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Spatiotemporal Regulation of the Anaphase-Promoting Complex in Mitosis

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Abstract

The appropriate timing of events that lead to chromosome segregation during mitosis and cytokinesis is essential to prevent aneuploidy, and defects in these processes can contribute to tumorigenesis. Key mitotic regulators are controlled through ubiquitylation and proteasome-mediated degradation. The Anaphase-Promoting Complex or Cyclosome (APC/C) is an E3 ubiquitin ligase that has a crucial function in the regulation of the mitotic cell cycle, particularly at the onset of anaphase and during mitotic exit. Co-activator proteins, inhibitor proteins, protein kinases and phosphatases interact with the APC/C to temporally and spatially control its activity and thus ensure accurate timing of mitotic events.

Cell cycle transitions are driven by oscillations in the activity of Cyclin-dependent kinases (Cdk). These oscillations in Cdk activity are often controlled by the production and degradation of Cyclins, which bind and activate Cdks. In higher eukaryotes, approximately 20 different Cdks and Cdk-related proteins (all of which are serine/threonine protein kinases) and four major Cyclin classes exist; different combinations of Cdks and Cyclins regulate cell phase-specific events such as DNA replication and mitosis¹. The abundance of Cyclins and other cell cycle regulators (such as Cdk inhibitors (CKIs)) oscillates during the cell cycle as a result of controlled expression and timely proteolysis mediated by the ubiquitin-proteasome pathway², and this drives the forward progression of the cell cycle.

The E3 ubiquitin ligase Anaphase-Promoting Complex/Cyclosome (APC/C) controls the order of events that ensures accurate chromosome segregation during mitosis, thus contributing to the maintenance of genomic integrity. Activity of the APC/C during mitotic progression is modulated in time and space by complex and multilayered regulatory events that include co-activator binding, post-translational modification, inhibition by the spindle checkpoint and compartmentalization in subcellular locations. These events regulate the activity of the APC/C to eventually promote the rapid and irreversible transition to anaphase and mitotic exit.

This review focuses on the spatiotemporal regulatory pathways that govern APC/C function in mitosis. Substantial recent advances in defining the structure of the APC/C, its associations with E2 enzymes, and the complex temporal and spatial regulation of its

activators and inhibitors make this an opportune time to summarize our current understanding.

THE APC/C UBIQUITYLATION PATHWAY

Ubiquitin-proteasome pathways involve the covalent attachment of multiple ubiquitin molecules to protein substrates that are targeted for degradation by the 26S proteasome complex³. The attachment of ubiquitin to target proteins is a 3-step process catalyzed by at least 3 enzymes: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3)⁴. Ubiquitin (a small 8kDa protein) is transferred to E1 in an ATP-dependent manner. This activated ubiquitin is then transferred to the E2, and the E3 ligase catalyzes the binding of ubiquitin to a lysine on target proteins. Binding of further ubiquitin molecules to either one of seven lysine residues of ubiquitin or its N terminus results in the formation of poly-ubiquitin chains⁵. Mono-ubiquitylation can impact protein localization or protein-protein interactions⁶. Poly-ubiquitin chains linked through different ubiquitin lysines have distinct structures and influence the fate of the modified protein differently. K11 and K48-linked chains target proteins for proteasomal degradation while K63-linked chains typically facilitate protein-protein interactions required for signaling. Poly-ubiquitin chains linked through K6, K27, K29, and K33 also exist but these are less understood^{4, 7–9}.

The human genome encodes two E1s, at least 35 E2s and ~600 E3s. Members of the cullin-RING family of E3 ligases have key roles in many aspects of cell cycle control¹⁰. Of these, the APC/C plays a prominent part, as it controls progression into, through, and out of mitosis by mediating degradation of key regulators at precise times. Although the APC/C is often discussed as becoming “activated” at the metaphase-anaphase transition, this is an oversimplification. The APC/C is active all through mitosis and through much of the rest of the cell cycle. Under exquisitely fine regulation, it is able to show strongest targeting of specific substrates at specific points during mitotic progression (Figure 1). In this review we discuss the many aspects of that regulation.

Structure of the APC/C

In 1995 the APC/C was discovered as a mitosis-specific E3 ubiquitin ligase in clam¹¹, *Xenopus laevis*¹² and budding yeast¹³. In recent years much progress has been made in understanding the structural organization of the APC/C by using insect cell expression systems to reconstitute the multi-subunit E3 ligase with or without its regulators^{14–19}.

The vertebrate 1.22 MDa APC/C is composed of 14 different protein subunits (19 subunits in total as 5 subunits are present in two copies) (Figure 2; Table 1). The complex is largely organized into three structural domains, called the ‘platform’, the ‘catalytic core’ and the ‘TPR lobe or arc lamp’^{14, 15, 17, 18, 20, 21}. The platform sub-complex forms a base to join the other subunits of the APC/C. The catalytic core sub-complex on its own cannot efficiently recruit substrates but along with an E2 enzyme can provide low ubiquitylation activity to the APC/C. The TPR lobe (also known as the ‘arc lamp’ owing to its overall shape) consists of several structurally related proteins with multiple tetratricopeptide (TPR) repeats. Three other subunits, the TPR accessory factors, are present in single copy and serve to stabilize

the APC/C subunits in the TPR lobe ^{14, 22}. The subunits in the TPR lobe account for more than 80% of the mass of the APC/C, exist as homodimers and are required to provide important scaffolding functions to the APC/C ^{15, 23}. Further, these subunits coordinate assembly of the APC/C and mediate important interactions with regulator proteins that modulate APC/C activity. Importantly, this region of the APC/C also interacts with the inhibitory complex called the Mitotic Checkpoint Complex (MCC), which plays a key role in regulating mitotic progression ^{15, 23}. Together this multi-subunit E3 ubiquitin ligase cooperates with at least two E2 enzymes and one of two co-activator proteins, Cdc20 or Cdh1 (in all eukaryotes), to recruit and ubiquitylate substrates for proteasomal degradation during mitosis.

Ubiquitin-conjugating enzymes (E2s) of the APC/C

In yeast and human cells, distinct E2s collaborate with the APC/C to initiate and then elongate ubiquitin chains. In yeast, Ubc1 and Ubc4 can both catalyze ubiquitin chain initiation and elongation in conjunction with the APC/C. However, Ubc4 functions preferentially in chain initiation whereas Ubc1 favors chain elongation ^{24, 25}. In higher eukaryotes including vertebrates, UbcH10 (also known as Ube2C and UbcX) links the first ubiquitin to substrates through binding to the RING domain of APC11 ^{26, 27}. At least *in vitro*, another initiating E2, UbcH5 (also called Ube2D) can also fulfill this role ^{27–32}. Chain elongation is catalyzed by Ube2S, which binds to a distinct surface of APC11 and also binds via its C terminus to other components of the catalytic core and platform ^{26, 31, 33–35}.

Though the use of two E2s is conserved throughout evolution, the linkage specificity of poly-ubiquitin is less conserved, and how specific linkages affect cell cycle progression in each species remains an active area of investigation. Budding yeast APC/C modifies substrates with K48-linked ubiquitin chains ²⁵. In contrast, in higher eukaryotes like *X. laevis* and humans the APC/C primarily generates K11-linked chains or mixed K11- and K48-linked chains, which are both recognized and degraded by the 26S proteasome ^{7, 30–33, 36}. Recently it has been shown that Ube2S can build branched ubiquitin chains by adding multiple K11-linked ubiquitins to existing ubiquitin chains linked through K48. These branched ubiquitin chains allowed efficient recognition by the proteasome and can promote substrate degradation when APC/C activity is limiting ³⁷.

Deubiquitylating enzymes (DUBs), which counteract APC/C-mediated ubiquitylation, have also been found to play important roles in mitotic control ³⁸. The DUBs shorten ubiquitin chain length thereby regulating order and timing of substrate degradation. For example, the DUB Usp37 removes polyubiquitin chains on Cyclin A at the G1/S transition. This allows entry into S phase ³⁹. The precise roles of DUBs in mitosis require further study.

Coactivators of the APC/C

The APC/C is largely inactive without one of its co-activators, Cdc20 or Cdh1. Their carboxy-termini contain a WD40 domain that forms a binding platform to recruit APC/C substrates ^{40, 41}. In addition, Cdc20 and Cdh1 promote ubiquitylation by enhancing the interaction of the APC/C with E2-Ub ^{14, 26, 35, 42}. Cdh1, and possibly Cdc20, bind to the subunits APC3 and APC8 through interaction with TPR motifs ¹⁴.

Although structurally related, Cdc20 and Cdh1 activate the APC/C at different times. Cdc20 associates with the phosphorylated APC/C in early mitosis and leads to the degradation of prometaphase and metaphase substrates^{41, 43–48}. Later, during anaphase and into G1, Cdc20 is replaced by Cdh1. Cdk1, Bub1 and MAPK phosphorylate Cdc20 on multiple residues. Some phosphorylations inhibit while others stimulate APC/C activity^{21, 45, 49–53}. Phosphorylation of Cdh1 by Cdk1 inhibits its association with the APC/C until mid to late anaphase^{45, 54–56}. At that time decreasing Cdk1 activity and increased phosphatase activity results in dephosphorylation of Cdh1, which then binds and activates the APC/C thereby causing substrate degradation in late mitosis and during G1. It was also shown that Cdh1 is sequestered in mitosis by the protein Mad212; degradation of this protein during anaphase frees Cdh1 to bind and activate the APC/C⁵⁷.

Recent structural studies have provided valuable insight into the APC/C-Cdh1- substrate-E2 complex^{14, 15, 1718}. The catalytic module of the APC/C, consisting of APC2-APC11, was found to be flexible¹⁴. Interestingly, the platform subunits of the APC/C were displaced upon coactivator-substrate binding. Coactivator binding disrupts the interaction between APC8 and APC1 causing a downward displacement of APC8 and other platform subunits concomitantly pushing the catalytic module (APC2 C-terminal domain -APC11) upwards¹⁴. This change in conformation possibly increases catalytic activity of the APC/C by bringing the initiating E2-Ub close to the substrate^{14, 15, 17} (Figure 3A). The coactivator Cdc20 binds the C terminal region (called the C-terminal peptide or CTP) of Ube2S, which might aid in recruiting Ube2S to the APC/C³⁵. Then the Ube2S CTP could be passed to the APC2-APC4 region of the platform toward which it shows strong affinity²⁶. At a site on the APC/C distinct from the chain initiation site that functions through UbcH10, the Ube2S-platform interaction generates a site for ubiquitin chain elongation. This region of the APC/C also interacts with specific residues on the terminal ubiquitin of the growing chain to position it as an acceptor for the addition of the next ubiquitin³⁵.

Substrate recognition sequences

Substrates have degradation sequences or degrons through which they bind specifically to the APC/C-coactivator complex. Most substrates have either a 9 residue D-box (RXXLXXI/VXN)^{58–61} and/or a KEN-box (KENxxxN/D)^{62–64}. The degrons interact with two distinct regions on the WD40 domain of coactivators^{21, 40, 65}. D box substrates bind to a bipartite receptor formed by APC10 and the lateral surface of the coactivator WD40 domain. APC10 enhances substrate binding and processivity of the ubiquitylation reaction^{62, 65–72}. The KEN box degrons interact with a region on the surface of the coactivator WD40 domain^{62, 73}.

Although these degrons are required they are not sufficient suggesting that substrates contain additional non-conserved sequences that are required to bind the APC/C-activator complex^{56, 62}. These additional recognition sites might be important for fine-tuning the timing of substrate degradation during the progression of mitosis⁷⁴. Other distantly related APC/C degron motifs exist such as the O-box in ORC1 (similar to D box), the G box in *Xenopus* kid (similar to KEN box), the A box found in Aurora kinase, CRY box in Cdc20, and less clearly defined degrons in Claspin and Iqg1^{36, 75}.

The timing of substrate degradation during mitosis is important to regulate proper mitotic progression. Regulators can modulate APC/C activity but in addition substrates themselves are post-translationally modified to regulate their precise timing of degradation. For example in vertebrates: phosphorylation of Cdc6 (licensing factor for DNA replication) prevents recognition by APC/C, phosphorylation of Securin enhances ubiquitylation by APC/C and phosphorylation of S phase kinase-associated protein 2 (SKP2) causes reduced binding to Cdh1^{36, 76–78}. Furthermore acetylation of the spindle checkpoint protein BubR1 at a lysine close to its KEN box inhibits ubiquitylation thereby inhibiting its degradation^{36, 79}. Localization of substrates is also important. APC/C substrates that promote mitotic spindle assembly are concentrated on spindle microtubules and hence protected from degradation⁸⁰. Thus substrates are post-translationally modified or differentially localized to regulate timing of their degradation in mitosis.

REGULATION OF THE APC/C IN EARLY MITOSIS

Although its most prominent roles after full activation are induction of anaphase onset and mitotic exit, the APC/C is regulated to be active towards distinct substrates even in early mitosis. This early activity has important consequences for mitotic progression.

Phosphorylation and Subcellular Localization of the APC/C

Phosphorylated APC/C can be detected in the prophase nucleus by immunofluorescence⁴⁶. APC/C is phosphorylated at approximately 34 sites located on multiple subunits, and some of these phosphorylation events enhance binding of the co-activator Cdc20^{46, 47}. Phosphorylation is predominantly catalyzed by Cyclin B1–Cdk1, the efficiency of which is increased when Cdk1 is bound to its small accessory subunit Cks^{44, 46, 47, 81, 82}. The Cks proteins are conserved through evolution, bind to Cdk1 and Cdk2 *in vitro*, and can allow binding to a previously phosphorylated Cdk consensus site through an anion-binding site^{83, 84}. Thus a Cyclin-Cdk-Cks complex can phosphorylate one site on a substrate and remain bound continuing to phosphorylate other nearby Cdk sites. Cdk1-mediated APC3 phosphorylation decreases when Cks proteins are depleted from mitotic *X. laevis* egg extracts^{81, 83}. Moreover, phosphorylated APC/C binds to a Cks affinity column⁸⁵, and Cks mutants in different organisms arrest in mitosis with elevated levels of mitotic Cyclins^{44, 83, 86, 87}.

The Cyclin B1-Cdk1-Cks complex is the primary but not the only kinase that phosphorylates and activates the APC/C in mitosis. Some studies suggested that Polo-like kinase 1 (Plk1) activates APC/C in mitosis though others indicated that inhibiting Plk1 activity does not prevent APC/C activation^{46, 88}. Although specific functions for individual phosphorylation sites have not been mapped, phosphorylation is likely to affect the structure, localization, and APC/C binding to activators, substrates, or inhibitors during mitosis^{46, 89, 90}.

Related to and perhaps controlled by phosphorylation, localization of the APC/C to different cellular compartments is likely to be important in mitotic progression but has received considerably less attention than other aspects of APC/C regulation. Concentration of APC/C and phosphorylation differences could produce spatial regulation of APC/C at different

subcellular locations. The APC/C has variously been reported to concentrate at centrosomes, microtubules, chromosomes and kinetochores during mitosis^{89–94}.

The APC/C inhibitor protein Early mitotic inhibitor 1 (Emi1), plays a major role during interphase to inhibit APC/C activity and allow accumulation of mitotic cyclins for mitotic entry. Most Emi1 is degraded through SCF-mediated ubiquitylation in early M phase, but a small pool persists and concentrates at spindle poles via interaction with NuMA and the Dynein-Dynactin complex. This complex then produces a concentrated pool of APC/C at the spindle poles. Retention of this APC/C at spindle poles requires activity of protein phosphatase 2A (PP2A) that maintains this population of APC/C in a hypophosphorylated state. This contrasts with the bulk of cytoplasmic APC/C, which is highly phosphorylated in mitotic cells prior to anaphase. It was hypothesized that inhibition of APC/C at spindle poles by Emi1 and hypophosphorylation blocks local Cyclin degradation hence promoting high activity of Cdk1 at spindle poles to enhance spindle assembly^{89, 92, 94, 95}.

Our recent study showed that the amount of hypophosphorylated APC/C bound to mitotic chromosomes increases as cells progress to metaphase⁹⁰. However, unexpectedly and in contrast to the predicted low activity of APC/C at centrosomes, the hypophosphorylated APC/C associated with mitotic chromosomes showed significantly higher ubiquitin-ligase activity than APC/C in the bulk mitotic cytoplasm.⁹⁰ Although these studies highlight a possible relationship between subcellular control of APC/C activity and the localization of protein kinase and phosphatase activities, they only begin to skim the surface of phosphoregulation. The roles of the phosphorylations at specific sites on APC/C subunits and their dynamic changes during mitosis remain unexplored and many questions and ambiguities remain. For example, despite the reported concentration of hypophosphorylated APC/C at spindle poles and chromosomes, an antibody made against a specific phosphorylated residue on APC1 (phospho S355) was reported to concentrate specifically at spindle poles and unattached kinetochores^{46, 93}. This suggests that much underlying complexity for spatial regulation of APC/C activity in mitosis remains to be investigated.

The spindle checkpoint

At metaphase, when the last chromosome bi-orient on the mitotic spindle, APC/C-mediated ubiquitylation of Securin and Cyclin B1 which are anaphase targets, becomes accelerated and these proteins are rapidly degraded resulting in chromatid separation and mitotic exit. An evolutionarily conserved mechanism called the spindle checkpoint, also termed the spindle assembly checkpoint or mitotic checkpoint, inhibits activity of APC/C-Cdc20 until all chromosomes are bi-oriented on the mitotic spindle and are under mechanical tension from kinetochore-microtubule interactions (Figure 1). The many protein interactions and kinase activities that catalyze spindle checkpoint signaling at kinetochores of unattached chromosomes will not be covered in detail here but is the subject of several recent reviews^{96–99}.

Prometaphase substrates of APC/C-Cdc20

Although the spindle checkpoint strongly inhibits the ubiquitylation of Securin and Cyclin B1, certain APC/C targets are efficiently degraded in prometaphase, or when the checkpoint

is fully activated by arresting cells in mitosis with microtubule drugs. Within minutes of nuclear envelope breakdown, Cyclin B1-Cdk1 activity reaches maximal levels, and APC/C-Cdc20 ubiquitylates prometaphase substrates such as Cyclin A and Nek2A (NIMA related kinase 2A), thereby targeting them for degradation by the 26S proteasome^{100–104} (Figure 1). In normal dividing cells 80% of Cyclin A and more than 50% of Nek2A is degraded before metaphase¹⁰⁰. How prometaphase targets are ubiquitylated in the presence of an active spindle checkpoint is an active area of study. The primary answer appears to be the ability of prometaphase targets to use alternatives to the canonical D-box and KEN-box motifs to bind the APC/C. Once these alternative substrates are modified with an initial ubiquitin moiety elongation of the chains is carried out through Ube2S. Importantly Ube2S activity is apparently not inhibited by spindle checkpoint signaling³⁵. This allows the APC/C to elongate chains on substrates that do not require canonical D box or KEN box interaction with the APC/C.

Cyclin A and Nek2A can bind the APC/C in multiple ways to promote their degradation in prometaphase. Cyclin A is bound to Cdc20 in G2 and early mitosis. Immediately after nuclear envelope breakdown, Cyclin A is targeted to the APC/C by Cks subunit of its Cdk partner which then promotes Cyclin A degradation^{83, 105}. Similarly, in yeast the degradation of S phase Cyclin Clb5 in early mitosis depends on its interaction with Cdk1-Cks1 and an N-terminal Cdc20 binding region¹⁰⁶. Degradation of Nek2A depends on an exposed carboxy-terminal methionine-arginine (MR) dipeptide tail. This MR tail facilitates direct binding of Nek2A to the APC/C even in the absence of Cdc20. Thus Cdc20 is required for degradation of Nek2A but not for the recruitment of Nek2A to the APC/C^{103, 104, 107}.

APC/C activity and mitotic duration

Rapid degradation of Securin and Cyclin B1 occurs after spindle checkpoint inactivation. However, regarding the APC/C to be “activated” at the metaphase-anaphase transition is an oversimplification, as it also degrades early mitotic substrates Cyclin A and Nek2A¹⁰⁸. Additionally, APC/C activity mediates slow degradation of Cyclin B1 in prometaphase. This is countered by Cyclin B1 production during mitosis¹⁰⁹. Continued Cyclin B1 synthesis is required to maintain cells in mitotic arrest induced with microtubule drugs^{109, 110}. Indeed some evidence suggests that the Cyclin B1 gene is transcribed during mitosis and that this transcription is required to sustain a mitotic arrest induced with microtubule drugs¹⁰⁹. The gradual degradation of Cyclin B1 might account for “mitotic slippage” where cells escape out of mitotic arrest induced by microtubule drugs^{108, 111–113}. The balance between synthesis and degradation likely differs among species and cell types, resulting in variation in the duration of mitotic arrest exhibited by different cells in the presence of microtubule inhibitors¹¹³. The type and concentration of microtubule drug also affects the strength of spindle checkpoint signaling thus affecting APC/C activity and the rate of degradation¹⁰⁸. While prometaphase APC/C targets are degraded in cells arrested in mitosis with microtubule drugs, the rate of their degradation is decreased by strong checkpoint activation. Cells treated with high concentrations of microtubule depolymerizing drugs such as nocodazole have maximal checkpoint signaling. In cells treated with low concentrations of nocodazole or in cells treated with microtubule stabilizers such as Taxol where microtubules

or small fragments persist and associate with kinetochores, checkpoint signals are weaker^{108, 114}. In addition the presence of intact microtubules might sequester substrates or promote the transport of the APC/C to favorable subcellular locations (e.g. to chromosomes) for activation^{80, 90}.

Inhibition of the APC/C by the MCC

The primary components of the spindle checkpoint include MAD1 (mitotic arrest deficient 1), MAD2, BUBR1 (budding uninhibited by benzimidazole related 1; MAD3 in yeast), BUB1 (budding uninhibited by benzimidazole 1), BUB3, and MPS1 (multipolar spindle 1) (reviewed in^{96–99}). Mad1-Mad2 heterodimers at unattached kinetochores catalyze a conformational change in additional Mad2 (to form closed Mad2 or C-Mad2) that allows it to bind and inhibit Cdc20¹¹⁵. Robust inhibition also requires the binding of C-Mad2-Cdc20 to BubR1 and Bub3^{116, 117}. This complex of spindle checkpoint proteins Mad2-Cdc20-BubR1-Bub3 forms the mitotic checkpoint complex (MCC)^{118–122}.

The crystal structure of the fission yeast MCC provided valuable information about interactions within the MCC components⁷³. BubR1 was found to interact through multiple residues with both closed-Mad2 and Cdc20. BubR1 has two KEN boxes, one in the N terminus and another in the C terminus. The N terminal KEN box of BubR1 binds Cdc20 and Mad2 thereby promoting assembly of the MCC. The C terminal KEN box is not required for MCC-APC/C interaction but it is required to inhibit substrate recruitment to the APC/C¹²³. It was initially proposed that the C terminal KEN box might bind a second copy of Cdc20²¹ and a recent experimental study supports that model¹²⁴.

MCC binding to APC/C sterically hinders APC/C activity by disrupting the substrate-binding site and preventing substrate recruitment (Figure 3B). Mad2 on its own competes with APC/C for the same binding site of Cdc20 and thus can inhibit Cdc20 association with the APC/C^{125, 126}. MCC binding positions Cdc20 downwards towards the APC/C platform thus disrupting the D box receptor formed between Cdc20 and APC10^{15, 21, 73}. This position of Cdc20 might also promote its own ubiquitylation in an APC15 dependent manner^{20, 127, 128}. The N-terminal KEN motif of BubR1 also binds and blocks the KEN box receptor on the surface of the Cdc20 WD-40 domain. A second Cdc20 molecule can bind to the MCC through BubR1's D box and C-terminal KEN box, and this interaction seems to be important for maximal checkpoint signaling¹²⁴. Lastly MCC interactions with the catalytic core of the APC/C also partially impair the binding or function of UbcH10^{15, 129}.

MCC turnover in mitosis

Several recent studies have indicated that continuous turnover of the MCC is an essential component for generating a system that can respond rapidly to the cessation of spindle checkpoint signaling after chromosome bi-orientation (Figure 4). Metaphase is normally very transient and delays at metaphase can lead to cohesion fatigue whereby spindle-pulling forces induce asynchronous chromatid segregation without mitotic exit^{130, 131}. Free MCC and MCC bound to APC/C have to be disassembled to fully activate APC/C after spindle checkpoint inactivation¹³². Although not completely understood, continuous assembly and disassembly [degradation of?] of the MCC during mitosis seems to prime the cell for rapid

and strong APC/C-mediated degradation of anaphase targets, Securin and Cyclin B1, once checkpoint signaling is switched off.

Cdc20 synthesis and degradation

During mitosis Cdc20 associated with the APC/C is continuously ubiquitylated and degraded. This is balanced by continuous synthesis of the protein hence ensuring constant steady state levels of Cdc20 during prometaphase¹³³. APC15, a subunit of the platform sub-complex of APC/C is required for Cdc20 ubiquitylation and degradation^{20, 127, 128}. Initially it was suggested that degradation of Cdc20 in prometaphase might be a mechanism to limit its accumulation and hence prevent premature APC/C activation in the presence of unattached kinetochores^{134–136}. More recent evidence suggests that continued synthesis and degradation of Cdc20 plays a key role in allowing rapid increase in APC/C activity to ubiquitylate [OK? Or 'ubiquitylate'?] anaphase targets when the spindle checkpoint is silenced^{20, 111, 127, 128}. Cdc20 synthesis and degradation is intimately connected to continued generation of MCC at unattached kinetochores and disassembly of the MCC in the cytoplasm (Figure 4A). Free MCC is in excess of MCC bound to APC/C-Cdc20^{124, 132}. During Cdc20 degradation and MCC turnover, this excess free MCC might rapidly bind APC/C-Cdc20 thereby promoting strong inhibition of APC/C activity in the presence of unattached kinetochores¹³². Inhibition of Cdc20 degradation or APC/C activity causes metaphase arrest subsequently followed by Cohesion Fatigue suggesting that APC/C activity is required to silence spindle checkpoint^{110, 137, 138}. Cohesion fatigue was then subsequently shown to reactivate the spindle checkpoint suggesting that inhibition of APC/C activation at metaphase can cause reactivation of spindle checkpoint¹³⁹. Thus MCC turnover is required for rapid anaphase onset and mitotic exit after checkpoint silencing¹³⁶ (Figure 4B). Lastly, while APC15 is required for Cdc20 ubiquitylation it is not required for APC/C-Cdc20 or APC/C-Cdh1 activity towards mitotic substrates Securin or Cyclin B1^{20, 127, 128}.

p31comet promotes MCC release from the APC/C

Another key component in MCC turnover is p31comet, a protein required for normal mitotic progression in vertebrates, but homologs have not been identified in lower eukaryotes. p31comet is a Mad2 paralogue that forms a dimer with closed Mad2^{140–143}. p31comet structurally mimics Mad2 and competes with BuBR1 for Mad2 binding. Its binding to Mad2 prevents conformational activation of Mad2¹⁴¹. Depletion of p31comet stabilizes the MCC, inhibits full activation of the APC/C, and delays mitotic exit^{111, 144–146}. Depletion of p31 also inhibits Cdc20 degradation during prometaphase and increases the amount of Mad2 in MCC^{111, 147}. Conversely overexpression of p31comet overrides a spindle checkpoint-mediated arrest¹⁴⁷. Using mitotic extracts from mammalian cells, it was found that p31 comet-mediated MCC disassembly required hydrolysis of β - γ bond of ATP^{148, 149}. Recently the AAA-ATPase TRIP13, which binds p31comet, was found to be required for MCC disassembly¹⁵⁰. TRIP13 and p31comet together release MCC from APC/C, promote MCC disassembly and inactivate the spindle checkpoint¹⁵⁰.

The p31comet protein binds to unattached kinetochores, and its activity might be modulated by the strength of checkpoint signaling¹⁴⁵. Strong checkpoint signaling resulting from high

concentrations of microtubule depolymerizers such as nocodazole result in unattached kinetochores and higher levels of Mad2 associated with the MCC. By comparison in cells treated with a microtubule stabilizer such as Taxol there is some microtubule association with kinetochore and this results in a weaker checkpoint signal. Because Mad2 is often present at sub-stoichiometric levels in MCC compared to the BubR1, controversy remains about whether the complete MCC is the key APC/C inhibitor or whether the MCC is an intermediary in the formation of a Cdc20–BubR1–Bub3 complex (known as BBC) which then serves as the primary inhibitor^{116, 123, 147, 151}. Levels of Mad2 in the MCC appear to correlate with the strength of checkpoint signaling^{116, 147, 151}, suggesting that the complete MCC, containing Mad2, is the more potent APC/C inhibitor. Finally the protein CUEDC2 has been implicated in releasing Mad2 from the APC/C¹⁵². Interestingly depletions of either p31comet or CUEDC2 results in transient delays at metaphase but cells generally progress to anaphase. One explanation is that these proteins or yet others not yet discovered have redundant essential roles in promoting anaphase onset. Alternatively these proteins might have evolved in higher eukaryotes to fine tune or amplify signals to promote anaphase onset after chromosome alignment at metaphase.

Silencing the spindle checkpoint

Bipolar attachment of spindle microtubules and the mechanical tension these impart on kinetochores result in molecular changes that quell checkpoint signaling. However loss of microtubule attachment in metaphase cells can reactivate the checkpoint. By severing microtubule attachments with a focused laser, it was determined that the “point of no return” after which the spindle checkpoint can not be reactivated is approximately 5 minutes before anaphase onset in HeLa cells¹⁵³. Several mechanisms participate in checkpoint silencing. Some checkpoint signaling proteins including Mad1, Mad2, Mps1, and BubR1 are depleted from kinetochore and moved to the spindle poles through the action of the minus end-directed microtubule motor, dynein^{96, 154–156}. In metazoans, this dynein-mediated protein “stripping” dampens spindle checkpoint signaling catalyzed at kinetochores⁹⁶ (Figure 4B).

Other proteins specifically accumulate in higher amounts at kinetochores of chromosomes as they achieve bipolar attachment and reach metaphase. One of these is protein phosphatase 1 (PP1) whose activity is required for checkpoint silencing¹⁵⁷. Reversible protein phosphorylation is a key regulatory mechanism of spindle checkpoint signaling^{97, 99, 157}. Bub1, Mps1, and Aurora B activities promote checkpoint signaling (reviewed in^{97–99}) (Figure 5). Aurora B kinase is also involved in destabilizing kinetochore-microtubule attachments, which results in checkpoint activation. Another element that accumulates at metaphase kinetochores is the Spindle and Kinetochore-Associated (SKA) complex. The SKA complex is composed of three proteins with both microtubule and kinetochore binding properties^{158, 159}. Depletion of the SKA complex generates a sustained metaphase arrest that eventually results in cohesion fatigue, where chromatids are pulled apart by spindle forces without anaphase onset¹⁶⁰. How the SKA complex promotes the metaphase-anaphase transition is not completely understood, but it appears to function, at least in part, by promoting APC/C accumulation on metaphase chromosomes¹⁶¹.

APC/C REGULATION AT THE METAPHASE-ANAPHASE TRANSITION

Spindle checkpoint silencing causes the cessation of kinetochore-based MCC assembly.[do you mean synthesis or accumulation?] Cdc20, freshly synthesized and/or free Cdc20 released by MCC disassembly rapidly amplifies APC/C-Cdc20 activity targeting Securin and Cyclin B1 for proteasomal-degradation (Figure 1). Securin degradation liberates the protease Separase, which cleaves the Rad21 component of the Cohesin complex and allows synchronous chromatid separation in anaphase. Cyclin B1 degradation results in Cdk1 inactivation. Reversal of the Cdk1 phosphorylation cascade by cellular phosphatases (such as PP1 and PP2A) induces cytokinesis and mitotic exit (Figure 5). There is strong evidence for positive feedback in Cdk1 inactivation during mitotic exit. Even when cells are arrested with high concentrations of microtubule inhibitors, the application of drugs that inhibit Cdk1 kinase rapidly induces many of the events of mitotic exit, including degradation of Cyclin B1 ¹⁶².

Phosphorylation changes during anaphase and mitotic exit are likely to be key regulators of the APC/C. Cdc20 binding to APC/C is controlled, at least in part, by removal of inhibitory phosphorylations ⁵⁰. During anaphase dephosphorylation of Cdh1 and degradation of the Cdh1-binding protein Mad2L2 allows Cdh1 to bind and activate APC/C ^{45, 54, 55, 57, 163}. APC/C-Cdh1 recognizes substrates including Cdc20, Polo and Aurora kinases, UbcH10, and Geminin (Figure 1). While APC/C-Cdh1 mediates degradation of these substrates at anaphase it might not be essential as depletion of Cdh1 stabilizes Aurora A and Aurora B but it does not affect the degradation of Plk1, Geminin and Cdc20 (although Cdc20 is degraded more slowly) ^{164–166}. Thus mitotic exit is largely unaffected when Cdh1 is deleted in budding yeast, fission yeast ¹⁶⁷, *Drosophila* ¹⁶⁸ or depleted from mammalian cells ^{36, 169, 170}. Cdc20 might persist and compensate for Cdh1 in its absence. Finally many APC/C subunits are highly phosphorylated in mitosis. Most of these phosphorylations are removed during anaphase and mitotic exit. Whether sites are dephosphorylated in specific order to regulate mitotic exit remains uncertain.

Subcellular compartmentalization of APC/C activity

While studies have focused on temporal control of APC/C activity, evidence suggests that APC/C within certain cellular compartments might be differentially regulated. Interestingly pools of APC/C associated with spindle poles and chromosomes are hypo-phosphorylated compared to the bulk APC/C in the mitotic cytoplasm. In the case of the spindle pole pool, it is hypothesized that the APC/C is specifically inactivated ⁹⁵. In the case of the chromosome-associated pool the APC/C was assayed and found to be more active compared to the cytoplasmic pool ¹⁶¹.

Indirect evidence suggests that APC/C-mediated degradation is compartmentalized, and Cyclin B1 degradation might be spatially regulated. In syncytial *D. melanogaster* embryos, Cyclin B1-GFP staining is lost first from the spindle poles suggesting that degradation begins there whereas in human cells it is lost simultaneously from the spindle poles and chromosomes^{171, 172}. Securin degradation is also spatially controlled. The majority of Securin protein appears to be free and phosphorylated in the cytoplasm and only a small dephosphorylated pool binds and inhibits Separase on chromosomes. PP2A

dephosphorylates the Securin bound to Separase⁷⁸. Upon full activation of APC/C-Cdc20 at anaphase onset, the bulk of the free cytoplasmic phosphorylated Securin is degraded before the small pool of Securin bound to Separase on chromatin¹⁷³. Auto-cleaved Separase is thought to inhibit Cdk1 on chromosomes after cohesion cleavage to further repress Cdk1 activity and hence initiate rapid poleward movement of sister chromatids^{173, 174}.

REGULATION OF THE APC/C IN INTERPHASE

After anaphase onset and mitotic exit, the two main substrates of the APC/C-Cdh1 are S phase and mitotic Cyclins, the levels of which are kept low to prevent cell cycle entry until a cell commits to another round of division. In the absence of Cdh1, mammalian cells accumulate Cyclin A early and begin DNA replication prematurely³⁶.

Post-translational modification

APC/C-Cdh1 must be inactivated for cells to re-enter the cell cycle and begin DNA replication. This is thought to occur by a combination of Cyclin-Cdk-mediated phosphorylation and inhibitor binding. G1 phase Cyclin E/A- Cdk complexes inactivate Cdh1 by phosphorylation and prevent it from binding to APC/C^{36, 54, 55}. APC/C-Cdh1 inactivation can also occur by degradation of its E2, UbcH10. By ubiquitylating UbcH10 and mediating its degradation, APC/C-Cdh1 inactivates itself¹⁷⁵. Finally, Cdh1 can be auto-ubiquitlated by the APC/C at the end of G1 to target itself for degradation and allow cell cycle re-entry^{10, 176}.

Inhibitor binding

Inactivation of APC/C-Cdh1 can also occur through binding of inhibitors. In budding yeast, Acm1 (APC/Cdh1 modulator 1) has been identified as an inhibitor of APC/C-Cdh1¹⁷⁷. Similarly RCA1 (F box protein regulator of Cyclin A) in *D. melanogaster*¹⁷⁸ and Emi1 (early mitotic inhibitor 1) in vertebrates also function as inhibitors of APC/C-Cdh1¹⁷⁹. In budding yeast Acm1 acts as a pseudo-substrate by competing with other substrates for Cdh1 binding thereby inhibiting their recruitment to the APC/C^{180, 181}. In anaphase APC/C-Cdc20 mediated degradation of Acm1 activates APC/C-Cdh1, and at the end of G1 phase, accumulation of Acm1 likely inactivates APC/C-Cdh1¹⁸¹. In vertebrate cells, Emi1 levels rise during S phase and decline at mitotic entry. *In vitro* Emi1 inhibits both APC/C-Cdc20 and APC/C-Cdh1 and *in vivo* Emi1 overexpression has been shown to result in accumulation of APC/C substrates^{179, 182}.

Structural evidence shows that Emi1 inhibits the APC/C in ways similar to MCC¹⁸³. The C terminus of Emi1 binds multiple sites on APC/C-Cdh1 to block the substrate-binding site¹⁸³. Emi2 (also called Erp1) is a protein closely related to Emi1 that functions in oocyte meiosis to inhibit APC/C activity. After ovulation and prior to fertilization oocytes are arrested at metaphase stage of meiosis II. Emi2 as a component of Cytostatic Factor mediates this arrest^{184–186}. Emi2 is also necessary for the early mitotic divisions of *Xenopus* embryos¹⁸⁷. Both Emi1 and Emi2 inhibit ubiquitin chain elongation by Ube2S. The Emi proteins have a functionally similar C terminal tail, through which they compete with Ube2S for APC/C binding^{183, 188–190}. Depletion of Emi1 leads to premature activation

of APC/C during G2 and destabilization of Geminin and Cyclin A^{191, 192}. When Emi1 does not inactivate APC/C-Cdh1 cells re-replicate their genomes and become polyploid³⁶.

At mitotic entry Emi1 is ubiquitinated and degraded by SCF- β -TrCP ubiquitin ligase^{193–196}. Expression of a non-degradable form of Emi1 does not prevent APC/C activation^{88, 192, 197, 198} suggesting that other mechanisms might also allow APC/C to escape inhibition by Emi1 during mitotic entry. A good candidate is Cdk1-mediated phosphorylation since phosphorylated Emi1 appears to be unable to bind and inhibit APC/C efficiently¹⁹⁹.

CONCLUSIONS AND CURRENT QUESTIONS

The APC/C serves as a central control node regulating transitions in mitosis and at other points in the cell cycle. It is subject to multiple activators and inhibitors that tune its activity and specificity to individual substrates. The temporal management of the APC/C by its regulators is well documented. More evidence for spatial regulation at the subcellular level is beginning to appear. To ensure proper mitotic progression the APC/C is positively regulated by mitotic protein kinases, co-activators and negatively regulated by the spindle checkpoint and inhibitors (Figure 5). Modulators of the APC/C ensure that the substrates are ubiquitinated and degraded at precise times in the appropriate sequence to ensure accurate chromatid segregation.

The localization of the APC/C or its substrates to mitotic organelles might aid in regulation of its activity during mitosis (Figure 6). The APC/C accumulates on chromosomes as cells reach metaphase and chromosome-associated pool has higher ubiquitylation activity⁹⁰. At metaphase, it has been observed that motor proteins on microtubules transport spindle checkpoint proteins away from the kinetochore. Thus after proper microtubule attachment, inhibitors of APC/C are hauled away from the kinetochore while the APC/C itself is accumulating on chromosomes. Final activation of the APC/C might occur on chromosomes to closely link Cohesin cleavage to synchronous chromatid separation at anaphase^{78, 173} (Figure 6). The compartmentalization of APC/C to chromosomes might be important for its final activation. It is possible that an active pool of APC/C is partitioned away from the cytosolic APC/C that is inhibited by the spindle checkpoint proteins. Recently, it has been shown that a second molecule of Cdc20 binds APC/C and is inhibited by MCC in the presence of unattached kinetochores¹²⁴. During MCC turnover, this active and primed pool of APC/C-Cdc20 might be responsible for the basal level of Cyclin B1 degradation in cells arrested in mitosis by microtubule-poisons. Also, an active pool of APC/C-Cdc20 might catalyze the rapid degradation of Cyclin B1 at metaphase upon spindle checkpoint inactivation. Moreover, localization to microtubules protects certain substrates from APC/C mediated degradation⁸⁰ while APC/C on centrosomes is anchored there and potentially kept inactive by Emi1–Nima–Dynein–Dynactin complex⁹⁴ (Figure 6). An important challenge in the future will be to understand how APC/C localized at specific compartments affect mitotic progression. Possibly endogenous inhibitors and activators of the APC/C regulate the ligase differentially in subcellular compartments, and tracking APC/C activities at the subcellular level will be challenging but important in understanding its control over cell cycle transitions.

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Glossary

Monoubiquitylation	the addition of a single ubiquitin to a target protein
Securin	protein inhibitor of the protease Separase
NuMA	Nuclear and Matrix Associated protein, partners with Dynein in assembly and maintenance of spindle poles
Dynein-Dynactin complex	microtubule motor complex involved in transport of spindle checkpoint proteins from kinetochores to spindle pole
Cohesin complex	protein complex that holds replicated sister chromatids together prior to anaphase

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Figure 1. Ordered degradation of APC/C substrates

The APC/C ubiquitylates proteins, marking their degradation at specific times and driving forward progression of the cell cycle. APC/C-Cdc20 ubiquitylates substrates during early and mid mitosis while APC/C-Cdh1 ubiquitylates substrates after anaphase onset, during mitotic exit and in G1. APC/C-Cdc20 ubiquitylates Cyclin A and Nek2A in prometaphase. During prometaphase APC/C-Cdc20 activity toward late substrates, Securin and Cyclin B1 is suppressed by the spindle checkpoint. At metaphase, the spindle checkpoint is silenced and ubiquitylation of Securin and Cyclin B1 is maximized. At mitotic exit, APC/C-Cdh1 ubiquitylates Cdc20, Aurora kinases and Plk1. At the G1-S transition, APC/C-Cdh1 is inactivated by a combination of binding to the APC/C inhibitor Emi1, degradation of its E2 UbcH10, Cdh1 phosphorylation, and ubiquitylation and degradation of Cdh1.

Figure 2. Structural organization of the APC/C

A) The subunits of the APC/C can be largely organized into three sub-complexes: the platform (APC1, APC4, APC5, APC15), the catalytic core (APC2, APC11, APC10/Doc1) and the TPR lobe or Arc lamp (APC8, APC6, APC3, APC7) sub-complex¹⁴. (For APC/C subunit nomenclature used in yeast see Table 1. The APC1 subunit in the platform is the largest APC/C subunit and acts to bridge the other sub-complexes: the catalytic core and TPR lobe^{19, 20}. APC2 acts as a scaffold for the catalytic core. APC11 potentiates interaction with E2 enzymes and APC10 forms part of the substrate-binding pocket^{68, 69}. The TPR lobe has multiple subunits that form homodimers and provide important scaffolding functions to the APC/C. Accessory proteins stabilize subunits in the TPR lobe: APC12 stabilizes APC6; APC13 interacts with the TPR repeats of APC3, APC6, APC8; APC16 interacts with TPR repeats of APC3 and APC7 subunits¹⁴. While most subunits exist as monomers, APC3, APC6, APC7, APC8 and APC12 are present as dimers. B) Cryo-electron microscopy reconstruction of the human APC/C-Cdh1 complex depicting the location of the individual subunits along with their underlying secondary structures. Figure 1B is reproduced with permission from reference¹⁴.

Figure 3. Conformational changes during APC/C activation and inactivation

The APC/C undergoes conformational changes upon coactivator-substrate binding to bring the E2-Ub close to the substrate and this conformational activation is inhibited by the Mitotic checkpoint complex (MCC). A) Diagram of the conformational activation of the APC/C upon coactivator and substrate binding. Coactivator binding disrupts interaction between APC8 and APC1 causing a downward shift of the platform that is accompanied by an upward shift of the catalytic module (APC2-APC11). This might bring the E2-Ub close to the substrate and potentiate attachment of the initiating ubiquitin^{14, 129}. Cdc20 is also required for the activity of the chain-elongating E2 (Ube2S)³⁵. A distinct region on APC2, near the APC2-APC4 junction is required to bind Ube2S²⁶. The APC/C also tethers the distal molecule of an emerging ubiquitin conjugate close to Ube2S thereby potentiating efficient ubiquitin chain elongation. B) Diagram of APC/C bound to MCC. MCC components inhibit recruitment of late mitotic substrates that rely upon recognition through D box and KEN box motif and hence inhibit APC/C activity toward these substrates. Mad2 and BubR1 bind Cdc20 and prevent its ability to recruit substrates. Cdc20 as part of the MCC is also pushed downwards towards platform subunits and prevented from forming the D box co-receptor with APC10¹⁵. This position of Cdc20 might also facilitate its own ubiquitylation and subsequent degradation during active spindle checkpoint signaling.

Figure 4. MCC turnover during mitosis

A) In the presence of unattached kinetochores, Mad2-BubR1-Bub3-Cdc20 interact to form a diffusible mitotic checkpoint complex (MCC) that binds and inhibits the APC/C. APC/C ubiquitylates and promotes degradation of its co-activator Cdc20. Cdc20 is continually synthesized during mitosis. In the continued presence of unattached kinetochores, spindle checkpoint proteins Mad2 and BubR1-Bub3 can be recycled to bind newly synthesized Cdc20, form MCC and inhibit APC/C. B) Once all sister kinetochores achieve bipolar attachment to spindle and are under mechanical tension, MCC formation is inhibited and MCC disassembly dominates. Cdc20 is released from MCC and/or freshly synthesized Cdc20 binds and generates the APC/C-Cdc20 with high activity toward late mitotic substrates^{20, 127, 128}. Several mechanisms contribute to loss of MCC activity. MCC catalysis at kinetochores is inhibited by transport of several checkpoint components, including Mad2 and BubR1 from kinetochores by the minus-end directed motor protein Dynein^{96, 154–156}. P31comet competes with BubR1 for binding Mad2 and prevents conformational activation of Mad2¹⁴¹. MCC disassembly allows APC/C activation leading to ubiquitylation and degradation of Securin and Cyclin B1 for anaphase onset and mitotic exit.

Figure 5. Positive and negative modulators control rapid changes in APC/C activity

Mitotic progression is primarily regulated through two main activators, Cdk1 kinase and the APC/C. These work through a feedback mechanism whereby Cdk1 activation of APC/C ultimately induces degradation of Cyclin B1 and Cdk1 inactivation (red arrows). APC/C activity is further modulated by a host of other components that are themselves regulated by posttranslational modification and by subcellular localization, particularly at kinetochores. The resulting regulatory networks control APC/C activity and allow the APC/C to respond to rapid changes in kinetochore attachment/detachment. The spindle checkpoint proteins Mad1, Mad2, BubR1, Bub3 inhibit APC/C activity. These spindle checkpoint proteins are themselves activated by mitotic protein kinases Mps1, Bub1, Aurora B, Cyclin B1/Cdk1 and inhibited by p31comet, protein phosphatases (PP1, PP2A) and Dynein. These regulators affect localization or activity of the spindle checkpoint proteins. While the spindle checkpoint inhibits APC/C, regulators of the spindle checkpoint also directly modulate APC/C activity. This results in complex regulatory networks that fine-tune APC/C activity during mitosis. In addition, some proteins have roles in both inhibiting and promoting APC/C activity. For example Cdk1 has inhibitory roles in phosphorylating Cdc20, Cdh1, and spindle checkpoint proteins. At the same time Cdk1 phosphorylation enhances APC/C-Cdc20 activity. The interplay of these regulators and the existence of subcellular pools of APC/C that differ in post-translational modification and inhibitor or activator binding is likely to play important roles in the dynamic regulation of APC/C activity during progressive stages of the cell cycle.

Figure 6. Hypothesis for the spatiotemporal regulation of the APC/C in mitosis

In prometaphase, APC/C activity is inhibited toward late mitotic substrates to prevent anaphase onset and mitotic exit until all kinetochores are bi-oriented on mitotic spindle and attached to microtubules properly. During mitosis subcellular localization of APC/C and its substrates might play important roles in mitotic progression. Some APC/C is concentrated at centrosomes where it is bound and potentially inhibited by binding to a protein complex containing Emi1, Numa and Dynein-Dynactin⁹⁴. Spindle assembly factors are localized to microtubules and thereby protected from APC/C-mediated degradation until completion of spindle formation⁸⁰. The spindle checkpoint generates the diffusible mitotic checkpoint complex (MCC) catalyzed at unattached kinetochores to inhibit the soluble cytosolic APC/C (intensity of red color denotes degree of APC/C inhibition, green indicates APC/C activation). A small pool of active APC/C-Cdc20 might remain associated with chromosomes in prometaphase, potentially escaping checkpoint inhibition and contributing to the basal Cyclin B1 degradation seen in cells arrested in mitosis with microtubule drugs. Upon proper microtubule attachment at metaphase, active APC/C-Cdc20 further accumulates on chromosomes dependent on the SKA complex⁹⁰. Loss of inhibition by spindle checkpoint proteins generates globally strong APC/C activity throughout the cytoplasm. Final activation of APC/C might occur on chromosomes to allow rapid Cohesin cleavage and synchronous anaphase chromatid separation¹⁷³. Thus APC/C activity is regulated spatially and temporally to control proper mitotic progression.

Table 1

Subunits of the APC/C

Subcomplex	Budding yeast protein	Vertebrate protein	Stoichiometry	Function
PLATFORM SUBCOMPLEX	Apc1	APC1	1	Scaffolding ¹⁴
	Apc4	APC4	1	Scaffolding and is required to bind elongating E2 Ube2S ³⁵
	Apc5	APC5	1	Scaffolding ¹⁴
	Mnd2	APC15	1	Promotes Cdc20 ubiquitylation and hence mediates disassembly of mitotic checkpoint complex ^{20, 127, 128}
CATALYTIC	Apc2	APC2	1	Catalytic and is required to bind elongating E2 Ube2S ²⁶ .
	Apc11	APC11	1	Catalytic: Binds initiating E2 enzyme interaction with and activation of elongating E2, recruitment of terminal ubiquitin ^{26, 35}
	Doc1	APC10	1	Part of degron (D box) receptor ^{66, 67, 69, 71, 72}
TPR LOBE OR ARC LAMP	Cdc27	APC3	2	Scaffolding: Binds APC10 and Cdh1/Cdc20 ^{14, 23, 72}
	Cdc16	APC6	2	Scaffolding ^{14, 22, 23, 200}
	-	APC7	2	Scaffolding ^{14, 23}
	Cdc23	APC8	2	Scaffolding: Binds Cdc20 ^{14, 23}
TPR ACCESSORY	Cdc26	APC12	2	TPR accessory: Stabilizes APC6 ^{14, 22}
	Swm1	APC13	1	TPR accessory: Stabilizes APC3, APC6 and APC8 ¹⁴
	-	APC16	1	TPR accessory: Stabilizes APC3 and APC7 ¹⁴