20 binding (Fig. 3B). In agreement with this model, mutation or deletion of the Apc1 loop permits Cdc20 binding regardless of APC/C phosphorylation status (Fujimitsu et al. 2016; Qiao et al. 2016; Zhang et al. 2016).

Interestingly, Apc1 phosphorylation is facilitated by an initial priming phosphorylation of the Apc3 subunit by Cdk1, which then recruits Cdk1–Cks complexes to further phosphorylate Apc3 and then Apc1. Moreover, the APC/C has been shown to be a weak substrate for Cdk1 in vitro and in vivo (i.e., it is only phosphorylated once high Cdk1 activity is achieved right before mitotic entry) (Lindqvist et al. 2007; Deibler and Kirschner 2010). These mechanisms may enforce a dependence on high Cdk1 activity

Figure 3. Control of APC/C^{Cdc20} activity by phosphorylation. (A) Schematic of apo-APC/C, showing auto-inhibition by the Apc1 loop. (B) The Cdk1 and Plk1 kinases phosphorylate the Apc1 loop, which releases the APC/C auto-inhibition mechanism. At the same time, Cdk1 phosphorylates the amino-terminal tail of Cdc20, which prevents its interaction with the APC/C. Dephosphorylation of Cdc20 by phosphatases (PPase) would cause its activation and binding to the APC/C.

and make APC/C^{Cdc20} activation kinetically lag behind initial Cdk1 activation, which may explain why APC/C^{Cdc20} only starts degrading substrates upon nuclear envelope breakdown. Although this model has good support from biochemical experiments in *Xenopus* egg extracts (Fujimitsu et al. 2016; Qiao et al. 2016), it will be important to assess whether phosphorylation of the Apc1 loop represents a conserved mechanism restraining activation of APC/C^{Cdc20} to mitosis in a cellular context. Interestingly, the interaction between APC/C and Cdh1 does not appear to be significantly affected by APC/C phosphorylation. This may be due to the fact that Cdh1 binds to the APC/C with higher affinity (Zhang et al. 2016), enabling it to efficiently displace the Apc1 loop from Apc8. This feature may explain the switch from APC/C^{Cdh1} to APC/C^{Cdc20} in late mitosis (see below).

Kinetochores-Mediated Cdc20 Activation through Dephosphorylation

As discussed above, Cdk1 activity promotes the interaction between APC/C and Cdc20. However, paradoxically Cdk1/2 proteins also block the binding of both Cdc20 and Cdh1 to the APC/C (Fig. 3B; Kramer et al. 2000; Yudkovsky et al. 2000). Phosphorylation sites near the amino-terminal C-box prevent the interaction of coactivators with the APC/C (Fig. 1C; Labit et al. 2012; Chang et al. 2015). Phosphorylated Cdc20 is found already in G₂ and, in human tissue culture cells, its phosphorylation may be important for the accumulation of cyclins and mitotic entry (Hein and Nilsson 2016). Interestingly, in *C. elegans* embryos, preventing Cdc20 phosphorylation significantly accelerates anaphase onset (Kim et al. 2017), indicating that Cdc20 phosphorylation is an important mechanism restraining APC/C^{Cdc20} activity in mitosis. These obser-

vations suggest that Cdc20 must be dephosphorylated to allow full APC/C activation (Fig. 3B).

In recent work, we showed that Cdc20 dephosphorylation, which contributes to APC/C activation, is promoted by kinetochores in *C. elegans* embryos (Fig. 4A; Kim et al. 2017). During mitosis, Cdc20 is recruited to kinetochores through its interaction with Bub1 (Di Fiore et al. 2015; Vleugel et al. 2015; Kim et al. 2017), a conserved component implicated in both the spindle assembly checkpoint and chromosome segregation (Bolanos-Garcia and Blundell 2011; Elowe 2011). Bub1, along with its binding partner Bub3, is recruited to kinetochores through the kinetochore scaffold Knl1, which is phosphorylated on “MELT” repeats in its amino terminus by the kinases Mps1 and Plk1 (London et al. 2012; Shepperd et al. 2012; Yamagishi et al. 2012; Espeut et al. 2015; von Schubert et al. 2015). At its extreme amino terminus, Knl1 possesses “SILK” and “RVxF” motifs that recruit the catalytic subunit of protein phosphatase 1 (PP1c) (Liu et al. 2010; Meadows et al. 2011; Rosenberg et al. 2011; Espeut et al. 2012). Our findings suggest that by bringing Cdc20 to the vicinity of Knl1-bound PP1, kinetochores catalyze Cdc20 activation by removing the inhibitory phosphorylation in its amino terminus (Fig. 4A). In support of this model, blocking Cdc20 or PP1 recruitment to kinetochores delays anaphase onset, an effect that can be bypassed by mutating the Cdk phosphorylation sites on Cdc20 (Kim et al. 2015, 2017). Notably, a role for kinetochore-localized Bub1-Bub3 in promoting APC/C^{Cdc20} activation has also been reported in budding yeast (Yang et al. 2015). As preventing Cdc20 recruitment to kinetochores does not result in a mitotic arrest, cytosolic phosphatases likely also promote Cdc20 dephosphorylation independently of kinetochores; alternatively, Cdc20 amino-terminal phosphorylation may not be sufficient to fully block its binding and activation of the APC/C.

In addition to its regulation by Cdk1/2, human Cdc20 is also inhibited by phosphorylation on Ser92 by Plk1 (Fig. 1C; Craney et al. 2016; Jia et al. 2016; Lee et al. 2017). This phosphorylation is facilitated by Bub1 and is suggested to inhibit the recruitment of the E2 ubiquitin conjugating enzyme Ube2S to the APC/C. In late mitosis, Ser92 phosphorylation is reversed by PP2A-B56 docked onto either BubR1 or the APC/C itself. Although this mechanism has biochemical support (Craney et al. 2016; Jia et al. 2016), its importance in an *in vivo* context is unclear, given that deletion of Ube2S (Wild et al. 2016) or mutation of Ser92 in Cdc20 (Lee et al. 2017) result in relatively mild effects on mitotic exit.

Kinetochores-Dependent APC/C^{Cdc20} Inhibition by the Spindle Assembly Checkpoint

Phosphorylation of the APC/C at mitotic entry that relieves inhibition of Cdc20 binding might explain why degradation of APC/C^{Cdc20} substrates such as cyclin A and Nek2A begins right at nuclear envelope breakdown, when mitotic kinases are active (van Zon and Wolthuis 2010). However, degradation of cyclin B and securin only occurs after microtubule binding to all kinetochores to

Figure 4. The two fates of Cdc20 at kinetochores. During mitosis, Cdc20 is recruited to kinetochores by Bub1/Bub3, which is bound to phospho-Knl1. (A) When kinetochores are attached by microtubules, kinetochores promote Cdc20 dephosphorylation by kinetochore-localized PP1c, which allows its activation. Cdc20 may also be dephosphorylated at the cytosol, likely through PP2A-B56. (B) When microtubules are unattached, signal from the spindle assembly checkpoint catalyzes the incorporation of Cdc20 into the mitotic checkpoint complex (MCC), which binds and inhibits APC/C^{Cdc20} activity. See text for more details.

prevent errors in chromosome segregation (Fig. 2). A large body of work has focused on how chromosomes regulate APC/C^{Cdc20} to prevent premature cyclin B and securin degradation, which is discussed below.

The spindle checkpoint is the mechanism that inhibits degradation of cyclin B and securin by APC/C^{Cdc20} in the presence of chromosomes with unattached kinetochores (Fig. 4B). When unattached, kinetochores catalyze the formation of an APC/C^{Cdc20} inhibitor known as the mitotic checkpoint complex or MCC, composed of BubR1 (Mad3 in yeast and nematodes), Bub3, Mad2, and Cdc20 (Sudakin et al. 2001). The spindle checkpoint has been subjected to extensive mechanistic analysis (for detailed reviews, see Lara-Gonzalez et al. 2012; Jia et al. 2013; Musacchio 2015; Etemad and Kops 2016; Corbett 2017). Here, we briefly summarize current understanding of how kinetochores control formation of the MCC and an interesting intertwining with the kinetochore-based APC/C activation mechanism that acts on Cdc20.

The initiation step in spindle checkpoint signaling is recruitment of the Mad1–Mad2 complex to kinetochores (Fig. 4B; Chen et al. 1996, 1998; Li and Benezra 1996; Maldonado and Kapoor 2011). Once localized, the Mad1–Mad2 complex recruits free, cytosolic Mad2, which is in an “open” conformation, and converts it into a “closed” form that captures Cdc20 (Mapelli and Musacchio 2007; Luo and Yu 2008; Rosenberg and Corbett 2015). The Mad2–Cdc20 dimer interacts with the BubR1–Bub3 complex to assemble the full MCC (Sudakin et al. 2001). The MCC then binds APC/C^{Cdc20} and inhibits the recruitment

of substrates; this is achieved by a conserved D-box–ABBA–KEN–ABBA cassette in BubR1 that prevents the formation of the bipartite Cdc20–Apc10 D-box substrate receptor, directly blocks the binding of E2 enzymes (Burton and Solomon 2007; Sczaniecka et al. 2008; Malureanu et al. 2009; Elowe et al. 2010; Lara-Gonzalez et al. 2011; Chao et al. 2012; Izawa and Pines 2015; Alfieri et al. 2016; Di Fiore et al. 2016; Yamaguchi et al. 2016; Sewart and Hauf 2017). Mps1, an essential spindle checkpoint kinase, is required for many steps in spindle checkpoint signaling, including the recruitment of the Bub1–Bub3 and Mad1–Mad2 complexes to kinetochores (Hewitt et al. 2010; Maciejowski et al. 2010; Santaguida et al. 2010; London et al. 2012; Sheppard et al. 2012; Yamagishi et al. 2012).

Microtubule attachment silences spindle checkpoint signaling employing at least three different mechanisms. First, microtubules promote the dynein motor-dependent “stripping” of spindle checkpoint proteins from the kinetochore (Howell et al. 2001; Wojcik et al. 2001). Second, microtubule attachment promotes PP1c recruitment to the kinetochore, which dephosphorylates the MELT repeats on Knl1 and therefore removes Bub1–Bub3 from kinetochores (Liu et al. 2010; Lesage et al. 2011; London et al. 2012; Espert et al. 2014; Nijenhuis et al. 2014). Third, microtubule binding by Ndc80 displaces Mps1 from kinetochores (Hiruma et al. 2015; Ji et al. 2015) (although this is not the case in budding yeast, where Mps1 persists at kinetochores even after microtubule attachment; Arava-

mudhan et al. 2015). In addition, cytosolic mechanisms such as p31-TRIP13 and Apc15-mediated Cdc20 autoubiquitination contribute to APC/C^{Cdc20} activation by catalyzing MCC disassembly (Habu et al. 2002; Xia et al. 2004; Reddy et al. 2007; Yang et al. 2007; Hagan et al. 2011; Jia et al. 2011; Mansfeld et al. 2011; Teichner et al. 2011; Westhorpe et al. 2011; Foster and Morgan 2012; Uzunova et al. 2012; Eytan et al. 2014; Ye et al. 2015; Yamaguchi et al. 2016; Zhang et al. 2016).

Integration of Mechanisms Activating and Inhibiting APC/C^{Cdc20} at the Kinetochore

As mentioned above, unattached kinetochores signal through the spindle checkpoint to inhibit APC/C^{Cdc20}. However, we have found that kinetochores also promote APC/C^{Cdc20} activation by removing inhibitory phosphates on the amino terminus of Cdc20 (Kim et al. 2017). How then can these opposing functions be reconciled? A key observation is that both mechanisms depend on the recruitment of Cdc20 to kinetochores (Fig. 4). Cdc20 is recruited through Bub1, which possesses a Cdc20-binding “ABBA” motif (Di Fiore et al. 2015; Vleugel et al. 2015; Kim et al. 2017). Notably, this recruitment is highly dynamic with kinetochore-bound Cdc20 showing a half-life of 0.5–2 sec (Kallio et al. 2002; Kim et al. 2017). Thus, Cdc20 is rapidly fluxing through kinetochores via interaction with Bub1’s ABBA motif. Mutation of the ABBA motif on Bub1 not only prevents the kinetochore-dependent anaphase promoting function but also abolishes spindle checkpoint signaling (Di Fiore et al. 2015; Vleugel et al. 2015; Kim et al. 2017). Bub1 is critical to recruit the Mad1–Mad2 complex to unattached kinetochores (Klebig et al. 2009; London and Biggins 2014; Moyle et al. 2014; Zhang et al. 2017), although this function is independent of the ABBA motif (Vleugel et al. 2015; Kim et al. 2017). Therefore, recruitment of Cdc20 to the ABBA motif of Bub1 likely promotes formation of the MCC by bringing it in close proximity to active Mad1–Mad2 that is also bound to Bub1 (Fig. 4B). Interestingly, Mps1 phosphorylation of the carboxyl terminus of Mad1, which is essential for Mad1–Mad2 activation (Faesen et al. 2017), may also create a binding site for Cdc20 (Ji et al. 2017). Thus, Bub1’s ABBA motif may help generate a locally high concentration of Cdc20 at kinetochores that, if Mad1–Mad2 is present and phosphorylated, places Cdc20 on the Mad1 carboxyl terminus in close proximity to the conformationally converting Mad2 and promotes formation of the Mad2–Cdc20 complex that matures into the MCC (Fig. 4B).

The above-mentioned data suggest that Cdc20 recruited to kinetochores on a single site has two opposite fates: APC/C activation through Cdc20 dephosphorylation and APC/C inhibition through its incorporation on the MCC (Kim et al. 2017). Given that the spindle assembly checkpoint is only active at unattached kinetochores, the choice between these two fates is dependent on the status of kinetochore-microtubule interactions (Fig. 4A,B). At unattached kinetochores, spindle checkpoint signaling would cause Cdc20 to be primarily incorporated onto

the MCC to prevent premature APC/C^{Cdc20} activation, whereas following microtubule attachment, when the spindle checkpoint is silenced, Cdc20 would be primarily dephosphorylated and activated to promote anaphase onset. The switch between these two fates could be further sharpened by PP1c recruitment, which may be promoted or dependent on microtubule attachment (Trinkle-Mulcahy et al. 2003; Liu et al. 2010; Kim et al. 2017). It is possible that Cdc20 dephosphorylation occurs throughout mitosis, regardless of kinetochore–microtubule interactions. Regardless, the responsiveness of checkpoint signaling to microtubule attachment would still shift the balance between the opposing Cdc20 fates.

APC/C^{Cdc20} INACTIVATION IN LATE MITOSIS

Once securin and cyclin B are degraded, the APC/C is thought to switch coactivators from Cdc20 to Cdh1 (Fig. 2). APC/C^{Cdh1} activity in late mitosis is essential for the degradation of Aurora kinases (Floyd et al. 2008). In addition, APC/C^{Cdh1} is required in G₁ for the degradation of cyclins in order to allow the loading of pre-replication complexes onto chromatin for the subsequent S phase (for review, see Sivaprasad et al. 2007).

The Cdc20–Cdh1 switch is likely explained by the decline in Cyclin B–Cdk1 activity, enabling phosphatases to dephosphorylate the APC/C and reduce its affinity for Cdc20. At the same time, Cdh1, which is kept inactivated by Cdk-dependent phosphorylation throughout most of the cell cycle, would become dephosphorylated and bind to and activate the APC/C (Peters 2006; Pines 2011). However, some APC/C^{Cdc20} activity persists in late mitosis and indeed, many late APC/C substrates, such as Plk1, survivin and Cenp-F are reliant on Cdc20 for their degradation (Floyd et al. 2008; Gurden et al. 2010). Regardless, at anaphase onset, Cdc20 itself becomes an APC/C substrate and therefore, by G₁, the APC/C is mostly Cdh1-bound.

CONCLUSION

Since its discovery in the early 1990s as the machine that drives mitotic exit (King et al. 1995; Sudakin et al. 1995), the APC/C and its coactivator Cdc20 have been extensively studied. In the last 5 years, advances in high-resolution cryo-EM combined with biochemical and cell-based assays have led to an explosive increase in our understanding of APC/C^{Cdc20} enzymology and mechanisms of its regulation.

Interestingly, the APC/C is not only required in dividing cells but also plays important roles in differentiated tissues, such as the nervous system (Huang and Bonni 2016). Although most of these functions depend on Cdh1, Cdc20 is expressed in some neuronal types and is required for their differentiation (Kim et al. 2009; Yang et al. 2009; Kowalski et al. 2014; Watanabe et al. 2014; Mao et al. 2015). These findings highlight the potential for new studies focused on understanding how postmitotic APC/C functions are regulated. For example, a cyclin-dependent kinase called Cdk5 is present in sensory neurons, where it regulates multiple signaling events (Kawauchi 2014);

therefore, Cdk5 may substitute for Cdk1 in neurons to regulate the interaction between APC/C and its coactivators in a manner similar to what has been observed during cell cycle progression (Maestre et al. 2008; Veas-Perez de Tudela et al. 2015). Given that Cdk5 has garnered a significant amount of interest for its role in Alzheimer's disease progression (Fuchsberger et al. 2017), its mechanistic connection with the APC/C in the nervous system is likely to be the focus of future work.

Finally, understanding of APC/C^{Cdc20} mechanism and regulation has opened the possibility for new therapies targeting the APC/C in cancer (Wang et al. 2015; Zhou et al. 2016). Current treatments use spindle poisons to activate the spindle assembly checkpoint and induce apoptosis but are limited by cells slipping out of mitosis because of residual APC/C activity (Brito and Rieder 2006; Gascoigne and Taylor 2008). A number of studies have shown that directly inhibiting mitotic exit is a more efficient approach to killing cancer cells (Huang et al. 2009; Manchado et al. 2010). Two small-molecule APC/C inhibitors have been developed, proTAME and Apcin (Zeng et al. 2010; Sackton et al. 2014), which block the interaction between coactivators and the APC/C. When added to cells in combination, proTAME and Apcin efficiently block mitotic exit (Sackton et al. 2014). Once optimized to act in a clinical context, these drugs have the potential to synergize with commonly used microtubule poisons that activate the spindle checkpoint (Giovinazzi et al. 2013; de Lange et al. 2015) and contribute to improving this widely used chemotherapeutic strategy.

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REFERENCES

- Alfieri C, Chang L, Zhang Z, Yang J, Maslen S, Skehel M, Barford D. 2016. Molecular basis of APC/C regulation by the spindle assembly checkpoint. *Nature* **536**: 431–436.
- Araki M, Yu H, Asano M. 2005. A novel motif governs APC-dependent degradation of *Drosophila* ORC1 in vivo. *Genes Dev* **19**: 2458–2465.
- Aravamudhan P, Goldfarb AA, Joglekar AP. 2015. The kinetochore encodes a mechanical switch to disrupt spindle assembly checkpoint signalling. *Nat Cell Biol* **17**: 868–879.
- Aristarkhov A, Eytan E, Moghe A, Admon A, Hershko A, Ruderman JV. 1996. E2-C, a cyclin-selective ubiquitin carrier protein required for the destruction of mitotic cyclins. *Proc Natl Acad Sci* **93**: 4294–4299.
- Barford D. 2015. Understanding the structural basis for controlling chromosome division. *Philos Trans A Math Phys Eng Sci* **373**: 20130392.
- Bolanos-Garcia VM, Blundell TL. 2011. BUB1 and BUBR1: Multifaceted kinases of the cell cycle. *Trends Biochem Sci* **36**: 141–150.
- Brito DA, Rieder CL. 2006. Mitotic checkpoint slippage in humans occurs via cyclin B destruction in the presence of an active checkpoint. *Curr Biol* **16**: 1194–1200.
- Burton JL, Solomon MJ. 2007. Mad3p, a pseudosubstrate inhibitor of APCC^{Cdc20} in the spindle assembly checkpoint. *Genes Dev* **21**: 655–667.
- Buschhorn BA, Petzold G, Galova M, Dube P, Kraft C, Herzog F, Stark H, Peters JM. 2011. Substrate binding on the APC/C occurs between the coactivator Cdh1 and the processivity factor Doc1. *Nat Struct Mol Biol* **18**: 6–13.
- Carroll CW, Enquist-Newman M, Morgan DO. 2005. The APC subunit Doc1 promotes recognition of the substrate destruction box. *Curr Biol* **15**: 11–18.
- Chang L, Zhang Z, Yang J, McLaughlin SH, Barford D. 2015. Atomic structure of the APC/C and its mechanism of protein ubiquitination. *Nature* **522**: 450–454.
- Chao WC, Kulkarni K, Zhang Z, Kong EH, Barford D. 2012. Structure of the mitotic checkpoint complex. *Nature* **484**: 208–213.
- Cheeseman IM. 2014. The kinetochore. *Cold Spring Harb Perspect Biol* **6**: a015826.
- Chen RH, Waters JC, Salmon ED, Murray AW. 1996. Association of spindle assembly checkpoint component XMAP2 with unattached kinetochores. *Science* **274**: 242–246.
- Chen RH, Shevchenko A, Mann M, Murray AW. 1998. Spindle checkpoint protein Xmad1 recruits Xmad2 to unattached kinetochores. *J Cell Biol* **143**: 283–295.
- Chu T, Henrion G, Haegeli V, Strickland S. 2001. Cortex, a *Drosophila* gene required to complete oocyte meiosis, is a member of the Cdc20/fizzy protein family. *Genesis* **29**: 141–152.
- Cooper KF, Mallory MJ, Egeland DB, Jarnik M, Strich R. 2000. Ama1p is a meiosis-specific regulator of the anaphase promoting complex/cyclosome in yeast. *Proc Natl Acad Sci* **97**: 14548–14553.
- Corbett KD. 2017. Molecular mechanisms of spindle assembly checkpoint activation and silencing. *Prog Mol Subcell Biol* **56**: 429–455.
- Craney A, Kelly A, Jia L, Fedrigo I, Yu H, Rape M. 2016. Control of APC/C-dependent ubiquitin chain elongation by reversible phosphorylation. *Proc Natl Acad Sci* **113**: 1540–1545.
- da Fonseca PC, Kong EH, Zhang Z, Schreiber A, Williams MA, Morris EP, Barford D. 2011. Structures of APC/C^{Cdh1} with substrates identify Cdh1 and Apc10 as the D-box co-receptor. *Nature* **470**: 274–278.
- Dawson IA, Roth S, Artavanis-Tsakonas S. 1995. 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* **129**: 725–737.
- Deibler RW, Kirschner MW. 2010. Quantitative reconstitution of mitotic CDK1 activation in somatic cell extracts. *Mol Cell* **37**: 753–767.
- de Lange J, Faramarz A, Oostra AB, de Menezes RX, van der Meulen IH, Rooimans MA, Rockx DA, Brakenhoff RH, van Beusechem VW, King RW, et al. 2015. Defective sister chromatid cohesion is synthetically lethal with impaired APC/C function. *Nat Commun* **6**: 8399.
- Diaz-Martinez LA, Tian W, Li B, Warrington R, Jia L, Brautigam CA, Luo X, Yu H. 2015. The Cdc20-binding Phe box of the spindle checkpoint protein BubR1 maintains the mitotic checkpoint complex during mitosis. *J Biol Chem* **290**: 2431–2443.
- Di Fiore B, Pines J. 2007. Emi1 is needed to couple DNA replication with mitosis but does not regulate activation of the mitotic APC/C. *J Cell Biol* **177**: 425–437.
- Di Fiore B, Davey NE, Hagting A, Izawa D, Mansfeld J, Gibson TJ, Pines J. 2015. The ABBA motif binds APC/C activators and is shared by APC/C substrates and regulators. *Dev Cell* **32**: 358–372.
- Di Fiore B, Wurzenberger C, Davey NE, Pines J. 2016. The mitotic checkpoint complex requires an evolutionary conserved cassette to bind and inhibit active APC/C. *Mol Cell* **64**: 1144–1153.
- Dong X, Zavitz KH, Thomas BJ, Lin M, Campbell S, Zipursky SL. 1997. Control of G₁ in the developing *Drosophila* eye: rcal regulates Cyclin A. *Genes Dev* **11**: 94–105.

- Eguren M, Manchado E, Malumbres M. 2011. Non-mitotic functions of the anaphase-promoting complex. *Semin Cell Dev Biol* **22**: 572–578.
- Elowe S. 2011. Bub1 and BubR1: At the interface between chromosome attachment and the spindle checkpoint. *Mol Cell Biol* **31**: 3085–3093.
- Elowe S, Dulla K, Uldschmid A, Li X, Dou Z, Nigg EA. 2010. Uncoupling of the spindle-checkpoint and chromosome-congression functions of BubR1. *J Cell Sci* **123**: 84–94.
- Espeut A, Uluocak P, Bastos RN, Mangat D, Graab P, Gruneberg U. 2014. PP2A-B56 opposes Mps1 phosphorylation of Knl1 and thereby promotes spindle assembly checkpoint silencing. *J Cell Biol* **206**: 833–842.
- Espeut J, Cheerambathur DK, Krenning L, Oegema K, Desai A. 2012. Microtubule binding by KNL-1 contributes to spindle checkpoint silencing at the kinetochore. *J Cell Biol* **196**: 469–482.
- Espeut J, Lara-Gonzalez P, Sassine M, Shiau AK, Desai A, Abrieu A. 2015. Natural loss of Mps1 kinase in nematodes uncovers a role for polo-like kinase 1 in spindle checkpoint initiation. *Cell Rep* **12**: 58–65.
- Etemad B, Kops GJ. 2016. Attachment issues: Kinetochore trans-formations and spindle checkpoint silencing. *Curr Opin Cell Biol* **39**: 101–108.
- Eytan E, Wang K, Miniowitz-Shemtov S, Sitry-Shevah D, Kaisari S, Yen TJ, Liu ST, Hershko A. 2014. Disassembly of mitotic checkpoint complexes by the joint action of the AAA-ATPase TRIP13 and p31^{comet}. *Proc Natl Acad Sci* **111**: 12019–12024.
- Faesen AC, Thanasoula M, Maffini S, Breit C, Müller F, van Gerwen S, Bange T, Musacchio A. 2017. Basis of catalytic assembly of the mitotic checkpoint complex. *Nature* **542**: 498–502.
- Fang G, Yu H, Kirschner MW. 1998. Direct binding of CDC20 protein family members activates the anaphase-promoting complex in mitosis and G₁. *Mol Cell* **2**: 163–171.
- Fay DS, Keenan S, Han M. 2002. fzr-1 and lin-35/Rb function redundantly to control cell proliferation in *C. elegans* as revealed by a nonbiased synthetic screen. *Genes Dev* **16**: 503–517.
- Floyd S, Pines J, Lindon C. 2008. APC/C^{Cdh1} targets aurora kinase to control reorganization of the mitotic spindle at anaphase. *Curr Biol* **18**: 1649–1658.
- Foster SA, Morgan DO. 2012. The APC/C subunit Mnd2/Apc15 promotes Cdc20 autoubiquitination and spindle assembly checkpoint inactivation. *Mol Cell* **47**: 921–932.
- Frye JJ, Brown NG, Petzold G, Watson ER, Grace CR, Nourse A, Jarvis MA, Kriwacki RW, Peters JM, Stark H, et al. 2013. Electron microscopy structure of human APC/C^{Cdh1}-EM1 reveals multimodal mechanism of E3 ligase shutdown. *Nat Struct Mol Biol* **20**: 827–835.
- Fuchsberger T, Lloret A, Vina J. 2017. New functions of APC/C ubiquitin ligase in the nervous system and its role in Alzheimer's disease. *Int J Mol Sci* **18**: 1057.
- Fujimitsu K, Grimaldi M, Yamano H. 2016. Cyclin-dependent kinase 1-dependent activation of APC/C ubiquitin ligase. *Science* **352**: 1121–1124.
- Funk LC, Zasadil LM, Weaver BA. 2016. Living in CIN: Mitotic infidelity and its consequences for tumor promotion and suppression. *Dev Cell* **39**: 638–652.
- Garcia-Higuera I, Manchado E, Dubus P, Cañamero M, Méndez J, Moreno S, Malumbres M. 2008. Genomic stability and tumour suppression by the APC/C cofactor Cdh1. *Nat Cell Biol* **10**: 802–811.
- Garnett MJ, Mansfield J, Godwin C, Matsusaka T, Wu J, Russell P, Pines J, Venkitaraman AR. 2009. UBE2S elongates ubiquitin chains on APC/C substrates to promote mitotic exit. *Nat Cell Biol* **11**: 1363–1369.
- Gascoigne KE, Taylor SS. 2008. Cancer cells display profound intra- and interline variation following prolonged exposure to antimitotic drugs. *Cancer Cell* **14**: 111–122.
- Giovinazzi S, Bellapu D, Morozov VM, Ishov AM. 2013. Targeting mitotic exit with hyperthermia or APC/C inhibition to increase paclitaxel efficacy. *Cell Cycle* **12**: 2598–2607.
- Glutzer M, Murray AW, Kirschner MW. 1991. Cyclin is degraded by the ubiquitin pathway. *Nature* **349**: 132–138.
- Golan A, Yudkovsky Y, Hershko A. 2002. The cyclin-ubiquitin ligase activity of cyclosome/APC is jointly activated by protein kinases Cdk1-cyclin B and Plk. *J Biol Chem* **277**: 15552–15557.
- Gopinathan L, Szmyd R, Low D, Diril MK, Chang HY, Coppola V, Liu K, Tessarollo L, Guccione E, van Pelt AMM, et al. 2017. Emi2 is essential for mouse spermatogenesis. *Cell Rep* **20**: 697–708.
- Grosskortenhaus R, Sprenger F. 2002. Rca1 inhibits APC-Cdh1^{Fzr} and is required to prevent cyclin degradation in G₂. *Dev Cell* **2**: 29–40.
- Gurden MD, Holland AJ, van Zon W, Tighe A, Vergnolle MA, Andres DA, Spielmann HP, Malumbres M, Wolthuis RM, Cleveland DW, et al. 2010. Cdc20 is required for the post-anaphase, KEN-dependent degradation of centromere protein F. *J Cell Sci* **123**: 321–330.
- Habu T, Kim SH, Weinstein J, Matsumoto T. 2002. Identification of a MAD2-binding protein, CMT2, and its role in mitosis. *EMBO J* **21**: 6419–6428.
- Hagan RS, Manak MS, Buch HK, Meier MG, Meraldi P, Shah JV, Sorger PK. 2011. p31^{comet} acts to ensure timely spindle checkpoint silencing subsequent to kinetochore attachment. *Mol Biol Cell* **22**: 4236–4246.
- He J, Chao WC, Zhang Z, Yang J, Cronin N, Barford D. 2013. Insights into degron recognition by APC/C coactivators from the structure of an Acm1-Cdh1 complex. *Mol Cell* **50**: 649–660.
- Hein JB, Nilsson J. 2016. Interphase APC/C-Cdc20 inhibition by cyclin A2-Cdk2 ensures efficient mitotic entry. *Nat Commun* **7**: 10975.
- Hewitt L, Tighe A, Santaguida S, White AM, Jones CD, Musacchio A, Green S, Taylor SS. 2010. Sustained Mps1 activity is required in mitosis to recruit O-Mad2 to the Mad1-C-Mad2 core complex. *J Cell Biol* **190**: 25–34.
- Hiruma Y, Sacristan C, Pachis ST, Adamopoulos A, Kuijt T, Ubbink M, von Castelmuir E, Perrakis A, Kops GJ. 2015. CELL DIVISION CYCLE. Competition between MPS1 and microtubules at kinetochores regulates spindle checkpoint signaling. *Science* **348**: 1264–1267.
- Holland AJ, Cleveland DW. 2012. Losing balance: The origin and impact of aneuploidy in cancer. *EMBO Rep* **13**: 501–514.
- Howell BJ, McEwen BF, Canman JC, Hoffman DB, Farrar EM, Rieder CL, Salmon ED. 2001. Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. *J Cell Biol* **155**: 1159–1172.
- Huang J, Bonni A. 2016. A decade of the anaphase-promoting complex in the nervous system. *Genes Dev* **30**: 622–638.
- Huang HC, Shi J, Orth JD, Mitchison TJ. 2009. Evidence that mitotic exit is a better cancer therapeutic target than spindle assembly. *Cancer Cell* **16**: 347–358.
- Izawa D, Pines J. 2012. Mad2 and the APC/C compete for the same site on Cdc20 to ensure proper chromosome segregation. *J Cell Biol* **199**: 27–37.
- Izawa D, Pines J. 2015. The mitotic checkpoint complex binds a second CDC20 to inhibit active APC/C. *Nature* **517**: 631–634.
- Ji Z, Gao H, Yu H. 2015. Cell division cycle. Kinetochore attachment sensed by competitive Mps1 and microtubule binding to Ndc80C. *Science* **348**: 1260–1264.
- Ji Z, Gao H, Jia L, Li B, Yu H. 2017. A sequential multi-target Mps1 phosphorylation cascade promotes spindle checkpoint signaling. *Elife* **6**: e22513.
- Jia L, Li B, Warrington RT, Hao X, Wang S, Yu H. 2011. Defining pathways of spindle checkpoint silencing: Functional redundancy between Cdc20 ubiquitination and p31^{comet}. *Mol Biol Cell* **22**: 4227–4235.
- Jia L, Kim S, Yu H. 2013. Tracking spindle checkpoint signals from kinetochores to APC/C. *Trends Biochem Sci* **38**: 302–311.
- Jia L, Li B, Yu H. 2016. The Bub1-Plk1 kinase complex promotes spindle checkpoint signalling through Cdc20 phosphorylation. *Nat Commun* **7**: 10818.
- Kallio MJ, Beardmore VA, Weinstein J, Gorbisky GJ. 2002. Rapid microtubule-independent dynamics of Cdc20 at kinetochores

- and centrosomes in mammalian cells. *J Cell Biol* **158**: 841–847.
- Kawauchi T. 2014. Cdk5 regulates multiple cellular events in neural development, function and disease. *Dev Growth Differ* **56**: 335–348.
- Kim AH, Puram SV, Bilimoria PM, Ikeuchi Y, Keough S, Wong M, Rowitch D, Bonni A. 2009. A centrosomal Cdc20-APC pathway controls dendrite morphogenesis in postmitotic neurons. *Cell* **136**: 322–336.
- Kim T, Moyle MW, Lara-Gonzalez P, De Groot C, Oegema K, Desai A. 2015. Kinetochores-localized BUB-1/BUB-3 complex promotes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* **161**: 507–517.
- Kim T, Lara-Gonzalez P, Prevo B, Meitinger F, Cheerambathur DK, Oegema K, Desai A. 2017. Kinetochores accelerate or delay APC/C activation by directing Cdc20 to opposing fates. *Genes Dev* **31**: 1089–1094.
- King RW, Peters JM, Tugendreich S, Rolfe M, Hieter P, Kirschner MW. 1995. A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* **81**: 279–288.
- Kitagawa R, Law E, Tang L, Rose AM. 2002. The Cdc20 homolog, FZY-1, and its interacting protein, IFY-1, are required for proper chromosome segregation in *Caenorhabditis elegans*. *Curr Biol* **12**: 2118–2123.
- Klebig C, Korinth D, Meraldi P. 2009. Bub1 regulates chromosome segregation in a kinetochore-independent manner. *J Cell Biol* **185**: 841–858.
- Kowalski JR, Dube H, Touroutine D, Rush KM, Goodwin PR, Carozza M, Didier Z, Francis MM, Juo P. 2014. The Anaphase-Promoting Complex (APC) ubiquitin ligase regulates GABA transmission at the *C. elegans* neuromuscular junction. *Mol Cell Neurosci* **58**: 62–75.
- Kraft C, Herzog F, Gieffers C, Mechtler K, Hagting A, Pines J, Peters JM. 2003. Mitotic regulation of the human anaphase-promoting complex by phosphorylation. *EMBO J* **22**: 6598–6609.
- Kraft C, Vodermaier HC, Maurer-Stroh S, Eisenhaber F, Peters JM. 2005. The WD40 propeller domain of Cdh1 functions as a destruction box receptor for APC/C substrates. *Mol Cell* **18**: 543–553.
- Kramer ER, Scheuringer N, Podtelejnikov AV, Mann M, Peters JM. 2000. Mitotic regulation of the APC activator proteins CDC20 and CDH1. *Mol Biol Cell* **11**: 1555–1569.
- Labit H, Fujimitsu K, Bayin NS, Takaki T, Gannon J, Yamano H. 2012. Dephosphorylation of Cdc20 is required for its C-box-dependent activation of the APC/C. *EMBO J* **31**: 3351–3362.
- Lahav-Baratz S, Sudakin V, Ruderman JV, Hershko A. 1995. Reversible phosphorylation controls the activity of cyclosome-associated cyclin-ubiquitin ligase. *Proc Natl Acad Sci* **92**: 9303–9307.
- Lara-Gonzalez P, Scott MI, Diez M, Sen O, Taylor SS. 2011. BubR1 blocks substrate recruitment to the APC/C in a KEN-box-dependent manner. *J Cell Sci* **124**: 4332–4345.
- Lara-Gonzalez P, Westhorpe FG, Taylor SS. 2012. The spindle assembly checkpoint. *Curr Biol* **22**: R966–R980.
- Lee SJ, Rodriguez-Bravo V, Kim H, Datta S, Foley EA. 2017. The PP2A^{B56} phosphatase promotes the association of Cdc20 with APC/C in mitosis. *J Cell Sci* **130**: 1760–1771.
- Lesage B, Qian J, Bollen M. 2011. Spindle checkpoint silencing: PP1 tips the balance. *Curr Biol* **21**: R898–R903.
- Li Y, Benezra R. 1996. Identification of a human mitotic checkpoint gene: hMAD2. *Science* **274**: 246–248.
- Li M, York JP, Zhang P. 2007. Loss of Cdc20 causes a securin-dependent metaphase arrest in two-cell mouse embryos. *Mol Cell Biol* **27**: 3481–3488.
- Lim HH, Goh PY, Surana U. 1998. Cdc20 is essential for the cyclosome-mediated proteolysis of both Pds1 and Clb2 during M phase in budding yeast. *Curr Biol* **8**: 231–234.
- Lindqvist A, van Zon W, Karlsson Rosenthal C, Wolthuis RM. 2007. Cyclin B1-Cdk1 activation continues after centrosome separation to control mitotic progression. *PLoS Biol* **5**: e123.
- Littlepage LE, Ruderman JV. 2002. Identification of a new APC/C recognition domain, the A box, which is required for the Cdh1-dependent destruction of the kinase Aurora-A during mitotic exit. *Genes Dev* **16**: 2274–2285.
- Liu D, Vleugel M, Backer CB, Hori T, Fukagawa T, Cheeseman IM, Lampson MA. 2010. Regulated targeting of protein phosphatase 1 to the outer kinetochore by KNL1 opposes Aurora B kinase. *J Cell Biol* **188**: 809–820.
- London N, Biggins S. 2014. Mad1 kinetochore recruitment by Mps1-mediated phosphorylation of Bub1 signals the spindle checkpoint. *Genes Dev* **28**: 140–152.
- London N, Ceto S, Ranish JA, Biggins S. 2012. Phosphoregulation of Spc105 by Mps1 and PP1 regulates Bub1 localization to kinetochores. *Curr Biol* **22**: 900–906.
- Lu D, Hsiao JY, Davey NE, Van Voorhis VA, Foster SA, Tang C, Morgan DO. 2014. Multiple mechanisms determine the order of APC/C substrate degradation in mitosis. *J Cell Biol* **207**: 23–39.
- Luo X, Yu H. 2008. Protein metamorphosis: The two-state behavior of Mad2. *Structure* **16**: 1616–1625.
- Machida YJ, Dutta A. 2007. The APC/C inhibitor, Emi1, is essential for prevention of rereplication. *Genes Dev* **21**: 184–194.
- Maciejowski J, George KA, Terret ME, Zhang C, Shokat KM, Jalileppalli PV. 2010. Mps1 directs the assembly of Cdc20 inhibitory complexes during interphase and mitosis to control M phase timing and spindle checkpoint signaling. *J Cell Biol* **190**: 89–100.
- Maestre C, Delgado-Esteban M, Gomez-Sanchez JC, Bolaños JP, Almeida A. 2008. Cdk5 phosphorylates Cdh1 and modulates cyclin B1 stability in excitotoxicity. *EMBO J* **27**: 2736–2745.
- Maldonado M, Kapoor TM. 2011. Constitutive Mad1 targeting to kinetochores uncouples checkpoint signalling from chromosome biorientation. *Nat Cell Biol* **13**: 475–482.
- Malureanu LA, Jeganathan KB, Hamada M, Wasilewski L, Davenport J, van Deursen JM. 2009. BubR1 N terminus acts as a soluble inhibitor of cyclin B degradation by APC/C^{Cdc20} in interphase. *Dev Cell* **16**: 118–131.
- Manchado E, Guillaumot M, de Cárcer G, Eguren M, Trickey M, Garcia-Higuera I, Moreno S, Yamano H, Cañamero M, Malumbres M. 2010. Targeting mitotic exit leads to tumor regression in vivo: Modulation by Cdk1, Mstl, and the PP2A/B55α,δ phosphatase. *Cancer Cell* **18**: 641–654.
- Mansfeld J, Collin P, Collins MO, Choudhary JS, Pines J. 2011. APC15 drives the turnover of MCC-CDC20 to make the spindle assembly checkpoint responsive to kinetochore attachment. *Nat Cell Biol* **13**: 1234–1243.
- Mao DD, Gujar AD, Mahlokozera T, Chen I, Pan Y, Luo J, Brost T, Thompson EA, Turski A, Leuthardt EC, et al. 2015. A CDC20-APC/SOX2 signaling axis regulates human glioblastoma stem-like cells. *Cell Rep* **11**: 1809–1821.
- Mapelli M, Musacchio A. 2007. MAD contortions: Conformational dimerization boosts spindle checkpoint signaling. *Curr Opin Struct Biol* **17**: 716–725.
- Matyskiela ME, Morgan DO. 2009. Analysis of activator-binding sites on the APC/C supports a cooperative substrate-binding mechanism. *Mol Cell* **34**: 68–80.
- Meadows JC, Shepperd LA, Vanoosthuysen V, Lancaster TC, Sochaj AM, Buttrick GJ, Hardwick KG, Millar JB. 2011. Spindle checkpoint silencing requires association of PP1 to both Spc7 and kinesin-8 motors. *Dev Cell* **20**: 739–750.
- Moyle MW, Kim T, Hattersley N, Espeut J, Cheerambathur DK, Oegema K, Desai A. 2014. A Bub1-Mad1 interaction targets the Mad1-Mad2 complex to unattached kinetochores to initiate the spindle checkpoint. *J Cell Biol* **204**: 647–657.
- Musacchio A. 2015. The molecular biology of spindle assembly checkpoint signaling dynamics. *Curr Biol* **25**: R1002–R1018.
- Musacchio A, Desai A. 2017. A molecular view of kinetochore assembly and function. *Biology (Basel)* **6**: 5.
- Nijenhuis W, Vallardi G, Teixeira A, Kops GJ, Saurin AT. 2014. Negative feedback at kinetochores underlies a responsive spindle checkpoint signal. *Nat Cell Biol* **16**: 1257–1264.
- Passmore LA, McCormack EA, Au SW, Paul A, Willison KR, Harper JW, Barford D. 2003. Doc1 mediates the activity of

- the anaphase-promoting complex by contributing to substrate recognition. *EMBO J* **22**: 786–796.
- Patra D, Dunphy WG. 1998. Xe-p9, a *Xenopus* Suc1/Cks protein, is essential for the Cdc2-dependent phosphorylation of the anaphase-promoting complex at mitosis. *Genes Dev* **12**: 2549–2559.
- Peters JM. 2006. The anaphase promoting complex/cyclosome: A machine designed to destroy. *Nat Rev Mol Cell Biol* **7**: 644–656.
- Peters JM, King RW, Hoog C, Kirschner MW. 1996. Identification of BIME as a subunit of the anaphase-promoting complex. *Science* **274**: 1199–1201.
- Pflegler CM, Kirschner MW. 2000. The KEN box: An APC recognition signal distinct from the D box targeted by Cdh1. *Genes Dev* **14**: 655–665.
- Pines J. 2011. Cubism and the cell cycle: The many faces of the APC/C. *Nat Rev Mol Cell Biol* **12**: 427–438.
- Primorac I, Musacchio A. 2013. Panta rhei: The APC/C at steady state. *J Cell Biol* **201**: 177–189.
- Prinz S, Hwang ES, Visintin R, Amon A. 1998. The regulation of Cdc20 proteolysis reveals a role for the APC components Cdc23 and Cdc27 during S phase and early mitosis. *Curr Biol* **8**: 750–760.
- Qiao R, Weissmann F, Yamaguchi M, Brown NG, VanderLinden R, Imre R, Jarvis MA, Brunner MR, Davidson IF, Litos G, et al. 2016. Mechanism of APC/C^{CDC20} activation by mitotic phosphorylation. *Proc Natl Acad Sci* **113**: E2570–E2578.
- Reddy SK, Rape M, Margansky WA, Kirschner MW. 2007. Ubiquitination by the anaphase-promoting complex drives spindle checkpoint inactivation. *Nature* **446**: 921–925.
- Reimann JD, Freed E, Hsu JY, Kramer ER, Peters JM, Jackson PK. 2001a. Emi1 is a mitotic regulator that interacts with Cdc20 and inhibits the anaphase promoting complex. *Cell* **105**: 645–655.
- Reimann JD, Gardner BE, Margottin-Goguet F, Jackson PK. 2001b. Emi1 regulates the anaphase-promoting complex by a different mechanism than Mad2 proteins. *Genes Dev* **15**: 3278–3285.
- Rodrigo-Brenni MC, Morgan DO. 2007. Sequential E2s drive polyubiquitin chain assembly on APC targets. *Cell* **130**: 127–139.
- Rosenberg SC, Corbett KD. 2015. The multifaceted roles of the HORMA domain in cellular signaling. *J Cell Biol* **211**: 745–755.
- Rosenberg JS, Cross FR, Funabiki H. 2011. KNL1/Sp105 recruits PP1 to silence the spindle assembly checkpoint. *Curr Biol* **21**: 942–947.
- Sackton KL, Dimova N, Zeng X, Tian W, Zhang M, Sackton TB, Meaders J, Pfaff KL, Sigoirol F, Yu H, et al. 2014. Synergistic blockade of mitotic exit by two chemical inhibitors of the APC/C. *Nature* **514**: 646–649.
- Santaguida S, Amon A. 2015. Short- and long-term effects of chromosome mis-segregation and aneuploidy. *Nat Rev Mol Cell Biol* **16**: 473–485.
- Santaguida S, Tighe A, D'Alise AM, Taylor SS, Musacchio A. 2010. Dissecting the role of MPS1 in chromosome biorientation and the spindle checkpoint through the small molecule inhibitor reversine. *J Cell Biol* **190**: 73–87.
- Schmidt A, Duncan PI, Rauh NR, Sauer G, Fry AM, Nigg EA, Mayer TU. 2005. *Xenopus* polo-like kinase Plx1 regulates XErp1, a novel inhibitor of APC/C activity. *Genes Dev* **19**: 502–513.
- Schwab M, Lutum AS, Seufert W. 1997. Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. *Cell* **90**: 683–693.
- Schwab M, Neutzner M, Möcker D, Seufert W. 2001. Yeast Hct1 recognizes the mitotic cyclin Clb2 and other substrates of the ubiquitin ligase APC. *EMBO J* **20**: 5165–5175.
- Sczaniecka M, Feoktistova A, May KM, Chen JS, Blyth J, Gould KL, Hardwick KG. 2008. The spindle checkpoint functions of Mad3 and Mad2 depend on a Mad3 KEN box-mediated interaction with Cdc20-anaphase-promoting complex (APC/C). *J Biol Chem* **283**: 23039–23047.
- Sewart K, Hauf S. 2017. Different functionality of Cdc20 binding sites within the mitotic checkpoint complex. *Curr Biol* **27**: 1213–1220.
- Sheppard LA, Meadows JC, Sochaj AM, Lancaster TC, Zou J, Buttrick GJ, Rappsilber J, Hardwick KG, Millar JB. 2012. Phosphodependent recruitment of Bub1 and Bub3 to Spc7/KNL1 by Mph1 kinase maintains the spindle checkpoint. *Curr Biol* **22**: 891–899.
- Shirayama M, Zachariae W, Ciosk R, Nasmyth K. 1998. The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in *Saccharomyces cerevisiae*. *EMBO J* **17**: 1336–1349.
- Shteinberg M, Protopopov Y, Listovsky T, Brandeis M, Hershko A. 1999. Phosphorylation of the cyclosome is required for its stimulation by Fizzy/cdc20. *Biochem Biophys Res Commun* **260**: 193–198.
- Sigrist SJ, Lehner CF. 1997. *Drosophila* fizzy-related down-regulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles. *Cell* **90**: 671–681.
- Sigrist S, Jacobs H, Stratmann R, Lehner CF. 1995. Exit from mitosis is regulated by *Drosophila* fizzy and the sequential destruction of cyclins A, B and B3. *EMBO J* **14**: 4827–4838.
- Sivaprasad U, Machida YJ, Dutta A. 2007. APC/C—the master controller of origin licensing? *Cell Div* **2**: 8.
- Sudakin V, Ganoh D, Dahan A, Heller H, Hershko J, Luca FC, Ruderman JV, Hershko A. 1995. The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis. *Mol Biol Cell* **6**: 185–197.
- Sudakin V, Chan GK, Yen TJ. 2001. Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2. *J Cell Biol* **154**: 925–936.
- Teichner A, Eytan E, Sitry-Shevah D, Miniowitz-Shemtov S, Dumin E, Gromis J, Hershko A. 2011. p31^{comet} Promotes disassembly of the mitotic checkpoint complex in an ATP-dependent process. *Proc Natl Acad Sci* **108**: 3187–3192.
- Tischer T, Hormanseder E, Mayer TU. 2012. The APC/C inhibitor XErp1/Emi2 is essential for *Xenopus* early embryonic divisions. *Science* **338**: 520–524.
- Trinkle-Mulcahy L, Andrews PD, Wickramasinghe S, Sleeman J, Prescott A, Lam YW, Lyon C, Swedlow JR, Lamond AI. 2003. Time-lapse imaging reveals dynamic relocalization of PP1γ throughout the mammalian cell cycle. *Mol Biol Cell* **14**: 107–117.
- Tung JJ, Hansen DV, Ban KH, Loktev AV, Summers MK, Adler JR III, Jackson PK. 2005. A role for the anaphase-promoting complex inhibitor Emi2/XErp1, a homolog of early mitotic inhibitor 1, in cytotstatic factor arrest of *Xenopus* eggs. *Proc Natl Acad Sci* **102**: 4318–4323.
- Uzunova K, Dye BT, Schutz H, Ladurner R, Petzold G, Toyoda Y, Jarvis MA, Brown NG, Poser I, Novatchkova M, et al. 2012. APC15 mediates CDC20 autoubiquitylation by APC/C^{MCC} and disassembly of the mitotic checkpoint complex. *Nat Struct Mol Biol* **19**: 1116–1123.
- van Zon W, Wolthuis RM. 2010. Cyclin A and Nek2A: APC/C-Cdc20 substrates invisible to the mitotic spindle checkpoint. *Biochem Soc Trans* **38**: 72–77.
- Veas-Perez de Tudela M, Maestre C, Delgado-Esteban M, Bolaños JP, Almeida A. 2015. Cdk5-mediated inhibition of APC/C-Cdh1 switches on the cyclin D1-Cdk4-pRb pathway causing aberrant S-phase entry of postmitotic neurons. *Sci Rep* **5**: 18180.
- Vleugel M, Hoek TA, Tromer E, Slidrecht T, Groenewold V, Omerzu M, Kops GJ. 2015. Dissecting the roles of human BUB1 in the spindle assembly checkpoint. *J Cell Sci* **128**: 2975–2982.
- Vodermaier HC, Gieffers C, Maurer-Stroh S, Eisenhaber F, Peters JM. 2003. TPR subunits of the anaphase-promoting complex mediate binding to the activator protein CDH1. *Curr Biol* **13**: 1459–1468.
- von Schubert C, Cubizolles F, Bracher JM, Slidrecht T, Kops GJ, Nigg EA. 2015. Plk1 and Mps1 cooperatively regulate the spindle assembly checkpoint in human cells. *Cell Rep* **12**: 66–78.
- Wang L, Zhang J, Wan L, Zhou X, Wang Z, Wei W. 2015. Targeting Cdc20 as a novel cancer therapeutic strategy. *Pharmacol Ther* **151**: 141–151.

- Watanabe Y, Khodosevich K, Monyer H. 2014. Dendrite development regulated by the schizophrenia-associated gene FEZ1 involves the ubiquitin proteasome system. *Cell Rep* **7**: 552–564.
- Weinstein J. 1997. Cell cycle-regulated expression, phosphorylation, and degradation of p55Cdc. A mammalian homolog of CDC20/Fizzy/slp1. *J Biol Chem* **272**: 28501–28511.
- Westhorpe FG, Tighe A, Lara-Gonzalez P, Taylor SS. 2011. p31comet-mediated extraction of Mad2 from the MCC promotes efficient mitotic exit. *J Cell Sci* **124**: 3905–3916.
- Wild T, Larsen MS, Narita T, Schou J, Nilsson J, Choudhary C. 2016. The spindle assembly checkpoint is not essential for viability of human cells with genetically lowered APC/C activity. *Cell Rep* **14**: 1829–1840.
- Williamson A, Wickliffe KE, Mellone BG, Song L, Karpen GH, Rape M. 2009. Identification of a physiological E2 module for the human anaphase-promoting complex. *Proc Natl Acad Sci* **106**: 18213–18218.
- Wojcik E, Basto R, Serr M, Scaërou F, Karess R, Hays T. 2001. Kinetochore dynein: Its dynamics and role in the transport of the Rough deal checkpoint protein. *Nat Cell Biol* **3**: 1001–1007.
- Wu T, Merbl Y, Huo Y, Gallop JL, Tzur A, Kirschner MW. 2010. UBE2S drives elongation of K11-linked ubiquitin chains by the anaphase-promoting complex. *Proc Natl Acad Sci* **107**: 1355–1360.
- Xia G, Luo X, Habu T, Rizo J, Matsumoto T, Yu H. 2004. Conformation-specific binding of p31^{comet} antagonizes the function of Mad2 in the spindle checkpoint. *EMBO J* **23**: 3133–3143.
- Yamagishi Y, Yang CH, Tanno Y, Watanabe Y. 2012. MPS1/Mph1 phosphorylates the kinetochore protein KNL1/Spc7 to recruit SAC components. *Nat Cell Biol* **14**: 746–752.
- Yamaguchi M, VanderLinden R, Weissmann F, Qiao R, Dube P, Brown NG, Haselbach D, Zhang W, Sidhu SS, Peters JM, et al. 2016. Cryo-EM of mitotic checkpoint complex-bound APC/C reveals reciprocal and conformational regulation of ubiquitin ligation. *Mol Cell* **63**: 593–607.
- Yang M, Li B, Tomchick DR, Machius M, Rizo J, Yu H, Luo X. 2007. p31comet blocks Mad2 activation through structural mimicry. *Cell* **131**: 744–755.
- Yang Y, Kim AH, Yamada T, Wu B, Bilimoria PM, Ikeuchi Y, de la Iglesia N, Shen J, Bonni A. 2009. A Cdc20-APC ubiquitin signaling pathway regulates presynaptic differentiation. *Science* **326**: 575–578.
- Yang Y, Tsuchiya D, Lacefield S. 2015. Bub3 promotes Cdc20-dependent activation of the APC/C in *S. cerevisiae*. *J Cell Biol* **209**: 519–527.
- Ye Q, Rosenberg SC, Moeller A, Speir JA, Su TY, Corbett KD. 2015. TRIP13 is a protein-remodeling AAA+ ATPase that catalyzes MAD2 conformation switching. *Elife* **4**: e07367.
- Yudkovsky Y, Shteinberg M, Listovsky T, Brandeis M, Hershko A. 2000. Phosphorylation of Cdc20/fizzy negatively regulates the mammalian cyclosome/APC in the mitotic checkpoint. *Biochem Biophys Res Commun* **271**: 299–304.
- Zeng X, Sigoillot F, Gaur S, Choi S, Pfaff KL, Oh DC, Hathaway N, Dimova N, Cuny GD, King RW. 2010. Pharmacologic inhibition of the anaphase-promoting complex induces a spindle checkpoint-dependent mitotic arrest in the absence of spindle damage. *Cancer Cell* **18**: 382–395.
- Zhang S, Chang L, Alfieri C, Zhang Z, Yang J, Maslen S, Skehel M, Barford D. 2016. Molecular mechanism of APC/C activation by mitotic phosphorylation. *Nature* **533**: 260–264.
- Zhang G, Kruse T, López-Méndez B, Sylvestersen KB, Garvan-ska DH, Schopper S, Nielsen ML, Nilsson J. 2017. Bub1 positions Mad1 close to KNL1 MELT repeats to promote checkpoint signalling. *Nat Commun* **8**: 15822.
- Zhou Z, He M, Shah AA, Wan Y. 2016. Insights into APC/C: From cellular function to diseases and therapeutics. *Cell Div* **11**: 9.

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