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 APC/C (anaphase-promoting complex; also known as the cyclosome) 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.

Monoubiquitylation
The addition of a single ubiquitin to a target protein.

Cell cycle transitions are driven by oscillations in the activity of cyclin-dependent kinases (CDKs). These oscillations in CDK activity are often controlled by the production and degradation of cyclins, which bind to and activate CDKs. In higher eukaryotes, there are approximately 20 different CDKs and CDK-related proteins (all of which are serine/threonine protein kinases) and 4 major cyclin classes; different combinations of CDKs and cyclins regulate cell-phase-specific events such as DNA replication and mitosis[1]. 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 pathway[2], and this drives the forward progression of the cell cycle.

The E3 ubiquitin ligase APC/C (anaphase-promoting complex; also known as the cyclosome) 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 (also termed the spindle assembly checkpoint or mitotic 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 spatiotemporal regulation of its activators and inhibitors make this an opportune time to summarize our current understanding.

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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 complex[3]. The attachment of ubiquitin to target proteins is a three-step process catalysed by at least three enzymes: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3)[4]. Ubiquitin (a small 8 kDa protein) is transferred to E1 in an ATP-dependent manner. This activated ubiquitin is then transferred to the E2 enzyme, and the E3 ligase catalyses 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 amino terminus results in the formation of polyubiquitin chains[5]. Monoubiquitylation can affect protein localization or protein–protein interactions[6]. Polyubiquitin 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, whereas K63-linked chains typically facilitate protein–protein interactions that are required for signalling. Polyubiquitin chains linked through K6, K27, K29 and K33 also exist, but these are less well understood[4,7–9].

The human genome encodes two E1 enzymes, at least 35 E2 enzymes and ~600 E3 enzymes. Members of the cullin–RING family of E3 ligases have key roles in many aspects of cell cycle control[10]. 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 described as becoming 'activated' at the metaphase–anaphase transition, this is an oversimplification. The APC/C is active throughout mitosis and 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 (FIG. 1). We discuss below the many aspects of this regulation.

Structure of the APC/C. In 1995, the APC/C was discovered as a mitosis-specific E3 ubiquitin ligase in clam[11], *Xenopus laevis*[12] and budding yeast[13]. 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 multisubunit 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 2 copies) (FIG. 2; TABLE 1). The complex is largely organized into three structural domains, called the platform, the catalytic core and the tetratricopeptide repeat (TPR) lobe (also known as the 'arc lamp' owing to its overall shape)[14,15,17,18,20,21]. The platform subcomplex forms a base to join the other subunits of the APC/C. The catalytic core subcomplex on its own cannot efficiently recruit substrates but, along with an E2 enzyme, it can provide low ubiquitylation activity to the APC/C. The TPR lobe consists of several structurally related proteins with multiple TPRs. Three other subunits, the TPR accessory factors, 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]. Furthermore, these subunits coordinate assembly of the APC/C and mediate important interactions with regulatory proteins that modulate APC/C activity. Importantly, this region of the APC/C also interacts with an inhibitory complex called the mitotic checkpoint complex (MCC), which has a key role in regulating mitotic progression[15,23]. Together, this multisubunit E3 ubiquitin ligase cooperates with at least two E2 enzymes and one of two co-activator proteins, CDC20 or CDC20 homologue 1 (CDH1; also known as FZR1) in all eukaryotes, to recruit and ubiquitylate substrates for proteasomal degradation during mitosis.

E2 enzymes of the APC/C. In yeast and human cells, distinct E2 enzymes collaborate with the APC/C to initiate and then elongate ubiquitin chains. In yeast, Ubc1 and Ubc4 can both catalyse ubiquitin chain initiation and elongation in conjunction with the APC/C. However, Ubc4 functions preferentially in chain initiation, whereas Ubc1 favours chain elongation[24,25]. In higher eukaryotes, including vertebrates, ubiquitin-conjugating enzyme E2C (UBE2C; also known as UBCH10 and UBCX) links the first ubiquitin to substrates by binding to the RING domain of APC11 (REFS 26,27). At least *in vitro*, another initiating E2 enzyme, UBE2D (also known as UBCH5), can also fulfil this role[27–32]. Chain elongation is catalysed by UBE2S, which binds to a distinct surface of APC11 and also binds via its carboxy terminus to other components of the catalytic core and platform[26,31,33–35].

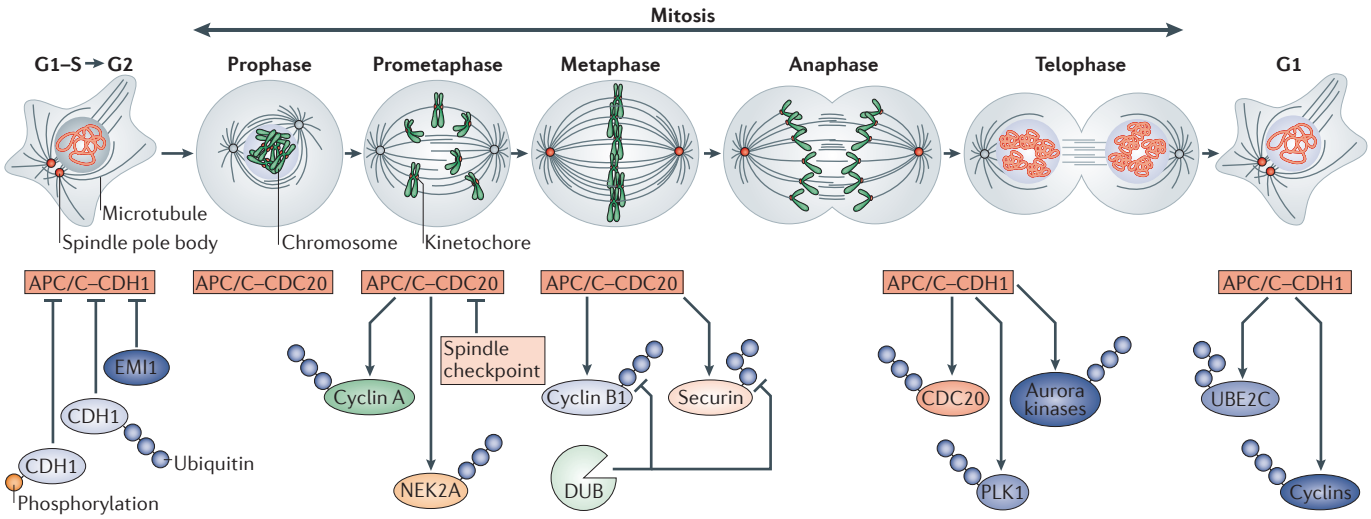


Figure 1 | **Ordered degradation of APC/C substrates.** The APC/C (anaphase-promoting complex; also known as the cyclosome) ubiquitylates proteins, marking their degradation at specific times and driving forward the progression of the cell cycle. APC/C–CDC20 ubiquitylates substrates during early and mid-mitosis, whereas APC/C–CDH1 (CDC20 homologue 1) ubiquitylates substrates after anaphase onset, during mitotic exit and in G1 phase. APC/C–CDC20 ubiquitylates cyclin A and NIMA-related kinase 2A (NEK2A) in prometaphase. During prometaphase APC/C–CDC20 activity towards 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 Polo-like kinase 1 (PLK1). At the G1–S transition, APC/C–CDH1 is inactivated by a combination of binding to the APC/C inhibitor early mitotic inhibitor 1 (EMI1), degradation of ubiquitin-conjugating enzyme E2C (UBE2C), CDH1 phosphorylation, and ubiquitylation and degradation of CDH1. DUB, deubiquitylating enzyme.

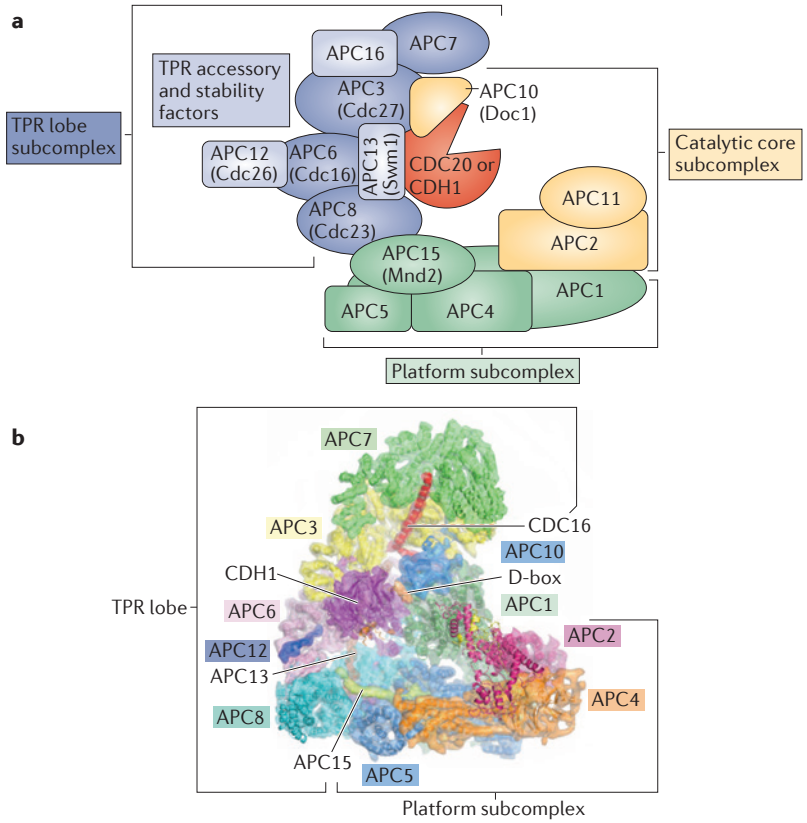


Figure 2 | **Structural organization of the APC/C.** **a** | The subunits of the APC/C (anaphase-promoting complex; also known as the cyclosome) can be largely organized into three subcomplexes: the platform (APC1, APC4, APC5 and APC15), the catalytic core (APC2, APC11 and APC10) and the tetratricopeptide repeat (TPR) lobe (APC8, APC6, APC3 and APC7) subcomplex[14]. APC/C subunit nomenclature used in yeast is shown in parentheses and TABLE 1. The APC1 subunit in the platform is the largest APC/C subunit and acts to bridge the other subcomplexes: the catalytic core and the TPR lobe[19,20]. APC2 acts as a scaffold for the catalytic core. APC11 potentiates the interaction with ubiqutin-conjugating enzymes (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 TPRs of APC3, APC6 and APC8; and APC16 interacts with the TPRs of APC3 and APC7 subunits[14]. Although 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 (CDC20 homologue 1) complex depicts the location of the individual subunits along with their underlying secondary structures. Part **b** reproduced from REF. 14, Nature Publishing Group.

Although the use of two E2 enzymes is conserved throughout evolution, the linkage specificity of polyubiquitin 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]. By contrast, in higher eukaryotes, such as *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 though K48. These branched ubiquitin chains allow 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 have important roles in mitotic control[38]. The DUBs shorten ubiquitin chain length, thereby regulating the order and timing of substrate degradation. For example, the DUB ubiquitin-specific protease 37 (USP37) removes polyubiquitin chains on cyclin A at the G1–S transition. This allows entry into the S phase[39]. The precise roles of DUBs in mitosis require further study.

Co-activators of the APC/C. The APC/C is largely inactive without one of its co-activators, CDC20 or CDH1. Their C 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-ubiquitin[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 phase, CDC20 is replaced by CDH1. CDK1, BUB1 and MAPK phosphorylate CDC20 on multiple residues. Phosphorylation of some residues inhibit, whereas 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 to and activates the APC/C, thereby causing substrate degradation in late mitosis and during G1 phase. It was also shown that CDH1 is sequestered in mitosis by mitotic arrest deficient 2-like protein 2 (MAD2L2); degradation of this protein during anaphase frees CDH1 to bind to and activate the APC/C[57].

Recent structural studies have provided valuable insights into the APC/C–CDH1–substrate–E2 enzyme complex[14,15,17,18]. The catalytic module of the APC/C, which consists of APC2–APC11, was found to be flexible[14]. Interestingly, the platform subunits of the APC/C were displaced upon co-activator–substrate binding. Co-activator binding disrupts the interaction between APC8 and APC1, which causes a downward displacement of APC8 and other platform subunits and concomitantly pushes the catalytic module (APC2 C-terminal domain APC11) upwards[14]. This change in conformation possibly increases the catalytic activity of the APC/C by bringing the initiating E2-ubiquitin close to the substrate[14,15,17] (FIG. 3a). The co-activator CDC20 binds to the C-terminal region (called the C-terminal peptide (CTP)) of UBE2S, which might aid in recruiting UBE2S to the APC/C[35]. The UBE2S CTP could then be passed to the APC2–APC4 region of the platform, towards which it shows strong affinity[26]. At a site on the APC/C that is distinct from the chain initiation site that functions through UBE2C, 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 ubiquitin[35].

Table 1 | **Subunits of the APC/C**

Budding yeast protein	Vertebrate protein	Stoichiometry	Functions	Refs
Platform subcomplex				
Apc1	APC1	1	Scaffolding	14
Apc4	APC4	1	Scaffolding; required for binding to UBE2S	35
Apc5	APC5	1	Scaffolding	14
Mnd2	APC15	1	Promotes CDC20 ubiquitylation and thus mediates disassembly of mitotic checkpoint complex	20, 127, 128
Catalytic core subcomplex				
Apc2	APC2	1	Catalytic; required for binding to UBE2S	26
Apc11	APC11	1	Catalytic; binds to initiating E2 enzyme; interacts with and activates elongating E2; recruits acceptor ubiquitin	26,35
Doc1	APC10	1	Part of degron (D-box) receptor	66,67, 69,71, 72
TPR lobe subcomplex				
Cdc27	APC3	2	Scaffolding; binds to APC10 and CDH1 or CDC20	14, 23,72
Cdc16	APC6	2	Scaffolding	14,22, 23,200
Not present	APC7	2	Scaffolding	14,23
Cdc23	APC8	2	Scaffolding; binds to CDC20	14,23
TPR accessory and stability factors				
Cdc26	APC12	2	Stabilizes APC6	14,22
Swm1	APC13	1	Stabilizes APC3, APC6 and APC8	14
–	APC16	1	Stabilizes APC3 and APC7	14

APC/C, anaphase-promoting complex (also known as the cyclosome); CDH1, CDC20 homologue 1; TPR, tetratricopeptide repeat; UBE2S, ubiquitin-conjugating enzyme E2S.

Substrate recognition sequences. Substrates have degradation sequences — known as degrons — through which they bind specifically to the APC/C–co-activator complex. Most substrates have 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 co-activators[21,40,65]. D-box substrates bind to a bipartite receptor formed by APC10 and the lateral surface of the co-activator WD40 domain. APC10 enhances substrate binding and the processivity of the ubiquitylation reaction[62,65–72]. The KEN-box degrons interact with a region on the surface of the co-activator WD40 domain[62,73].

Although these degrons are required, they are not sufficient, which suggests that substrates contain additional non-conserved sequences that are required for binding to the APC/C–co-activator complex[56,62]. These additional recognition sites might be important for fine-tuning the timing of substrate degradation during the progression of mitosis[74]. There are other distantly related APC/C degron motifs, such as the O-box in ORC1 (similar to the D-box), the G-box in *X. laevis* kinesin-like DNA-binding protein (kid; similar to the KEN-box), the A-box found in Aurora kinase, the CRY-box in CDC20, and less clearly defined degrons in claspin and Iqg1 (REFS 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, the substrates themselves are post-translationally modified to regulate their precise timing of degradation. For example, in vertebrates, phosphorylation of CDC6 (a 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 (REFS 36,76–78). Furthermore, acetylation of the spindle checkpoint protein BUBR1 (also known as BUB1β) at a lysine residue 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 are thus protected from degradation[80]. Therefore, substrates are post-translationally modified or differentially localized to regulate the timing of their degradation in mitosis.

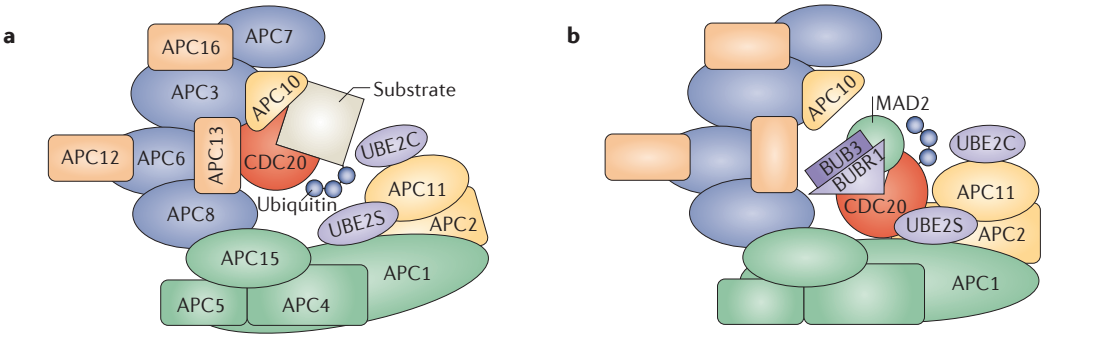
Regulation of the APC/C in early mitosis

Although the most prominent roles of the APC/C after full activation are induction of anaphase onset and mitotic exit, it 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 immunofluorescence[46]. The 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 (REFS 46,47). Phosphorylation is predominantly catalysed 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 column[85], and CKS mutants in different organisms arrest in mitosis with elevated levels of mitotic cyclins[44,83,86,87].

The complex composed of cyclin B1, CDK1 and CKS is the primary but not the only kinase that phosphorylates and activates the APC/C in mitosis. Some studies

Securin
A protein inhibitor of the protease separase.



Mitotic APC/C: active towards D-box and Ken-box substrates

Mitotic APC/C bound to MCC: inhibits recruitment of D-box and Ken-box substrates but active in ubiquitylating CDC20

Figure 3 | **Conformational changes during APC/C activation and inactivation.** The APC/C (anaphase-promoting complex; also known as the cyclosome) undergoes conformational changes upon co-activator and substrate binding to bring the E2-ubiquitin close to the substrate, and this conformational activation is inhibited by the mitotic checkpoint complex (MCC). **a** | Conformational activation of the APC/C upon co-activator and substrate binding is shown. Co-activator binding disrupts the 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-ubiquitin close to the substrate and potentiate attachment of the initiating ubiquitin[14,129]. CDC20 is also required for the activity of the chain-elongating ubiquitin-conjugating enzyme UBE2S[35]. A distinct region on APC2, near the APC2–APC4 junction, is required to bind to UBE2S[26]. The APC/C also tethers the distal molecule of an emerging ubiquitin conjugate close to UBE2S, thereby potentiating efficient ubiquitin chain elongation. **b** | APC/C bound to the MCC is shown. The MCC components inhibit the recruitment of late mitotic substrates that rely on recognition though D-box and KEN-box motifs, and hence inhibit APC/C activity towards these substrates. Mitotic arrest deficient 2-like protein 1 (MAD2) and BUBR1 bind to 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 APC10 (REF. 15). This position of CDC20 might also facilitate its own ubiquitylation and subsequent degradation during active spindle checkpoint signalling.

have suggested that Polo-like kinase 1 (PLK1) activates APC/C in mitosis, although others have 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. The concentration of APC/C and differences in its phosphorylation could give rise to spatial regulation of APC/C at different subcellular locations. The APC/C has been reported to concentrate at centrosomes, microtubules, chromosomes and kinetochores during mitosis[89–94].

The APC/C inhibitor protein early mitotic inhibitor 1 (EMI1; also known as FBXO5) plays a major part during interphase to inhibit APC/C activity and allow accumulation of mitotic cyclins for mitotic entry. Most EMI1 is degraded through SKP1–cullin-1–F-box (SCF)-mediated ubiquitylation in the early M phase, but a small pool persists and concentrates at spindle poles via its interaction with nuclear mitotic apparatus protein (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 the activity of protein phosphatase 2A (PP2A), which 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 before anaphase. It was hypothesized that inhibition of the APC/C at spindle poles by EMI1 blocks hypophosphorylation 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 metaphase[90]. 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 did 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 considerable underlying complexity associated with spatial regulation of APC/C activity in mitosis remains to be investigated.

Nuclear mitotic apparatus protein
(NUMA). A protein that partners with dynein in the assembly and maintenance of spindle poles.

Dynein–dynactin complex
A microtubule motor complex involved in the transport of spindle checkpoint proteins from kinetochores to the spindle pole.

The spindle checkpoint. At metaphase, when the last chromosome bi-orients on the mitotic spindle, APC/C-mediated ubiquitylation of securin and cyclin B1 — which are anaphase targets — is accelerated, and these proteins are rapidly degraded, resulting in chromatid separation and mitotic exit. An evolutionarily conserved mechanism called the spindle checkpoint inhibits the activity of APC/C–CDC20 until all chromosomes are bi-oriented on the mitotic spindle and are under mechanical tension from kinetochore–microtubule interactions (FIG. 1). The many protein interactions and kinase activities that catalyse spindle checkpoint signalling at kinetochores of unattached chromosomes are not discussed in detail here but have been discussed in 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 NIMA-related kinase 2A (NEK2A; also known as NEK2), thereby targeting them for degradation by the 26S proteasome[100–104] (FIG. 1). In normal dividing cells, 80% of cyclin A and more than 50% of NEK2A are degraded before metaphase[100]. How prometaphase targets are ubiquitylated in the presence of an active spindle checkpoint is an active area of study. The primary mechanism seems to be the ability of prometaphase targets to use alternatives to the canonical D-box and KEN-box motifs to bind to 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 signalling[35]. 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 to the APC/C in multiple ways to promote their degradation in prometaphase. Cyclin A is bound to CDC20 in G2 phase and early mitosis. Immediately after nuclear envelope breakdown, cyclin A is targeted to the APC/C by the CKS subunit of its CDK partner, which then promotes cyclin A degradation[83,105]. Similarly, in yeast the degradation of the S phase cyclin Clb5 in early mitosis depends on its interaction with Cdk1–Cks1 and an N-terminal Cdc20-binding region[106]. Degradation of NEK2A depends on an exposed C-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, describing the APC/C as 'activated' at the metaphase–anaphase transition is an oversimplification, as the APC/C also degrades early mitotic substrates cyclin A and NEK2A[108]. Additionally, APC/C activity mediates slow degradation of cyclin B1 in prometaphase. This is countered by cyclin B1 production during mitosis[109]. 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 drugs[109]. The gradual degradation of cyclin B1 might account for 'mitotic slippage' in which cells escape out of mitotic arrest induced by microtubule drugs[108,111–113]. The balance between synthesis and degradation is likely to differ among species and cell types, resulting in variation in the duration of mitotic arrest exhibited by different cells in the presence of microtubule inhibitors[113]. The type and concentration of microtubule drugs also influence the strength of spindle checkpoint signalling, thus affecting APC/C activity and the rate of degradation[108]. Although prometaphase APC/C targets are degraded in cells that are 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 signalling. In cells treated with low concentrations of nocodazole or in cells treated with microtubule stabilizers such as Taxol, in which 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 favourable subcellular locations (for example, 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-like protein 1), MAD2, BUBR1 (Mad3 in yeast), BUB1, BUB3 and monopolar spindle protein 1 (MPS1) (reviewed in REFS 96–99). MAD1–MAD2 heterodimers at unattached kinetochores catalyse a conformational change in an additional MAD2 (to form closed MAD2 or C-MAD2) that allows it to bind to and inhibit CDC20 (REF. 115). Robust inhibition also requires the binding of C-MAD2–CDC20 to BUBR1 and BUB3 (REFS 116,117). This complex of spindle checkpoint proteins — MAD2–CDC20–BUBR1–BUB3 — forms the MCC[118–122].

The crystal structure of the fission yeast MCC provided valuable information about interactions within the MCC components[73]. BUBR1 was found to interact through multiple residues with both C-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 to CDC20 and MAD2, thereby promoting assembly of the MCC. The C-terminal KEN-box is not required for the MCC–APC/C interaction but is required to inhibit substrate recruitment to the APC/C[123]. It was initially proposed that the C-terminal KEN-box might bind to a second copy of CDC20 (REF. 21), and a recent experimental study supports that model[124].

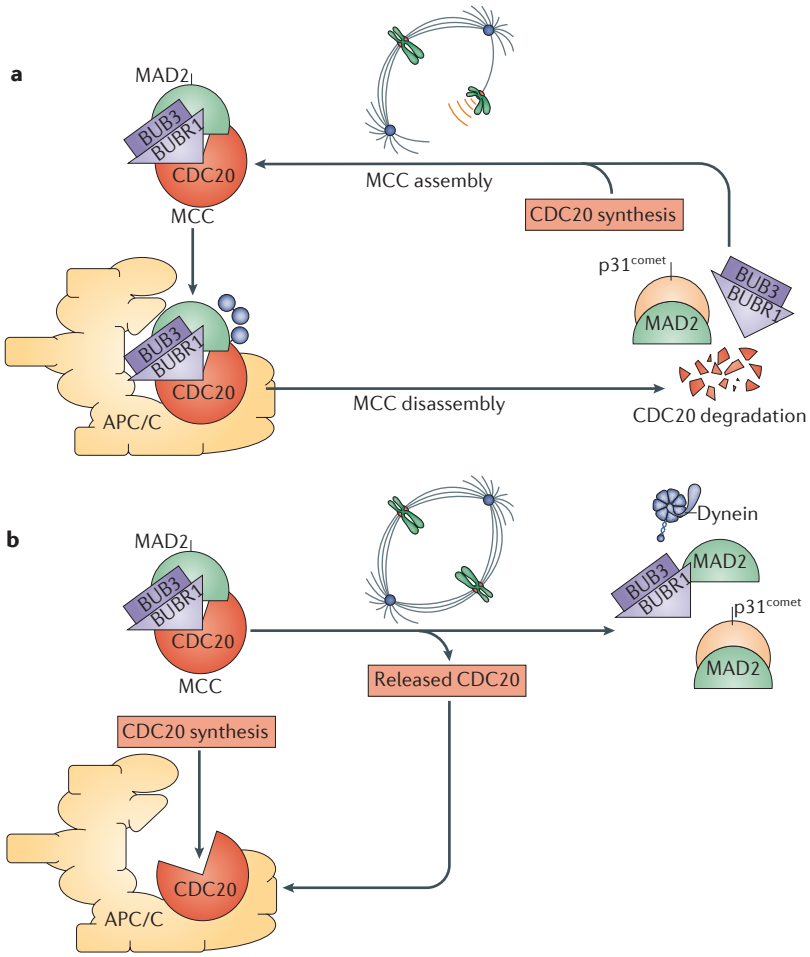


Figure 4 | **MCC turnover during mitosis. a** | In the presence of unattached kinetochores, mitotic arrest deficient 2-like protein 1 (MAD2), BUBR1, BUB3 and CDC20 interact to form a diffusible mitotic checkpoint complex (MCC) that binds to and inhibits the APC/C (anaphase-promoting complex; also known as the cyclosome). The APC/C ubiquitylates and promotes the degradation of its co-activator CDC20. CDC20 is continuously synthesized during mitosis. In the continued presence of unattached kinetochores, spindle checkpoint proteins MAD2 and BUBR1–BUB3 can be recycled to bind to newly synthesized CDC20, form the MCC and inhibit the APC/C. **b** | Once all sister kinetochores achieve bipolar attachment to the spindle and are under mechanical tension, MCC formation is inhibited and MCC disassembly dominates. CDC20 released from the MCC and freshly synthesized CDC20 generate the APC/C–CDC20 complex with high activity towards late mitotic substrates[20,127,128]. Several mechanisms contribute to the loss of MCC activity. MCC catalysis at kinetochores is inhibited by the transport of several checkpoint components, including MAD2 and BUBR1 from kinetochores by the minus-end-directed motor protein dynein[96,154–156]. p31^{comet} competes with BUBR1 for binding to MAD2 and prevents conformational activation of MAD2 (REF. 141). MCC disassembly allows APC/C activation, leading to ubiquitylation and degradation of securin and cyclin B1 for anaphase onset and mitotic exit.

MCC binding to the APC/C sterically hinders APC/C activity by disrupting the substrate-binding site and preventing substrate recruitment (FIG. 3b). MAD2 on its own competes with the APC/C for the same binding site of CDC20 and can thus inhibit the association of CDC20 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 (REFS 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 to and blocks the KEN-box receptor on the surface of the CDC20 WD40 domain. A second CDC20 molecule can bind to the MCC through the D-box and C-terminal KEN-box of BUBR1, and this interaction seems to be important for maximal checkpoint signalling[124]. Last, the interactions of the MCC with the catalytic core of the APC/C also partially impair the binding or function of UBE2C (REFS 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 signalling after chromosome bi-orientation (FIG. 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]. Both free MCC and MCC bound to the APC/C have to be disassembled to fully activate the APC/C after spindle checkpoint inactivation[132]. Although not completely understood, continuous assembly and disassembly 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 signalling is switched off.

CDC20 synthesis and degradation. During mitosis, CDC20 associated with the APC/C is continuously ubiquitylated and degraded. This is balanced by the continuous synthesis of the protein, hence ensuring constant steady-state levels of CDC20 during prometaphase[133]. APC15, a subunit of the platform subcomplex 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 has a key role in rapidly increasing APC/C activity to ubiquitylate anaphase targets when the spindle checkpoint is silenced[20,111,127,128]. CDC20 synthesis and degradation is intimately connected to continued generation of the MCC at unattached kinetochores and disassembly of the MCC in the cytoplasm (FIG. 4a). Free MCC is in excess of MCC bound to APC/C–CDC20 (REFS 124,132). During CDC20 degradation and MCC turnover, this excess free MCC might rapidly bind to APC/C–CDC20, thereby promoting strong inhibition of APC/C activity in the presence of unattached kinetochores[132]. Inhibition of CDC20 degradation or APC/C activity causes metaphase arrest, which is subsequently followed by cohesion fatigue; this suggests that APC/C activity is required to silence the spindle checkpoint[110,137,138]. Cohesion fatigue has been subsequently shown to reactivate the spindle checkpoint, which suggests that inhibition of APC/C activation at metaphase can cause reactivation of the spindle checkpoint[139]. MCC turnover is thus required for rapid anaphase onset and mitotic exit after checkpoint silencing[136] (FIG. 4b). Last, although APC15 is required

for CDC20 ubiquitylation, it is not required for APC/C–CDC20 or APC/C–CDH1 activity towards the mitotic substrates securin or cyclin B1 (REFS 20,127,128).

p31^{comet} promotes MCC release from the APC/C. Another key component in MCC turnover is p31^{comet}, a protein that is required for normal mitotic progression in vertebrates, but homologues have not been identified in lower eukaryotes. p31^{comet} is a MAD2 paralogue that forms a dimer with C-MAD2 (REFS 140–143). p31^{comet} structurally mimics MAD2 and competes with BUBR1 for MAD2 binding. Its binding to MAD2 prevents conformational activation of MAD2 (REF. 141). Depletion of p31^{comet} stabilizes the MCC, inhibits full activation of the APC/C and delays mitotic exit[111,144–146]. Depletion of p31^{comet} also inhibits CDC20 degradation during prometaphase and increases the amount of MAD2 in the MCC[111,147]. Conversely, overexpression of p31^{comet} overrides a spindle checkpoint-mediated arrest[147]. Using mitotic extracts from mammalian cells, it was found that p31^{comet}-mediated MCC disassembly required hydrolysis of the β-γ bond of ATP[148,149]. Recently, the AAA-ATPase thyroid hormone receptor interactor 13 (TRIP13), which binds to p31^{comet}, was found to be required for MCC disassembly[150]. TRIP13 and p31^{comet} together release MCC from the APC/C, promote MCC disassembly and inactivate the spindle checkpoint[150].

The p31^{comet} protein binds to unattached kinetochores, and its activity might be modulated by the strength of checkpoint signalling[145]. Strong checkpoint signalling that results from high concentrations of microtubule depolymerizers such as nocodazole leads to 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. As MAD2 is often present at substoichiometric 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 BUBR1–BUB3–CDC20 complex (known as BBC), which then serves as the primary inhibitor[116,123,147,151]. Levels of MAD2 in the MCC seem to correlate with the strength of checkpoint signalling[116,147,151], which suggests that the complete MCC, containing MAD2, is the more potent APC/C inhibitor. Finally, the protein CUE domain-containing protein 2 (CUEDC2) has been implicated in releasing MAD2 from the APC/C[152]. Interestingly, depletion of either p31^{comet} or CUEDC2 results in transient delays at metaphase, but these cells generally progress to anaphase. One explanation for this is that these proteins or others that have not yet been 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 they impart on kinetochores result in molecular changes that quell checkpoint signalling. 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 cannot be reactivated is approximately 5 minutes before anaphase onset in HeLa cells[153]. Several mechanisms participate in checkpoint silencing. Some checkpoint signalling proteins, including MAD1, MAD2, MPS1 and BUBR1, are depleted from the 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 signalling catalysed at kinetochores[96] (FIG. 4b).

Other proteins specifically accumulate in higher amounts at kinetochores of chromosomes as they achieve bipolar attachment and reach metaphase. One of these is PP1, the activity of which is required for checkpoint silencing[157]. Reversible protein phosphorylation is a key regulatory mechanism of spindle checkpoint signalling[97,99,157]. The kinases BUB1, MPS1 and Aurora B promote checkpoint signalling (reviewed in REFS 97–99) (FIG. 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 has 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 onset[160]. How the SKA complex promotes the metaphase–anaphase transition is not completely understood, but it seems to function, at least in part, by promoting APC/C accumulation on metaphase chromosomes[161].

APC/C at the metaphase–anaphase transition

Spindle checkpoint silencing causes the cessation of kinetochore-based MCC assembly. Newly synthesized CDC20 and/or free CDC20 released by MCC disassembly rapidly amplifies APC/C activity targeting securin and cyclin B1 for proteasomal degradation (FIG. 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 (FIG. 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 rapidly induces many of the events associated with mitotic exit, including degradation of cyclin B1 (REF. 162).

Changes in phosphorylation during anaphase and mitotic exit are likely to be key regulators of the APC/C. Binding of CDC20 to the APC/C is controlled, at least in part, by the removal of inhibitory phosphorylations[50]. During anaphase, dephosphorylation of CDH1 and

Cohesin complex
A protein complex that holds replicated sister chromatids together before anaphase.

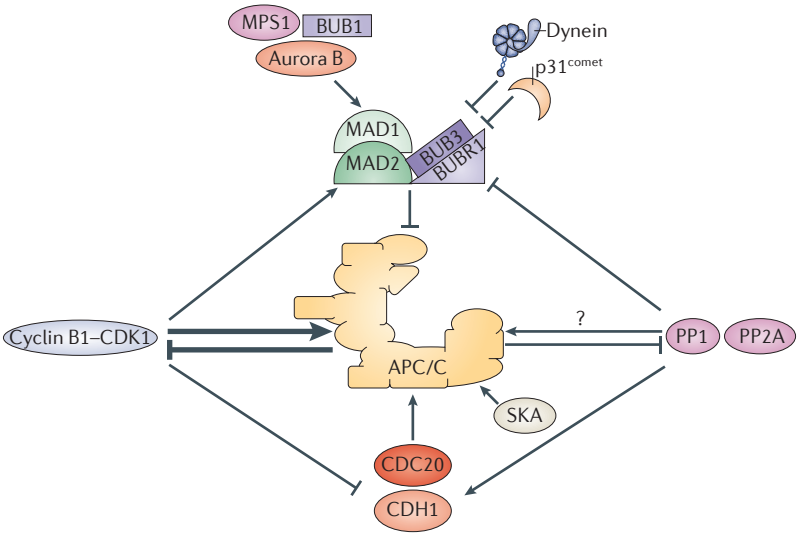


Figure 5 | **Positive and negative modulators control rapid changes in APC/C activity.** Mitotic progression is primarily regulated through two main activators, cyclin-dependent kinase 1 (CDK1) and the APC/C (anaphase-promoting complex; also known as the cyclosome). These work through a feedback mechanism whereby CDK1-mediated activation of APC/C ultimately induces the degradation of cyclin B1 and CDK1 inactivation (thick black arrows). APC/C activity is further modulated by a host of other components that are themselves regulated by post-translational 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 and detachment. The spindle checkpoint proteins mitotic arrest deficient 1-like protein 1 (MAD1), MAD2, BUBR1 and BUB3 inhibit APC/C activity. These spindle checkpoint proteins are themselves activated by the mitotic protein kinases monopolar spindle protein 1 (MPS1), BUB1, Aurora B, cyclin B1 and CDK1, and inhibited by $p31^{comet}$, protein phosphatases (PP1 and PP2A) and dynein. These regulators affect the localization or activity of the spindle checkpoint proteins. Although the spindle checkpoint inhibits the 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, CDC20 homologue 1 (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 have important roles in the dynamic regulation of APC/C activity during progressive stages of the cell cycle. SKA, spindle and kinetochore-associated.

degradation of the CDH1-binding protein MAD2L2 allows CDH1 to bind to and activate the APC/C[45,54,55,57,163]. APC/C–CDH1 recognizes substrates such as CDC20, Polo and Aurora kinases, UBE2C and geminin (FIG. 1). Although 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 does not affect the degradation of PLK1, geminin and CDC20 (although CDC20 is degraded more slowly)[164–166]. Mitotic exit is thus largely unaffected when CDH1 is deleted in budding yeast, fission yeast[167], *Drosophila melanogaster*[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 a specific order to regulate mitotic exit remains uncertain.

Subcellular compartmentalization of APC/C activity. Although 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 hypophosphorylated 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 than 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, which suggests 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 seems to be free and phosphorylated in the cytoplasm, and only a small dephosphorylated pool binds to and inhibits separase on chromosomes. PP2A dephosphorylates the securin bound to separase[78]. 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 chromatin[173]. Autocleaved 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 prematurely[36].

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–CDK or cyclin A–CDK complexes inactivate CDH1 by phosphorylation and prevent it from binding to the APC/C[36,54,55]. APC/C–CDH1 inactivation can also occur by degradation of its E2 enzyme, UBE2C. By ubiquitylating UBE2C and mediating its degradation, APC/C–CDH1 inactivates itself[175]. Finally, CDH1 can be auto-ubiquitylated by the APC/C at the end of G1 phase 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–Cdh1 (REF. 177). Similarly, Rca1 (F-box protein regulator of Cyclin A) in *D. melanogaster*[178] and EMI1 in vertebrates also function as inhibitors of APC/C–CDH1 (REF. 179). In budding yeast, Acm1 acts as a pseudosubstrate by competing with other substrates for

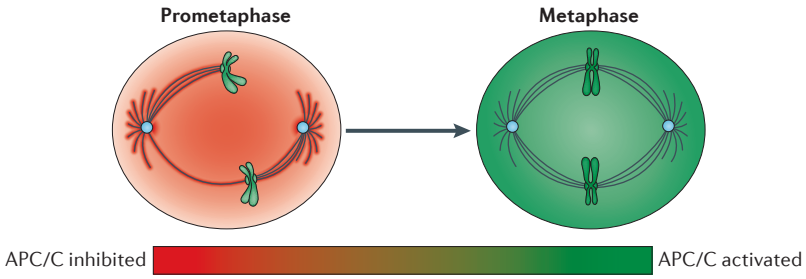


Figure 6 | **Hypothesis for the spatiotemporal regulation of the APC/C in mitosis.** In prometaphase, APC/C (anaphase-promoting complex; also known as the cyclosome) activity is inhibited towards late mitotic substrates to prevent anaphase onset and mitotic exit until all kinetochores are bi-oriented on the mitotic spindle and properly attached to microtubules. During mitosis, subcellular localization of the APC/C and its substrates might have 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 early mitotic inhibitor 1 (EMI1), nuclear mitotic apparatus protein (NUMA) and dynein–dynactin[94]. Spindle assembly factors are localized to microtubules and thereby protected from APC/C-mediated degradation until completion of spindle formation[80]. The spindle checkpoint generates the diffusible mitotic checkpoint complex (MCC) catalysed at unattached kinetochores to inhibit the soluble cytosolic APC/C (the intensity of the red colour denotes the 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, and this is dependent on the spindle and kinetochore-associated (SKA) complex[90]. 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 separation[173]. Therefore, APC/C activity is regulated spatiotemporally to control proper mitotic progression.

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 probably inactivates APC/C–Cdh1 (REF. 181). In vertebrate cells, EMI1 levels rise during the 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 the accumulation of APC/C substrates[179,182].

Structural evidence shows that EMI1 inhibits the APC/C in ways similar to the MCC[183]. The C terminus of EMI1 binds to multiple sites on APC/C–CDH1 to block the substrate-binding site[183]. EMI2 (also known as ERP1) is a protein closely related to EMI1 that functions in oocyte meiosis to inhibit APC/C activity. After ovulation and before fertilization, oocytes are arrested at the 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* embryos[187]. 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 phase and destabilization of geminin and cyclin A[191,192]. When EMI1 does not inactivate APC/C–CDH1, cells re-replicate their genomes and become polyploid[36].

At mitotic entry EMI1 is ubiquitylated and degraded by the SCF–β-TRCP (β-transducin repeat-containing protein) ubiquitin ligase[193–196]. Expression of a non-degradable form of EMI1 does not prevent APC/C activation[88,192,197,198], which suggests that other mechanisms also allow the APC/C to escape inhibition by EMI1 during mitotic entry. A good candidate is CDK1-mediated phosphorylation, as phosphorylated EMI1 seems unable to bind to and inhibit the APC/C efficiently[199].

Conclusions and current questions

The APC/C serves as a central control node that regulates 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 and co-activators, and negatively regulated by the spindle checkpoint and inhibitors (FIG. 5). Modulators of the APC/C ensure that the substrates are ubiquitylated 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 (FIG. 6). The APC/C accumulates on chromosomes as cells reach metaphase, and the chromosome-associated APC/C pool has a higher ubiquitylation activity[90]. At metaphase, it has been observed that motor proteins on microtubules transport spindle checkpoint proteins away from the kinetochore. Therefore, after proper microtubule attachment, inhibitors of the APC/C are hauled away from the kinetochore, whereas 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] (FIG. 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. 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. An active pool of APC/C–CDC20 might also catalyse the rapid degradation of cyclin B1 at metaphase upon spindle checkpoint inactivation. Moreover, localization to microtubules protects certain substrates from APC/C-mediated degradation[80] whereas, until metaphase, APC/C on centrosomes is anchored and potentially kept inactive by the EMI1–NUMA–dynein–dynactin complex[94] (FIG. 6). An important challenge in the future will be to understand how APC/C localized at specific compartments affects mitotic progression. It is possible that 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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