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Review

Mitotic regulation of the anaphase-promoting complex

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Abstract. Orderly progression through mitosis is regulated by the anaphase-promoting complex/cyclosome (APC/C), a large multiprotein E3 ubiquitin ligase that targets key mitotic regulators for destruction by the proteasome. APC/C has two activating subunits, Cdc20 and Cdh1. The well-established view is that Cdc20 activates APC/C from the onset of mitosis through the metaphase-anaphase transition, and that Cdh1 does so from anaphase through G1. Recent work, however, indicates that Cdh1 also activates APC/C in early mitosis and that this APC/C pool targets the anaphase inhibitor securin. To prevent premature degradation of securin, the nuclear transport factors Nup98 and Rae1 associate with APC/C^{Cdh1}-securin complexes. In late metaphase, when all kinetochores are attached to spindle microtubules and the spindle assembly checkpoint is satisfied, Nup98 and Rae1 are released from these complexes, thereby allowing for prompt ubiquitination of securin by APC/C^{Cdh1}. This, and other mechanisms by which the catalytic activity of APC/C is tightly regulated to ensure proper timing of degradation of each of its mitotic substrates, are highlighted.

Keywords. Anaphase-promoting complex, APC/C, spindle assembly checkpoint, Cdc20, Cdh1.

Introduction

Progression through the eukaryotic cell cycle is dependent on the temporally controlled degradation of cell cycle regulatory proteins by the ubiquitin-proteasome system. Proteins degraded by this system are first tagged with a chain of at least four lysine 48-linked ubiquitin molecules. The addition of ubiquitin, a highly conserved 76-amino acid protein, requires the concerted activation of three enzymes: a ubiquitin-activating enzyme (E1) [1], a ubiquitin-conjugating enzyme (E2) [2], and a ubiquitin ligase (E3) [2, 3]. In a reaction that consumes ATP, E1 functions to create a high-energy thioester bond between its active-site cysteine and the C-terminal glycine residue of ubiquitin, resulting in the activation of ubiquitin [1, 4, 5]. Ubiquitin is then transferred to the active-site cysteine residue of the E2 molecule, with a new thioester linkage [2]. Lastly, ubiquitin is coupled to a lysine side chain of a target substrate via an isopeptide linkage [3]. The final transfer of ubiquitin to a targeted substrate is performed by the joint activities of E2 and one of several E3 ligases, conferring substrate specificity [3, 6]. Two structurally related multiprotein E3 ligases, the anaphase-promoting complex/cyclosome (APC/C) and the Skp1/Cullin/F-box protein (SCF) complexes, drive progression through the eukaryotic cell cycle [7–11]. These complexes differ in that the activity of SCF ligases mainly controls the transition from G1/S and G2/M [7], while APC/C is primarily required for mitotic progression and exit [8, 12–14].

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APC/C-mediated ubiquitination of its substrates requires one of two coactivators, Cdc20 (cell division cycle 20) or Cdh1/Hct1, and the recruitment and transient association of one of two specific E2 enzymes: UbcH10 or UbcH5 [11, 15]. APC/C activity needs to be tightly controlled to prevent unscheduled substrate degradation. A variety of APC/C inhibitory mechanisms seem to exist to mediate proper substrate degradation and control the catalytic activity of APC/C. Here, we will review these mechanisms with an emphasis on the actions of the spindle assembly checkpoint, a complex multiprotein network that inhibits cyclin B and securin destruction until the cell is poised to properly segregate its chromosomes at the onset of anaphase.

Composition of the vertebrate APC/C

The initial discovery of APC/C resulted from observations that certain cyclins are synchronously degraded as cells pass through mitosis [16]. Cdk1 functions to bring cells into mitosis, but its activity needs to be quenched during anaphase and telophase. If Cdk1 remains in its active state, chromosomes will not decondense, the nuclear envelope will not reassemble, and cell division is precluded [11]. Partial inactivation of Cdk1 is also required for the separation of sister chromatids during anaphase [17]. The major mechanism for Cdk1 inactivation is the destruction of its activating cyclins, cyclin A or B [11]. APC/C, the E3 ligase required to target cyclin B for destruction by the ubiquitin-proteasome pathway, was discovered nearly simultaneously in *Saccharomyces cerevisiae* [18], *Xenopus* eggs and clam oocytes [19, 20].

Subsequent work revealed that the vertebrate APC/C is a multiprotein complex consisting of at least 11 core subunits [21–23]. The largest APC/C subunit is Apc1, which was initially discovered in *S. cerevisiae* [24] and *Xenopus* eggs [25]. Apc1 is expressed at constant levels throughout the cell cycle and is specifically phosphorylated in mitosis [25, 26]. Although the function of Apc1 is largely unclear, it has been suggested that this subunit acts either as a scaffolding protein or as a protein required for the interaction with polyubiquitinated proteins [27]. Two APC/C subunits, Apc2 and Apc11, contain cullin [21] and RING-H2 finger domains [28], respectively, which are also found in subunits of SCF complexes. The cullin domain of Apc2 associates with the RING-H2 finger domain of Apc11 [29]. The RING-H2 finger of Apc11 further mediates interaction with the E2 ligases UbcH10 and UbcH5 [30]. An unexpected finding was that, *in vitro*, Apc11 and UbcH5 are sufficient for polyubiquitination of cyclin B [31]. Apc2/Apc11 along with UbcH10 can catalyze ubiquitination of securin and cyclin B *in vitro* [32]. Four subunits of APC/C, Apc3/Cdc27, Apc6/Cdc16, Apc7, and Apc8/Cdc23, all contain a 34-residue tetratricopeptide (TPR) motif [33, 34]. This motif, which is found in many other proteins, is known to mediate protein-protein interactions in large multiprotein complexes [35]. Phosphorylation of Apc3/Cdc27, Apc6/Cdc16, Apc7 and Apc8/Cdc23 during mitosis is required for APC/C activation and mitotic progression [25, 36]. Apc3/Cdc27 and Apc7 have increased affinity for the APC/C coactivator Cdc20 when they are phosphorylated [37]. Apc3/Cdc27 and Apc7 bind to Cdc20 and Cdh1 through an isoleucine-arginine (IR) dipeptide motif in the C terminus of the latter proteins [38, 39]. In addition, a sequence in the N terminus of both Cdc20 and Cdh1 called the C-box, is necessary for coactivator-APC/C association in yeast [40]. The C-box is not required for Cdc20 binding to APC/C in human cells [41] but is thought to play a role in substrate recognition [42]. Apc10/Doc1 is a subunit of APC/C that contains a Doc domain which is also found in several other proteins of the ubiquitin-proteasome system, including HECT and cullin family members [43, 44]. Apc10 is not required for the stable interaction of other APC/C subunits, which is somewhat surprising because Apc10 interacts directly with Apc3/Cdc27, Apc7, and Apc11 [30, 45]. Mutants of Apc10 are known to prevent substrate binding to APC/C^{Cdh1}, suggesting that this subunit may play a role in substrate recognition [38, 46]. It is not yet clear whether interaction of the substrate with Cdh1 and APC/C is directly or indirectly linked to Apc10. Currently, little is known about the function of the APC/C subunits Apc4, Apc5, Cdc26, and Apc13. Apc4 and Apc5 bind to Apc2 and Apc11, perhaps mediating the interaction of these proteins with the TPR motif-containing APC/C subunits [39]. Cdc26 plays a role in maintaining the structure of APC/C [21, 22, 47]. Apc13, which binds to Apc5 and Apc8/Cdc23 [23], presumably plays a more critical role in meiosis than in mitosis, although its exact function is unclear [48].

The fully assembled vertebrate APC/C consists of two large domains [49–51]. These domains, referred to as the 'platform' and the 'arc lamp', have a large amount of flexibility relative to each other [52]. When APC/C associates with the cofactor Cdh1, a conformational change occurs in the relative positions of the 'platform' and 'arc lamp', perhaps mediating the activation of APC/C [51]. It is not yet known where Cdc20 binds to APC/C or if this binding results in a similar change of APC/C conformation.

Disruption of APC/C subunits by genetic manipulation leads to early lethality in every species examined

thus far, from simple organisms like fungi to advanced vertebrates like mice [18, 53–55]. It is commonly believed that this lethality is due to the accumulation of securin and mitotic cyclins which inhibits chromosome segregation and mitotic exit [11]. However, APC/C is responsible for degradation of several other proteins as well, and it is entirely possible that overabundance of another substrate may result in cell death [52].

Control of APC/C-mediated substrate recognition and degradation

Phosphorylation of APC/C subunits regulates both the function and the assembly of the mature complex. Three kinases mediate APC/C subunit phosphorylation: protein kinase A (PKA), Polo-like kinase 1 (Plk1), and cyclin B/Cdk1 [56–59]. Phosphorylation of Apc3/Cdc27, Apc6/Cdc16, and Apc8/Cdc23 by cyclin B/Cdk1 leads to binding of Cdc20 to APC/C [19, 56, 59, 60]. Plk1 also promotes APC/C-mediated ubiquitination, but only in synergy with cyclin B/Cdk1 [56, 57]. In contrast to the activation of APC/C by these two kinases, PKA phosphorylation of APC/C inhibits the destruction of cyclin B, even when all activating cofactors are present [59]. It remains to be established which phosphatase functions to remove these inhibitory modifications.

The coactivators Cdc20 and Cdh1 are only transiently associated with APC/C. Substrates that have a destruction box (D-box) or a KEN-box are recognized and ubiquitinated by the APC/C [61, 62]. D-box recognition elements, with the consensus amino acid sequence RXXLXXXN, are found in several proteins, including mitotic cyclins, and are essential for ubiquitin-mediated destruction [62]. The KEN-box, which contains a consensus KEN amino acid sequence, is found in several APC/C substrates and is preferentially, but not exclusively, recognized by APC/C^{Cdh1} [61]. Most substrates only bind to the APC/C when it is activated by Cdc20 or Cdh1 [49]. Binding of Cdc20 to APC/C is tightly regulated to prevent premature APC/C-mediated ubiquitination. Regulation of Cdc20 occurs at various levels, with the protein being transcribed and translated during S and G2 phases, and phosphorylation occurring in a cell cycle-dependent fashion [63–65]. Phosphorylation of Cdc20 does not induce the activation of APC/C, but results in coactivator recognition by components of the spindle assembly checkpoint in mitosis, resulting in APC/C inhibition and anaphase prevention [66]. Regulation of APC/C activity by this cell cycle checkpoint will be expanded upon below.

Mitotic substrates of APC/C

Current models propose that APC/C^{Cdc20} is active during the early stages of mitosis, whereas APC/C^{Cdh1} is active in late mitosis and G1 (Table 1). In early mitosis, Cdh1 is phosphorylated by cyclin B/Cdk1 which precludes its association with APC/C [67–69]. Only after Cdk1 has been inactivated, by APC/C^{Cdc20}-mediated destruction of cyclin A and B, can inhibitory phosphates be removed from Cdh1 by Cdc14, thereby allowing the coactivator to associate with APC/C [67, 70]. However, recent findings, which will be discussed later, challenge this view. A summary of known mitotic substrates of APC/C can be found in Table 1.

Various mechanisms control the catalytic activity of APC/C. Early mitotic inhibitor 1 (Emi1) prevents premature activation of the APC/C by interacting with newly synthesized Cdc20 [71–73]. In prophase, Plk1 phosporylates Emi1. This allows it to be targeted for destruction by SCF$^{\beta TrCP}$ E3 ligase [74], which, in turn, leads to formation of active APC/C^{Cdc20}. Overexpression of Emi1 in cells lacking p53 has been shown to promote proliferation, tetraploidy, and chromosomal instability [75], underscoring that Emi1 is a key mitotic regulator. A recent study suggests that following Emi1 destruction in prophase, Cdc20 continues to be inhibited through prometaphase by the tumor suppressor protein Ras association domain family 1 (Rassf1A) [76]. At the end of prometaphase, when this inhibition ceases, APC/C^{Cdc20} becomes active and begins to ubiquitinate Nek2A and cyclin A, resulting in the complete destruction of these substrates in metaphase [77–79]. Nek2A binds to APC/C directly, without any need for adapter proteins or coactivator molecules [79]. Its subsequent destruction can then ensue upon APC/C activation by coactivator binding. Perhaps cyclin A is degraded in a similar fashion, but this remains to be confirmed.

In order for a cell to transit from metaphase to anaphase, several key substrates of APC/C need to be degraded, including cyclin B and securin [11, 20, 80–83]. Anaphase onset is marked by the separation of sister chromatids, which are held together by a large multiprotein complex called cohesin [84, 85]. Separase is the enzyme that mediates cohesin cleavage [84]. Until the metaphase to anaphase transition, securin binding and cyclin B/Cdk1-mediated phosphorylation inhibit the enzymatic activity of separase, thereby preventing premature sister chromatid separation (PMSCS) [86, 87]. A recent study has demonstrated that cyclin B binding to separase alone, without a requirement for cyclin B/Cdk1-mediated phosphorylation, is sufficient to inhibit separase activity [88]. The view that APC/C^{Cdc20} regulates degradation of both cyclin B and securin at the

Table 1. Mitotic substrates of APC/C

Substrate	Start of degradation	APC/C activity involved	Reference
Cyclin A	prophase	APC/C^{Cdc20} (early mitosis); APC/C^{Cdh1} (G1)	78
Nek2A	prophase	APC/C^{Cdc20}	77, 79
Cyclin B	metaphase	APC/C^{Cdc20} and APC/C^{Cdh1}	20, 80, 83
Securin	metaphase	APC/C^{Cdc20} and APC/C^{Cdh1}	80–82
Xkid	metaphase	APC/C^{Cdc20} and APC/C^{Cdh1}	90–92
Prc1	metaphase	APC/C^{Cdh1}	95, 96
Kip1	metaphase	APC/C^{Cdc20}	93
Cin8	metaphase	APC/C^{Cdh1}	94
Geminin	metaphase	currently unkown	98
Tpx2	anaphase	APC/C^{Cdh1}	97
Plk1	before mitotic exit	APC/C^{Cdh1}	100
Aurora A	before mitotic exit	APC/C^{Cdh1}	105–107
Cdc20	before mitotic exit	APC/C^{Cdh1}	61, 103, 104
Aurora B	before mitotic exit	APC/C^{Cdh1}	101
Anillin	before mitotic exit	APC/C^{Cdh1}	102

metaphase/anaphase transition has been challenged by gene knockout studies showing that premature activation of APC/C^{Cdc20} leads to unscheduled degradation of cyclin B, but not securin [80]. Premature activation of APC/C^{Cdh1}, on the other hand, leads to the precocious degradation of securin, but not cyclin B, *in vivo* [80, 89]. The latter finding has established a critical role for the activity of APC/C^{Cdh1} in mitosis much earlier than originally thought.

In addition to securin and cyclin B, several other proteins need to be degraded to allow for anaphase entry and progression. *Xenopus* Xkid is implicated in generating the polar ejection force that makes the chromosomes align during metaphase [90, 91]. In order for chromosomes to migrate to the poles in anaphase, Xkid needs to be degraded. Both APC/C^{Cdc20} and APC/C^{Cdh1} have been shown to ubiquitinate Xkid *in vitro* [92]. The motor proteins Kip1 and Cin8 are degraded during anaphase by APC/C^{Cdc20} and APC/C^{Cdh1}, respectively, to allow polar movement of chromosomes [93, 94]. Another protein whose degradation is required for progression through anaphase is Prc1. This protein associates with the spindle midzone and is subjected to ubiquitination by APC/C^{Cdh1} [95, 96]. APC/C^{Cdh1} also degrades the microtubule-associated protein Tpx2. Its degradation starts in anaphase and continues through cytokinesis [97]. Geminin, a protein that inhibits DNA replication, is targeted by APC/C beginning at metaphase and continuing until the cell exits from mitosis [98]. It is currently unclear which APC/C coactivator drives geminin destruction. Complete ablation of geminin results in endoreduplication [99], demonstrating that APC/C-mediated destruction of this protein requires strict temporal regulation. The mitotic kinases Plk1 [100] and Aurora B [101] are also destroyed late in mitosis. Their destruction by APC/C^{Cdh1} allows entry into G1. Anillin, which controls the spatial contractility of myosin at the cleavage furrow during cytokinesis, is targeted by APC/C^{Cdh1} for destruction late in cytokinesis and G1 [102]. However, this destruction is not required for mitotic exit in mammalian cells. The activity of Cdh1-bound APC/C continues through G1, where it regulates the destruction of Cdc20 [61, 103, 104], Aurora A, and mitotic cyclins to prevent the reaccumulation of these proteins [105–107].

Regulation of APC/C by the spindle assembly checkpoint

The spindle assembly checkpoint, alternatively referred to as the mitotic checkpoint, is a molecular system that ensures accurate segregation of mitotic chromosomes by delaying anaphase onset until each kinetochore has properly attached to the mitotic spindle [108–110]. Kinetochores that are not yet attached to mitotic microtubules and chromosome pairs that lack tension across sister chromatids generated by the spindle poles activate the spindle assembly checkpoint [111, 112]. The established view is that various mitotic checkpoint proteins, including Bub1, BubR1, Bub3, Mad1, and Mad2, bind to kinetochores that lack attachment or tension to generate a “stop anaphase” signal that diffuses into the mitotic cytosol [108, 112–115] (Fig. 1A). This signal is believed to consist of complexes of Bub3, BubR1, and Mad2, which bind and inhibit APC/C^{Cdc20} [11, 30, 116, 117]. As each pair

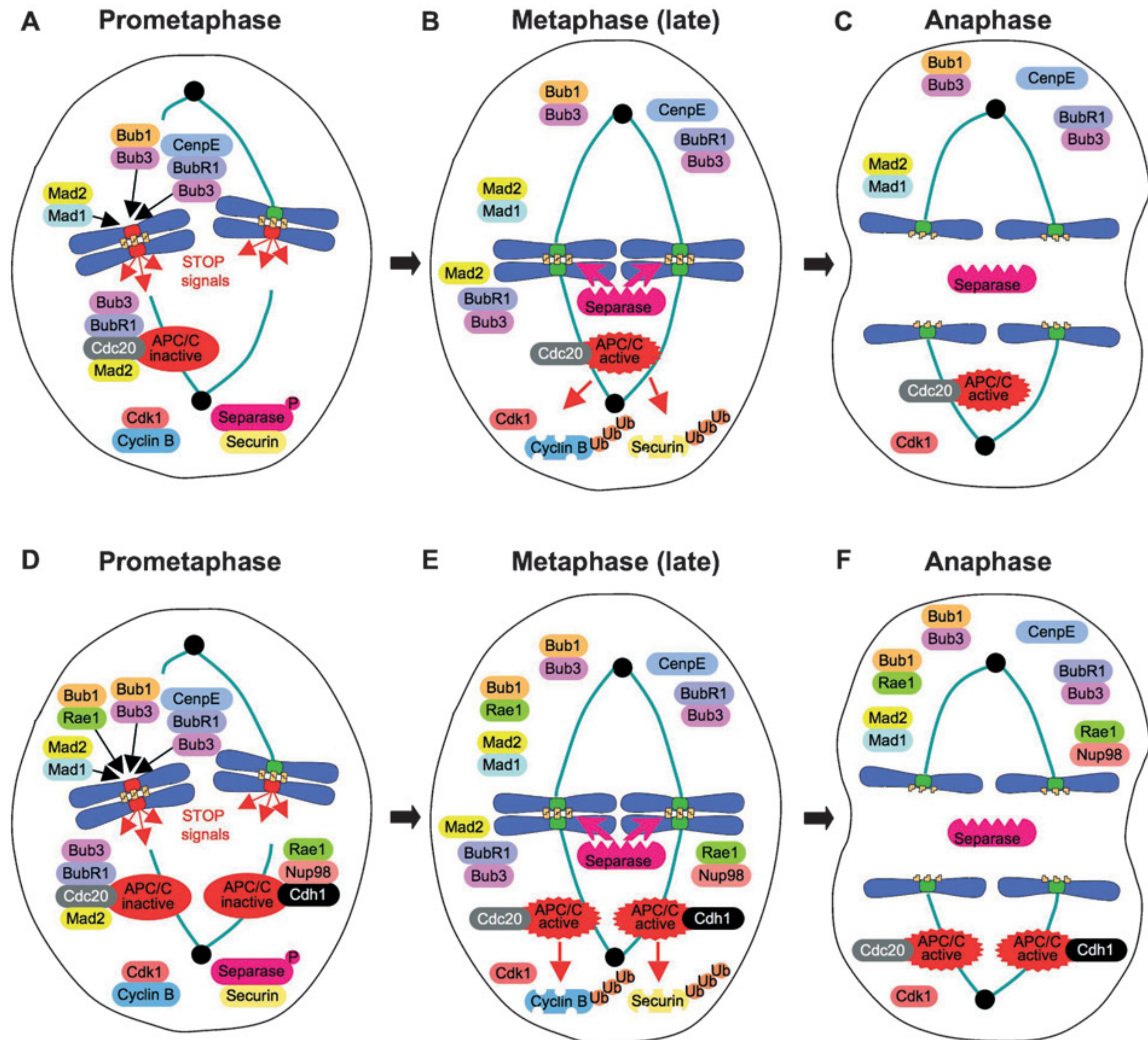

Figure 1. Regulation of APC/C during the metaphase-to-anaphase transition. For details see text. (**A–C**) Established model for spindle assembly checkpoint control where APC/C^{Cdc20} regulates both securin and cyclin B in a checkpoint-dependent manner. (**D–F**) Modified model for spindle assembly checkpoint function that includes the nuclear transport factors. In this model, the degradation of cyclin B and securin is mainly mediated by APC/C^{Cdc20} and APC/C^{Cdh1}, respectively.

of sister kinetochores attaches to microtubules, and microtubule motors generate tension that stretches them, production of inhibitory 'stop anaphase' signals at those kinetochores quenches. Silencing of the "stop anaphase" signal, which occurs when the final kinetochore is captured by spindle microtubules [118], triggers the release of inhibitory mitotic checkpoint protein complexes from APC/C^{Cdc20} (Fig. 1B). This then allows for APC/C^{Cdc20}-mediated destruction of cyclin B and securin. Separase, which in mammalian cells is inhibited through its association with securin and by cyclin B/Cdk1-mediated phosphorylation, subsequently triggers sister chromatid disjunction by cleavage of the cohesin subunit Scc1 [84, 119]. This allows cells to progress into anaphase (Fig. 1C).

Recent work involving the nuclear transport factors Nup98 and Rae1 has challenged several aspects of the above model [80]. Mutant mice that express low levels of both Nup98 and Rae1 exhibit PMSCS and massive aneuploidy. In cells from these mice, securin undergoes ubiquitin-mediated destruction in prometaphase instead of at anaphase onset. On the other hand, the timing of cyclin B destruction is normal in these cells. In prometaphase, Rae1 and Nup98 were observed to interact specifically with APC/C^{Cdh1} to prevent degradation of securin, but not APC/C^{Cdc20} (Fig. 1D), which was surprising as previous studies suggested that the formation of APC/C^{Cdh1} in early mitosis is inhibited through phosphorylation of Cdh1. However, this mechanism does not completely prevent APC/C^{Cdh1}

formation in early mitosis. In fact, comparative coimmunoprecipitation experiments suggest that there are very similar amounts of APC/C^{Cdc20} and APC/C^{Cdh1} in early mitosis [89]. Dissociation of Rae1 and Nup98 from APC/C^{Cdh1} coincides with the release of BubR1 from APC/C^{Cdc20} [80]. Because the release of BubR1, and its coinhibitors Bub3 and Mad2, occurs at the metaphase/anaphase transition to activate APC/C^{Cdc20} and drive cells into anaphase, it is likely that the dissociation of Rae1 and Nup98 from APC/C^{Cdh1} also occurs at this mitotic stage and for the same purpose. If APC/C^{Cdc20} promotes anaphase through ubiquitination of both cyclin B and securin, one would expect to observe premature degradation of both of these proteins in cells in which BubR1 is deficient. However, only cyclin B is prematurely degraded in such cells, suggesting that Cdc20 is the primary coactivator for degradation of cyclin B, but not for degradation of securin *in vivo* [80]. Conversely, APC/C^{Cdh1} activated by release of Rae1 and Nup98 might have a more important role in the destruction of securin (Fig. 1E). It is currently not understood how the Rae1-Nup98 complex is targeted to Cdh1 in response to lack of attachment at kinetochores and how it senses kinetochore capture to release its inhibition of APC/C^{Cdh1}. Rae1 is known to form a complex with Bub1 and localize to unattached kinetochores [120, 121]. One possibility is that these Bub1-Rae1 complexes regulate Nup98-Rae1 binding to APC/C^{Cdh1}, perhaps in much the same way as other kinetochore-associated mitotic checkpoint proteins promote binding of Bub3-BubR1-Mad2 complexes to APC/C^{Cdc20}.

Additional means of regulating the ubiquitin ligase activity of APC/C during mitosis are beginning to be elucidated (Table 2). A complex of CBP and p300, two transcription factors, is essential for APC/C activity in mitosis [122, 123]. Through reciprocal immunoprecipitation assays, it has been shown that CBP/p300 interacts with three structural components of APC/C, in addition to the coactivators Cdc20 and Cdh1 [123]. Furthermore, CBP colocalizes with APC/C and siRNA-mediated depletion of CBP leads to reduced APC/C E3 ligase activity and the accumulation of cyclin B and Plk1 [123]. Together these findings implicate CBP/p300 in activation of both coactivator-bound forms of APC/C during mitosis. This regulation may be through CBP/p300-mediated acetylation of APC/C subunits and/or the coactivators Cdh1 and Cdc20, although the details are not yet clearly established. It will be interesting to examine in future experiments whether the actions of CBP/p300 in mitosis are regulated by the spindle assembly checkpoint. The implication of Rae1-Nup98 and CBP/p300 complexes in mitotic regulation of APC/C highlights that controlling APC/C activity in mitosis is far more complex than once thought.

Consequences of deregulated APC/C activity in mitosis

Proper spindle assembly checkpoint function and timely activation of APC/C and destruction of its substrates are essential for accurate chromosome segregation. Aberrant checkpoint signaling leads to defects in inhibition of APC/C activity, which results in untimely entry into anaphase with a high risk of segregation defects (Table 2). Several chromosomal segregation defects are often seen in checkpoint-defective cells. PMSCS is caused by separation of duplicated chromatids before entry into anaphase and is seen in Mad2 [124, 125], Rae1 [120], BubR1 [126, 127], and Bub3 [120, 128] haploinsufficient mice and BubR1 hypomorphic mice [129, 130] (Table 2). Cells that have insufficient amounts of the APC/C^{Cdh1} inhibitors Rae1 and Nup98 have an increased incidence of PMSCS, perhaps due to premature destruction of securin [80]. PMSCS can ultimately contribute to chromosomal instability. Kinetochores that lack microtubule attachment and/or tension but fail to activate the spindle assembly checkpoint and inhibit APC/C activity will produce lagging chromosomes. Additionally, merotelic kinetochore attachment causes lagging chromosomes [131]. During segregation in anaphase, this chromosome does not move towards the spindle poles along with other chromosomes. It is distributed randomly in one of the two daughter cells, which may result in a gain or loss of a whole chromosome. Other missegregation defects, including anaphase bridges may also be present when the spindle checkpoint is defective [130].

Studies of mitotic checkpoint proteins in mice have revealed that insufficiency of almost every component of the spindle assembly checkpoint not only causes chromosomal instability but also increased predisposition to chemical- or mutagen-induced tumorigenesis [120, 126, 130, 132] (Table 2). Furthermore, several other studies have reported alterations in various mitotic checkpoint protein genes, including BubR1, Bub1, Mad2, and Mad1, in human cancers [108, 133–135]. These findings link deregulated mitotic checkpoint inhibition of APC/C to chromosomal instability and cancer, suggesting that control of APC/C in mitosis indeed has physiological consequences, which reinforces the need for its proper and timely regulation.

APC/C coactivators have also been observed to be deregulated in human cancers. For example, overexpression of Cdc20 is observed in a variety of

Table 2. Mitotic regulators of APC

Class of proteins	Members	Function	Consequences of insufficiency	References
Mitotic checkpoint proteins	BubR1	A kinase that interacts with Bub3 and CENP-E at the kinetochores. Inhibited by CENP-E upon microtubule attachment. Part of the APC/C inhibitory complex MCC. Required for ensuring proper tension generation at kinetochores.	PMSCS, lagging chromosomes, and aneuploidy. In mice, complete loss is embryonic lethal, but reduced levels result in sensitivity to AOM and DMBA-induced tumorigenesis and premature aging.	126, 127, 129, 130
	Bub1	A kinase that binds to Bub3 and Rae1. Involved in direct phosphorylation and inhibition of APC/C^{Cdc20}. Also required for recruitment of BubR1, Mad2 and CENP-E to kinetochores	Aneuploidy and anchorage-independent growth in culture.	114, 115
	Bub3	Interacts with Bub1 at kinetochores. Also binds with cytosolic BubR1 to form the APC/C^{Cdc20} inhibitory complex MCC.	Lagging chromosomes and aneuploidy. In mice, complete loss is embryonically lethal. Haploinsufficiency causes sensitivity to DMBA-induced tumorigenesis. Compound haploinsufficiency of Bub3 and Rae1 implicated in early aging.	120, 128
	Mad1	Required for recruitment of Mad2 to kinetochores.		
	Mad2	Recruited by Mad1 to kinetochores. Key component of MCC. Required for ensuring proper microtubule capture at kinetochores.	PMSCS, lagging chromosomes, and anueploidy. Accelerated chromosome loss, apoptosis, and embryonic lethality due to complete loss in mice.	124, 125
	CENP-E	A kinase that localizes to the kinetochores and interacts with BubR1. Involved in inhibition of BubR1 upon microtubule attachment.	Abberant chromosome alignment. Aneuploidy.	118, 134
	Rae1	An mRNA export factor with mitotic functions. It resembles Bub3 in function and interacts with Bub1. Also forms an APC/C^{Cdh1} inhibitory complex with Nup98. Shown to be involved in spindle formation as well.	Deficiency is implicated in aberrant spindle formation. In mice, insufficiency leads to aneuploidy and susceptibility to DMBA-induced tumorigenesis. Synergizes with Bub3 in early onset of aging phenotypes.	80, 89, 120, 121, 132
Mitotic kinases	Plk1	Activates APC/C by phosphorylating its various subunits. Also phosphorylates Emi1 and targets it for degradation.	siRNA-mediated depletion leads to impaired spindle formation and prometaphase arrest.	56, 57
	Cdkl	Activated by cyclin B. Multiple phosphorylation targets in mitosis including APC/C components. It also phosphorylates and inhibits separase.		19, 56, 59, 60
	PKA	Inhibits APC/C by phosphorylating its various substrates.		59
Other complexes	Rae1-Nup98	Binds Cdh1 and inbhits APC/C^{Cdh1} activity in early mitosis.	Double haploinsufficiency of the complex components leads to increased PMSCS, aneuploidy, and predisposition to DMBA-induced tumorigenesis in mice.	80, 89
	CBP-p300	Interacts with both Cdh1 and Cdc20. Required for proper activation of both forms of APC/C in mitosis.	Double depletion of both components leads to loss of APC/C E3 ligase activity resulting in arrest of cells in mitosis.	122, 123

human malignancies, including pancreatic [136], lung [137], and gastric [138] cancers. Cdh1 down-regulation is observed in murine lymphomas, whereas overexpression of Cdh1 acts to suppress B cell tumorigenesis [139]. In this regard, Cdc20 appears to be a potential oncogene, whereas Cdh1 is more likely to be a tumor suppressor. Critical testing of this hypothesis would require the use of both transgenic and knockout mice for these genes. The physiological relevance of Cdh1 or Cdc20 is unclear and needs further investigation.

Conclusions

An emerging theme in the regulation of APC/C-mediated degradation of substrates in mitosis is that three key components associate with APC/C. These three components are: (i) APC/C coactivators; (ii) inhibitory protein complexes that prevent unscheduled APC/C-mediated ubiquitination, and (iii) the substrate that needs to be degraded. In this way, APC/C is loaded with the target substrate before the transfer of ubiquitin takes place through the associa-

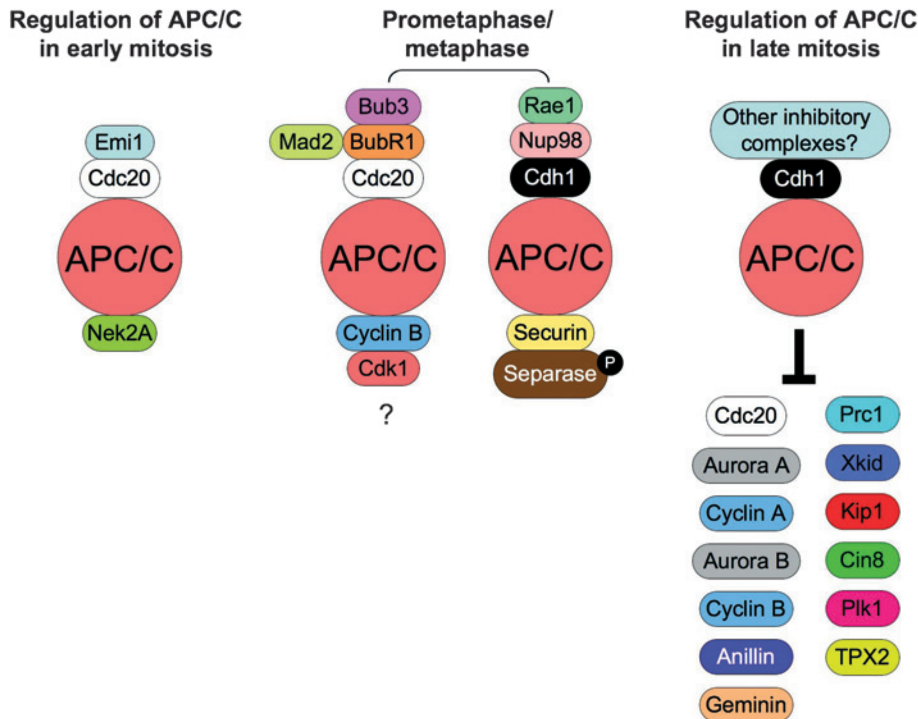

Figure 2. Inhibitory mechanism of APC/C^{Cdc20} and APC/C^{Cdh1}. In early mitosis, Emi1 regulates the activity of APC/C^{Cdc20}. The well-established MCC and the newly found Nup98-Rae1 complex inhibit the catalytic activity of APC/C during prometaphase, preventing substrate destruction. *In vitro* evidence suggests that APC/C^{Cdc20} also regulates the destruction of securin, but it is not known if MCC also binds to this pool of APC/C. Perhaps additional inhibitory complexes are found later in mitosis to prevent the unscheduled degradation of other key mitotic regulator proteins. The existence of inhibitory complexes may explain how the cell discriminates between early and late mitotic APC/C substrates.

tion of an E2 enzyme. Once the inhibitory complex is removed from APC/C, swift degradation of bound substrates can ensue because the APC/C is primed for destruction by having the coactivator and the substrate existing in a large complex.

The first example that supports this hypothesis occurs in prophase. Two early mitotic substrates of APC/C are Nek2A and cyclin A. To prevent the unscheduled destruction of these proteins, Emi1 binds to both Cdc20 and Cdh1 before these two coactivators can associate with APC/C [71, 72]. Both Cdc20-Emi1 and Cdh1-Emi1 complexes can associate *in vitro* with APC/C, but only Cdc20-Emi1 loads onto APC/C *in vivo* in early mitosis [72]. Emi1 inhibits APC/C until Plk1 phosphorylates Emi1 and allows it to be recognized and ubiquitinated by SCF$^{\beta TrCP}$ ubiquitin ligase [74]. Degradation of Emi1 allows Cdc20 activation of preformed APC/C-Nek2A complexes, which in turn leads to prompt destruction of Nek2A [72, 79]. It is therefore possible that Nek2A binds to APC/C while Cdc20-Emi1 complexes are forming and loaded onto APC/C.

The second example that supports our model occurs during prometaphase. The well-established MCC inhibitory complex consists of Mad2, Bub3, and BubR1 bound to Cdc20. Association of this complex with APC/C^{Cdc20} inhibits the ligase activity until the spindle checkpoint is satisfied. Once the checkpoint is silenced, the inhibitory MCC complex dissociates from APC/C^{Cdc20}, which results in APC/C activation and swift destruction of cyclin B and, potentially, securin. Importantly, cyclin B already associates with APC/C in prometaphase even though its destruction takes place much later at the metaphase-to-anaphase transition [89]. Thus, both cyclin B and the MCC may bind to APC/C^{Cdc20} similarly to the way Nek2A and Emi1 associate with APC/C^{Cdc20} (Fig. 2).

The third example that supports our model also occurs during prometaphase. The Rae1-Nup98 inhibitory complex binds to securin-bound APC/C^{Cdh1} complexes [89]. The rapid degradation of securin occurs at a similar rate to cyclin B destruction when the spindle assembly checkpoint is satisfied. Release of Rae1-Nup98 and MCC inhibitory complexes occurs at nearly the same rate, but the mechanism behind the release of Rae1-Nup98 is not known. The rapid and synchronous nature of cyclin B and securin degradation suggests that APC/C is primed for the destruction of these two key mitotic regulators. Having the substrate bound to inactivated APC/C could explain this precise regulation.

Currently, the events of late mitosis are not well understood at the mechanistic level. Following metaphase-anaphase transition, ubiquitin-mediated destruction of substrates primarily occurs through APC/C^{Cdh1}, but it is not known if other inhibitory complexes prevent the premature degradation of substrates during late mitosis. Does the degradation follow a sequential pattern? Are there additional checkpoints/inhibitory complexes that exist after

metaphase to insure that APC/C target proteins are not degraded early in mitosis but before mitotic exit? Do substrates that regulate the exit of mitosis also bind to APC/C before they are scheduled for destruction? What is the benefit of binding and sequestering substrates of APC/C prior to their destruction? These are but a few of the questions that remain in the ever-expanding field of APC/C regulation during mitosis.

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