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Authors
Lara-Gonzalez, Pablo
Kim, Taekyung
Desai, Arshad

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Taming the beast: control of APC/C^{Cdc20}–dependent destruction

Pablo Lara-Gonzalez[1,2], **Taekyung Kim**[1], and **Arshad Desai**[1,2,@]

[1]Ludwig Institute for Cancer Research

[2]Department of Cellular & Molecular Medicine, University of California San Diego, La Jolla, CA 92093

Abstract

The anaphase promoting complex/cyclosome (APC/C) is a large multi-subunit 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 towards these two key substrates requires the co-activator 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 prior to 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.

Introduction

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 prior to 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 (Peters 2006; Pines 2011; Primorac and Musacchio 2013; Barford 2015) (Fig. 1A&B). 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.

[@]:Corresponding author abdesai@ucsd.edu, Phone:(858)-534-9698, Fax: (858)-534-7750, Address: CMM-E Rm 3052, 9500 Gilman Dr, La Jolla, CA 92093-0653.

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

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). Co-activator binding is essential for APC/C activity and for the recruitment of substrates. All APC/C co-activators possess a C-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 co-activator-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 co-activators promote APC/C activity and how different regulators control APC/C activity (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) (Fig. 1B).

The APC/C recognizes substrates that possess degrons (Fig. 1D) known as the D-box [RXXL] and KEN box (Glotzer 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, such as for cyclin A (Di Fiore et al. 2015). When bound by co-activators, the APC/C forms a bi-partite receptor for D-box substrates that comprises the side of the WD40 barrel and the subunit Apc10/Doc1 (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) (Fig. 1B). In addition, the WD40 barrel serves as the receptor for KEN box and ABBA substrates (Chao et al. 2012; He et al. 2013) (Fig. 1D). 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 utilizes 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 co-activator 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 in order 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; Reimann et al. 2001b; Grosskortenhaus and Sprenger 2002). However, while *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 exhibits 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 in order 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 towards substrates such as cyclin A and Nek2A (van Zon and Wolthuis 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 90s 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 (Fujimitsu et al. 2016; Qiao et al. 2016; Zhang et al. 2016) (Fig. 3). 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 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. While 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^{Cdc20} to APC/C^{Cdh1} in late mitosis (*see below*).

Kinetochore-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 N-terminal C-box prevent the interaction of co-activators with the APC/C (Labit et al. 2012; Chang et al. 2015) (Fig. 1C). 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 observations suggest that Cdc20 must be dephosphorylated in order 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 (Kim et al. 2017) (Fig. 4A). 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 repeats in its N-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 N-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 N-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; Kim et al. 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 N-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 (Craney et al. 2016; Jia et al. 2016; Lee et al. 2017) (Fig. 1C). 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. While 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.

Kinetochore-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 in order to 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 (Chen et al. 1996; Li and Benezra 1996; Chen et al. 1998; Maldonado and Kapoor 2011) (Fig. 4B). 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 bi-partite Cdc20–Apc10 D-box substrate receptor, directly blocks the binding of KEN-box substrates, and partially prevents the recruitment 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; Shepperd 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; Aravamudhan 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 N-terminus of Cdc20 (Kim et al. 2017). How can then 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 exhibiting a half-life of 0.5-2 seconds (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 C-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 C-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 suggests 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 (reviewed in 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.

Final remarks

Since its discovery in the early 90s as the machine that drives mitotic exit (King et al. 1995; Sudakin et al. 1995), the APC/C and its co-activator Cdc20 have been extensively studied. In the last five years, advances in high-resolution cryo-EM, combined with biochemical and cell-based assays have lead 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). While most of these functions dependent 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 post-mitotic 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 co-activators 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 employ spindle poisons to activate the spindle assembly checkpoint and induce apoptosis but are limited by cells slipping out of mitosis due to 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 co-activators 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 employed 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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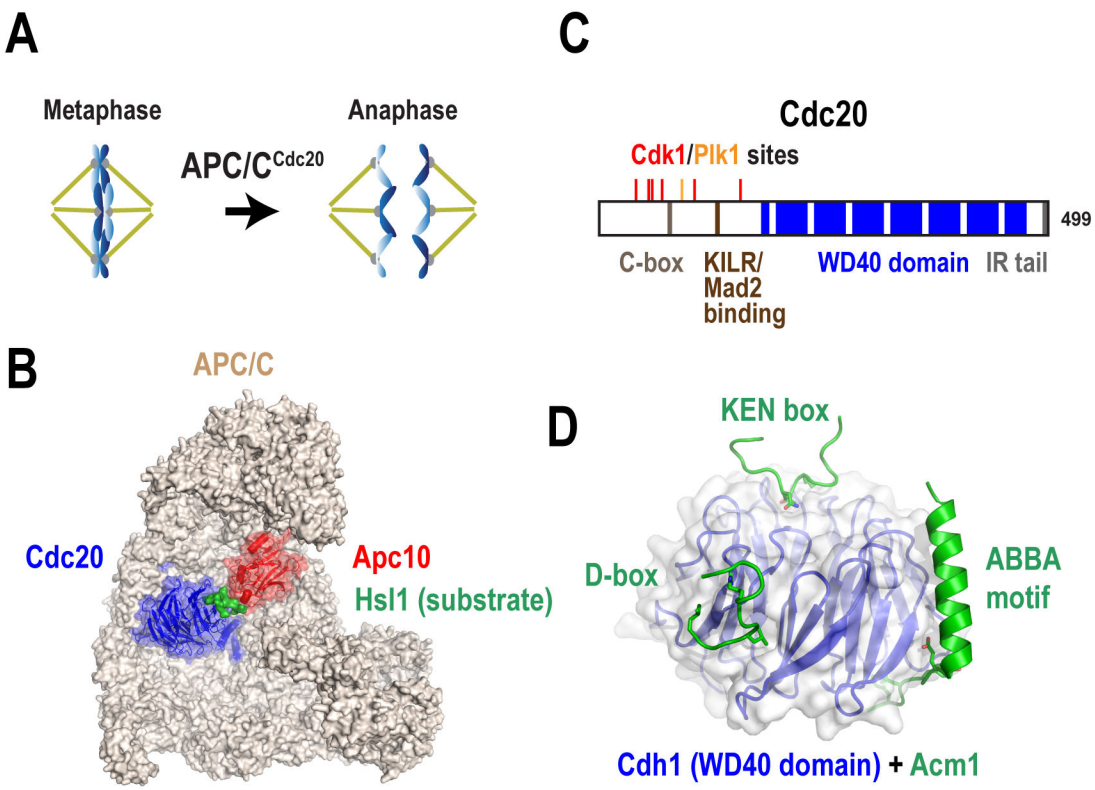

Figure 1. APC/C structure and mechanism of substrate recognition.

(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 grey. **(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 *S.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).

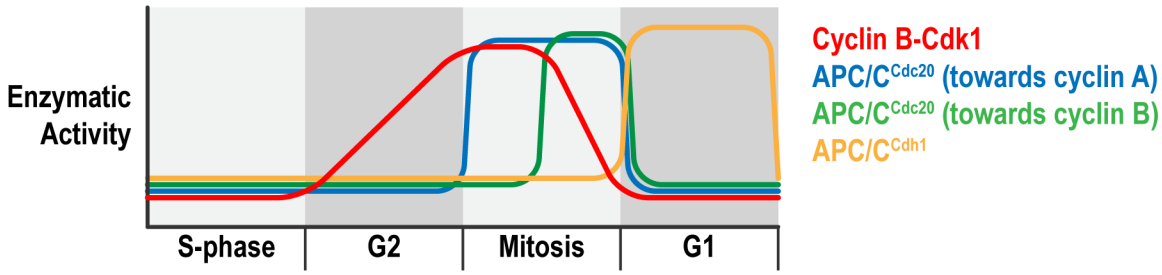

Figure 2. Regulation of APC/C activity during the cell cycle.

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} towards cyclin A (blue) or cyclin B (green).

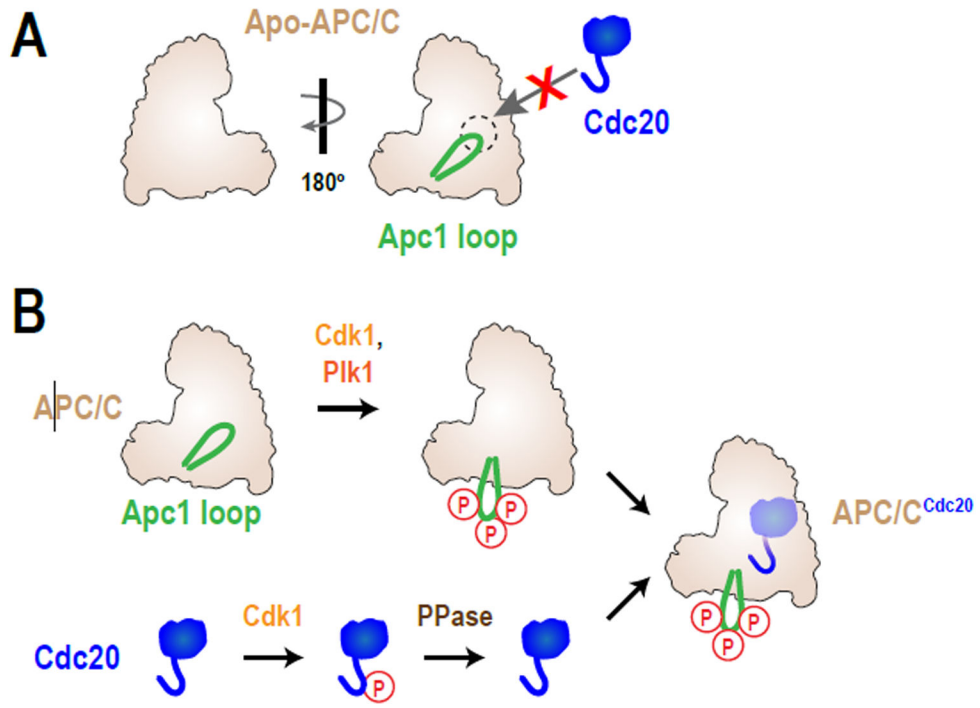

Figure 3. Control of APC/C^{Cdc20} activity by phosphorylation.

(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 N-terminal tail of Cdc20, which prevents its interaction with the APC/C. De-phosphorylation of Cdc20 by phosphatases (PPase) would cause its activation and binding to the APC/C.

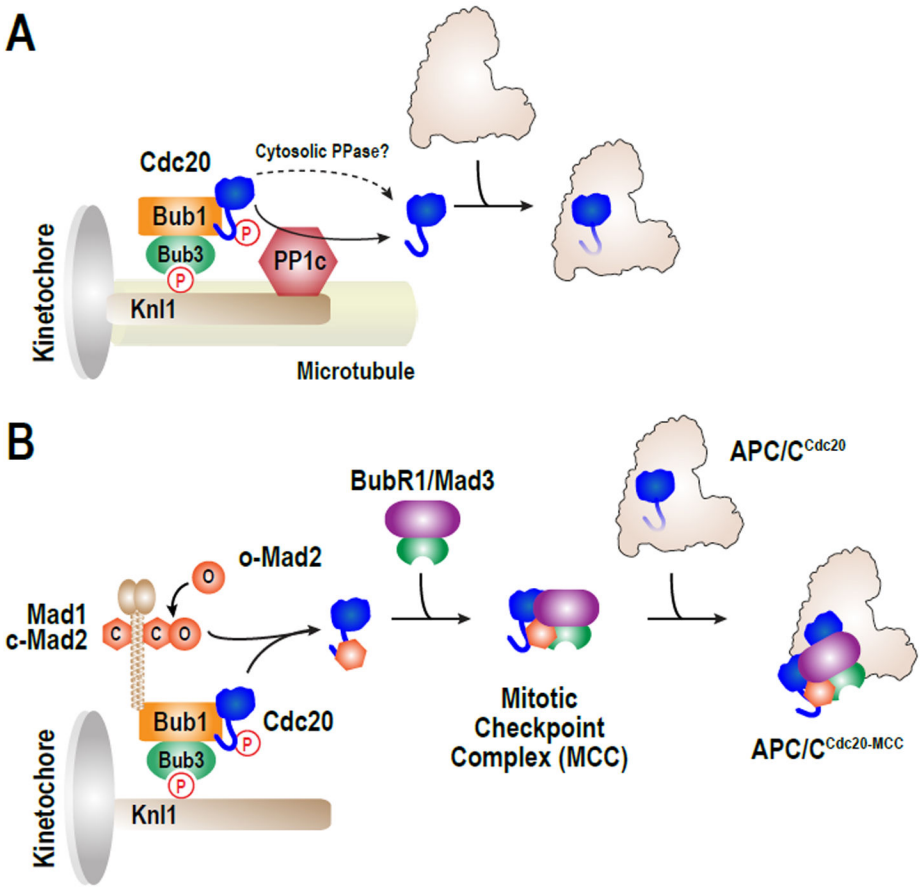

Figure 4. The two fates of Cdc20 at kinetochores.

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 de-phosphorylation 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.