The anaphase promoting complex/cyclosome: a machine designed to destroy

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Abstract | The anaphase promoting complex/cyclosome (APC/C) is a ubiquitin ligase that has essential functions in and outside the eukaryotic cell cycle. It is the most complex molecular machine that is known to catalyse ubiquitylation reactions, and it contains more than a dozen subunits that assemble into a large 1.5-MDa complex. Recent discoveries have revealed an unexpected multitude of mechanisms that control APC/C activity, and have provided a first insight into how this unusual ubiquitin ligase recognizes its substrates.

Ubiquitin ligase (E3)
The third enzyme in a series — the first two are designated ubiquitin-activating (E1) and ubiquitin-conjugating (E2) — that is responsible for the ubiquitylation of target proteins. E3 enzymes provide platforms for binding E2 enzymes and specific substrates, thereby coordinating the ubiquitylation of selected substrates.

Polyubiquitin chains
Protein assemblies that are composed of several copies of the small protein ubiquitin. The ubiquitin residues are covalently attached to each other through isopeptide bonds.

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Cell proliferation in all eukaryotes depends strictly on the ubiquitin ligase (E3) activity of the anaphase promoting complex/cyclosome (APC/C). APC/C is a 1.5-MDa protein complex that is found in the nucleus of interphase cells, and that spreads throughout the cytoplasm and associates with parts of the spindle apparatus during mitosis. Without APC/C, cells cannot separate their sister chromatids in anaphase, they cannot exit from mitosis and divide into two daughter cells, and they cannot initiate the steps that are necessary for DNA replication later in S phase. APC/C seems to have similarly important functions in meiosis, and a number of observations imply that APC/C has also adopted new roles during the evolution of multicellular organisms in postmitotic differentiated cells (reviewed in REFS 1,2).

In all known cases, APC/C performs its various functions by assembling polyubiquitin chains on substrate proteins (Supplementary information S1 (table)), which targets these proteins for destruction by the 26S proteasome. Proteolysis is inherently associated with inactivation, which is what APC/C does in a number of cases in which its substrate protein is either a catalytic protein or an essential activator of such an enzyme. Prominent examples of these types of APC/C substrate are the mitosis-specific Aurora kinases and Polo-like kinase-1 (Plk1), and mitotic A- and B-type cyclins, the activating subunits of cyclin-dependent kinases-1 and -2 (Cdk1 and Cdk2). However, APC/C can also activate proteins, in this case, by targeting inhibitors of these proteins for destruction. Famous examples are securin, an inhibitor of the protease separase, and geminin, a protein that inactivates the replication factor CTD1.

In this article I discuss how APC/C might be able to selectively recognize its substrates, and review recent discoveries that have provided new insights into the essential mitotic functions of APC/C and its regulation during the cell cycle. But first I describe the APC/C complex and its cofactors, and their roles in APC/C-mediated ubiquitylation.

The APC/C complex and its cofactors

APC/C is composed of at least a dozen different subunits (TABLE 1), but it can only ubiquitylate substrates with the help of three cofactors, the ubiquitin-activating (E1) enzyme, a ubiquitin-conjugating (E2) enzyme and a co-activator protein.

APC/C cofactors. Like all E3 enzymes, APC/C uses ubiquitin residues that have been activated by E1 and then transferred to E2 enzymes. APC/C can collaborate with two E2 enzymes, UBCH5 and UBCH10 (which is also known as E2-C or UbcX)[3,4]. Although each of these is sufficient to support APC/C-mediated ubiquitylation reactions *in vitro*, UBCH10 orthologues in human cells, *Drosophila melanogaster* and fission yeast are essential for the initiation of anaphase, indicating that UBCH5 alone cannot fully support APC/C activity *in vivo*[5–7]. A possible exception is budding yeast, in which Ubc5 alone seems to be sufficient for APC/C function[8].

UBCH5 and UBCH10 associate with APC/C only transiently[9]. However, it is unknown whether the dynamic nature of this interaction is required for ubiquitylation reactions, as has been proposed to be the case for the ubiquitin ligase SCF[10], or whether the transient nature of APC/C–E2 interactions is simply a

Table 1 | **APC/C subunits and co-activators**

Vertebrates	*Drosophila melanogaster*	Budding yeast	Structural motifs	Functions
Subunits				
APC1/TSG24	Shattered	Apc1	RPN1 and RPN2 homology*	-
APC2	Morula	Apc2	Cullin homology	APC11 and DOC1 binding
CDC27/APC3	Makos	Cdc27	TPRs	CDH1 binding
APC4	-	Apc4	WD40 repeats	-
APC5	Ida	Apc5	TPRs	-
CDC16/APC6	-	Cdc16	TPRs	-
APC7	-	-	TPRs	-
CDC23/APC8	-	Cdc23	TPRs	-
DOC1/APC10	-	Doc1/Apc10	Doc domain	Substrate recognition, processivity
APC11	Lemming	Apc11	RING-H2 finger	E2 recruitment, E3 activity
CDC26	-	Cdc26	-	-
SWM1/APC13	-	Swm1/Apc13	-	-
-	-	Apc9	-	-
-	-	Mnd2	-	Ama1 inhibition
Co-activators				
CDC20/p55^{CDC}	Fizzy	Cdc20	C-box, WD40 repeats and IR-tail	Substrate recognition
CDH1 A–D	Fizzy-related	Cdh1/Hct1	C-box, WD40 repeats and IR-tail	Substrate recognition
-	Rap	-	C-box, WD40 repeats and IR-tail	Substrate recognition
-	-	Ama1	C-box, WD40 repeats and IR-tail	Substrate recognition
-	Cortex	-	C-box, WD40 repeats and IR-tail	Substrate recognition

*RPN1 and RPN2 are subunits of the 26S proteasome. Ama1, activator of meiotic APC/C protein-1; APC/C, anaphase promoting complex/cyclosome; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase; Mnd2, meiotic nuclear division protein-2; swm/SWM, spore wall maturation; TPRs, tetratrico peptide repeats; TSG, testis-specific gene.

26S proteasome
A large multisubunit protease complex that selectively degrades multi-ubiquitylated proteins. It contains a 20S particle that carries the catalytic activity and two regulatory 19S particles.

Cyclin-dependent kinase
(Cdk). A protein kinase that has activity that depends on an association with a cyclin subunit. Cdks are essential for DNA replication and entry into mitosis.

Ubiquitin-activating (E1) enzyme
An enzyme that activates the C-terminal glycine residue of the small protein ubiquitin, allowing it to form a high-energy thioester bond to a specific cysteine residue of the E1. E1 then transfers this activated form of ubiquitin onto ubiquitin-conjugating (E2) enzymes.

Ubiquitin-conjugating (E2) enzyme
An enzyme that forms a thioester bond with a ubiquitin residue, which is transferred to the E2 enzyme from ubiquitin-activating (E1) enzyme. E2 uses the high energy from the thioester bond to generate an isopeptide bond between the ubiquitin residue and a lysine residue on a substrate protein.

SCF
A multisubunit ubiquitin ligase complex that is composed of two scaffolding subunits (cullin and Skp1), a RING-finger subunit that binds ubiquitin-conjugating (E2) enzymes and one of many F-box subunits that recruit substrates.

C-box
A sequence element (consensus DRF/YIPXR) that was first found in the N-terminal region of Cdc20. It is conserved in all known APC/C co-activators.

IR-tail
A sequence element (consensus IR) at the extreme C terminus of APC/C co-activators and the APC/C subunit Doc1.

reflection of the modularity of the ubiquitin system, in which a few E2 enzymes can interact with different E3 enzymes. Consistent with the possibility that the transient interaction is a reflection of the modularity of the ubiquitin system, UBCH5 is a highly promiscuous enzyme that can interact with several E3 enzymes, possibly in contrast to UBCH10, which so far is only known to support APC/C.

In addition to E2 enzymes, APC/C activity is also strictly dependent on one of several co-activator proteins that associate with APC/C during specific periods of the cell cycle. The best studied of these are Cdc20 and Cdh1, which are encoded by all known eukaryotic genomes, but additional meiosis-specific APC/C co-activators have been identified in yeast and *D. melanogaster* (TABLE 1). All of these proteins are characterized by the presence of sequence elements, known as the C-box[11] and the IR-tail[12,13], that mediate their binding to APC/C[11–13]. Also, all APC/C co-activators contain a C-terminal WD40 domain that is predicted to fold into a propeller-like structure, and that is now believed to recognize APC/C substrates by interacting with specific recognition elements in these substrates[14], called D-boxes[15] and KEN-boxes[16].

APC/C: a cullin–RING-finger ubiquitin ligase. APC/C is thought to be a distant relative of the ubiquitin ligase SCF because both contain subunits with cullin and RING-finger domains[17–19]. In the APC/C, the cullin domain of Apc2 is associated with the RING-finger domain of Apc11 (REFS 13,20), which in turn interacts with E2 enzymes[21,22]. Remarkably, Apc11 and the E2 enzyme UBCH5 alone can efficiently catalyse ubiquitylation reactions *in vitro*, albeit with reduced substrate specificity, indicating that other APC/C subunits are not absolutely essential for the E3 activity of APC/C[20–22]. There is no evidence that Apc11 participates directly in the transfer of ubiquitin residues onto substrates, and it is therefore generally believed that APC/C serves as a scaffold that brings E2 enzymes and substrates into close proximity (reviewed in REF. 23).

It remains a big mystery why APC/C is composed of many different subunits (12 have been identified in humans and 13 in budding yeast; TABLE 1), although it takes only four proteins to build a functional SCF (reviewed in REF. 24). Biochemical fractionation and scanning transmission electron microscopy (EM) experiments indicate that APC/C from animal cells has a mass of 1.4–1.5 MDa[25,26] (with a sedimentation

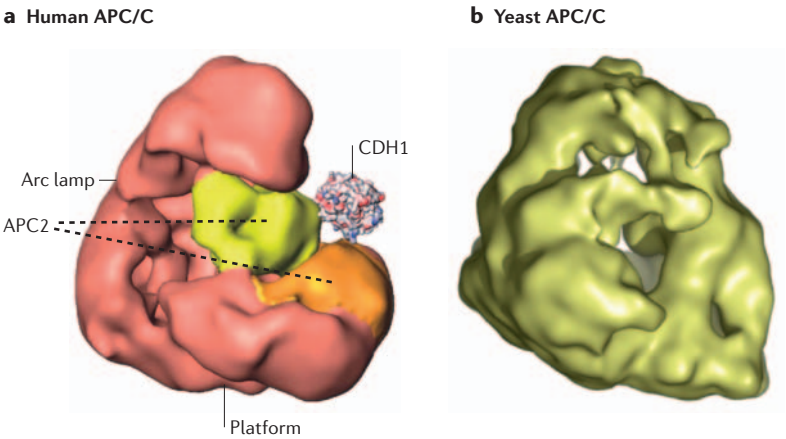


Figure 1 | **The three-dimensional structure of APC/C. a** | A three-dimensional (3D) model of human anaphase promoting complex/cyclosome (APC/C) obtained by cryo-negative staining electron microscopy (EM) and angular reconstitution. Human APC/C is composed of two large domains, known as 'platform' and 'arc lamp', that are flexible with respect to their relative positions to each other. Two candidate positions where the APC/C subunit APC2 might be located (as identified by antibody labelling) are shown, and a propeller domain similar to the one found in the co-activator CDH1 has been projected into the position where CDH1 is thought to bind. It is not known where CDC20 binds to APC/C. Reproduced with permission from REF. 26 © (2005) Elsevier. **b** | A 3D model of budding yeast APC/C obtained by cryo-EM and angular reconstitution. The models of human and yeast APC/C have similar triangular shapes, similar dimensions and both contain an internal cavity, but they differ in many structural details. Reproduced with permission from REF. 28 © (2005) Elsevier.

coefficient of 22S[27]), which places APC/C in the same size class as the ribosome, the 26S proteasome and chaperone complexes. Yeast APC/C is estimated to have a mass of 1.7 MDa[28] and can form even larger 36S dimers[12,167]. *In vitro*, the specific activity of these dimers is higher than that of monomers[28], which raises the interesting possibility that APC/C might function as a gigantic 3.4-MDa dimer *in vivo*.

APC/C architecture. The first structural insight into APC/C was obtained by cryo-EM of complexes purified from human cells, *Xenopus laevis* egg extracts[26,27] and budding yeast[28]. Three-dimensional modelling showed in all three cases that APC/C is an asymmetric triangular complex (200 by 230 Å in size) that is composed of an outer wall that encloses an internal cavity (FIG. 1). It has been speculated that substrate ubiquitylation might occur inside this cavity[27], but recent labelling experiments have shown that the co-activator CDH1 and the cullin domain of APC2 are located on the outside of the complex[26]. Because these proteins have been implicated in substrate recognition and ubiquitylation, respectively, it is now more plausible to think that ubiquitylation reactions occur on the outside. Vertebrate APC/C is composed of two large domains, known as 'platform' and 'arc lamp', that show a large degree of flexibility relative to each other. Interestingly, a change in their relative positions can also be observed when APC/C associates with CDH1, raising the possibility that co-activator binding might induce conformational changes in APC/C[26].

Topology of APC/C subunits. APC/C can be artificially dissociated into smaller subcomplexes[13,29,30], which has provided the first insights into the topology of its subunits (BOX 1). These studies have confirmed that Apc2 and Apc11 are essential for ubiquitylation activity[13,30], and have shown that yeast Apc2 also interacts with the small subunit Doc1 (REF. 30). However, in human cells, APC2 might not be the only binding partner of DOC1, because DOC1 binding is only reduced and not abolished if APC2 and APC11 are dissociated from human APC/C[13].

Like Doc1, Cdh1 seems to interact with more than one APC/C subunit. The TPR-domain protein Cdc27 binds to Cdh1 via its IR-tail[13,14,30], whereas Apc2 seems to interact with the C-box domain of Cdh1, either directly, or indirectly via an Apc2-binding partner, Apc11 or Doc1 (REF. 30). Interestingly, both Doc1 and Cdh1 have been implicated in substrate recognition and APC/C processivity, and their proximity to Apc2, Apc11 and the ubiquitin-charged E2 enzyme might therefore be important for catalysis of the ubiquitylation reaction.

Selective substrate recognition by APC/C

How are substrates recognized by APC/C? It has long been suspected that the essential role of co-activator proteins in APC/C activation might be to recruit substrates to APC/C, analogously to the role of adaptor proteins in SCF complexes. This view was supported by the observations that Cdc20 and Cdh1 can confer a limited degree of substrate specificity to APC/C[31–33], and that they can bind APC/C substrates, at least under certain conditions[11,34–38]. Also, the fact that Cdc20 and Cdh1 contain WD40 domains was consistent with this notion, because similar domains are found in the SCF adaptor proteins Cdc4 and β-Trcp, for which co-crystallization experiments have clearly shown that these domains bind substrates (reviewed in REF. 24).

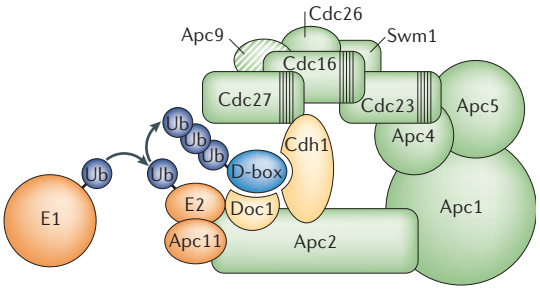
However, there were also observations and concerns that questioned the substrate–adaptor hypothesis. First, there was no agreement about which domains of the co-activators would bind to substrates[11,34–38], and, more importantly, there was no evidence that the detected association between substrates and co-activators was required for substrate ubiquitylation. The lack of evidence indicating that substrate–co-activator interactions are necessary for substrate ubiquitylation was a particular concern with regard to cyclins, because these proteins are not only substrates of APC/C but are also subunits of Cdks that are known to phosphorylate Cdc20 and Cdh1. Furthermore, it was discovered that the association of the yeast cyclin Clb2 with Cdh1 does not depend on the D-box domain of Clb2, although Clb2 degradation does[11], raising the question of whether the Clb2–Cdh1 interaction is required for Clb2 destruction. It was therefore also possible that cyclins would bind to Cdc20 and Cdh1 because the co-activators are Cdk1 substrates, and not vice versa. Last, it has been shown that cyclin B and the mitotic kinase Nek2A can bind to APC/C in *X. laevis* egg extracts in a manner that does not depend on Cdc20, the only co-activator that is present in significant amounts in these extracts[39,40], casting further doubt on the substrate–adaptor hypothesis.

WD40 domain
A propeller-shaped protein domain that is composed of sequence repeats that are ~40-amino-acid residues long and contain tryptophan (W) and aspartate (D) residues in conserved positions. In most cases, seven WD40 repeats fold into a seven-bladed propeller structure.

D-box
(Destruction-box). A sequence element (consensus RXXLXXXN) that was first discovered in the N terminus of mitotic cyclins that is required for their destruction. D-boxes can be recognized by APC/C^{Cdc20} and by APC/C^{Cdh1}.

KEN-box
A sequence element (consensus KEN) that is present in many APC/C substrates. KEN-boxes are preferentially, but not exclusively, recognized by APC/C^{Cdh1}.

Box 1 | **Model of how APC/C might recruit and ubiquitylate substrates**



Ubiquitin (Ub) is first activated and covalently bound through a thioester bond by the ubiquitin-activating (E1) enzyme and then transferred to the ubiquitin-conjugating (E2) enzyme with which the ubiquitin residue again forms a thioester bond. The ubiquitin-charged E2 enzyme interacts with anaphase promoting complex protein-11 (Apc11). This anaphase promoting complex/cyclosome (APC/C) subunit has ubiquitin ligase (E3) activity and promotes the transfer of the ubiquitin residue from the E2 enzyme to the substrate protein on which the C terminus of ubiquitin forms a covalent isopeptide bond with a lysine residue. In subsequent reactions, the attached ubiquitin can itself become ubiquitylated, resulting in the formation of a polyubiquitin chain. All proteins that are known to be involved in the catalysis of ubiquitylation reactions are shown in orange. Substrates are recruited to the APC/C if they contain a D-box or a KEN-box. Both of these sequences are recognized by an APC/C co-activator, such as Cdh1 or Cdc20. Cdh1 binds to APC/C by interacting with two subunits, Cdc27 and Apc2. Cdc27 is one of several TPR proteins that are present in the APC/C (TPR domains are shown as vertical stripes), and Apc2 is a scaffold subunit that binds to Apc11 via a cullin domain. The small globular protein Doc1 is required for processive ubiquitylation of substrates and might also interact with the D-box of substrates, although direct evidence for such an interaction is lacking. The APC/C subunits that are implicated in substrate recognition are shown in yellow. The topology of subunits is based on biochemical data in REFS 13,29,30. Note that this model illustrates subunit interactions but does not represent a structural map of where subunits are located in the three dimensional models that are shown in FIG. 1. Apc9 is hatched because so far it has only been detected in budding yeast APC/C. Human APC/C also contains another TPR subunit, APC7, and human DOC1 interacts not only with APC2 but also with another, unidentified subunit[13] (not shown here). Swm, spore wall maturation. Modified from REF. 30 © (2006) Cold Spring Harbor Laboratory Press.

How then does APC/C recognize its substrates? Several recent studies indicate that both co-activators and APC/C have important roles in this process. As in *X. laevis*[39], binding of substrates to APC/C can also be detected in budding yeast, but in this case the interaction depends on the presence of co-activators[12,41,42]. If, however, the small subunit Doc1 is removed from APC/C, the resulting APC/C$^{doc1\Delta}$ complex can no longer stably bind to substrates[12] and ubiquitylate them in a processive manner[9,43], despite the fact that Cdc20 and Cdh1 can still associate with APC/C[12]. These observations indicate that the co-activators are required but not sufficient for stable APC/C–substrate interactions, and that Doc1, and possibly other APC/C subunits, are also needed for this process. Similar observations have recently been reported for human APC/C, which can bind and ubiquitylate substrates more efficiently in the presence of Cdc20 than in its absence[44].

Co-activators, Doc1 and D-boxes: a 'ménage à trois'? How do co-activators and APC/C contribute to substrate recognition? In the case of co-activators, it has recently been found that the D-box of substrates directly contacts the WD40 domain of Cdh1 (REF. 14). Mutation of the WD40 domain compromises the capability of Cdh1 to bind substrates, and importantly, the resulting mutant APC/C^{Cdh1} complexes cannot ubiquitylate substrates in a processive manner[14]. These observations indicate that the binding of substrates to co-activators is indeed essential for efficient substrate ubiquitylation. A stoichiometric role for co-activators in substrate recruitment is also supported by the observations that APC/C, co-activator and substrate form a ternary complex[41,42], and that the amount of substrate that binds to APC/C is directly proportional to the amount of co-activator that is associated with APC/C[41].

The role of APC/C subunits in substrate recognition is more mysterious. Surprisingly, not only the interactions between substrates and co-activators but also those between substrates and APC/C seem to be D-box dependent[39,44], but the identity of the APC/C subunits that mediate these interactions is unknown. The best candidate so far is Doc1, the crystal structure of which has shown that this protein is composed of a compact globular domain with a surface that is predicted to interact with a ligand of unknown identity[45,46]. Mutational analyses have shown that this surface is essential for the capability of APC/C to ubiquitylate substrates in a processive manner[43]. Interestingly, domains that are homologous to Doc1 have also been identified in other ubiquitin ligases, implying that such 'Doc domains' might contribute to processive substrate ubiquitylation in other E3 enzymes[47,48]. In the case of APC/C, it is tempting to speculate that the unidentified ligand of Doc1 might be the substrate itself, but there are a number of other possibilities.

So far, it has been impossible to detect Doc1–substrate interactions. Indirect support for their existence, however, comes from the observation that the residual ubiquitylation activity of APC/C$^{doc1\Delta}$ is not further reduced by mutation of the D-box in substrates[43], which is consistent with the possibility that Doc1 contributes either directly or indirectly to the recognition of the D-box. The D-box might therefore interact with both the propeller domain of co-activators and with APC/C subunits such as Doc1, either sequentially or simultaneously, and therefore facilitate the formation of a ternary APC/C–co-activator–substrate complex (BOX 1). If correct, this arrangement would be strikingly similar to the domain structure of otherwise completely unrelated sugar-hydrolysing enzymes of the sialidase family. Also in

Cullin
A member of the cullin family of proteins. All cullins are subunits of SCF ubiquitin ligases or APC/C, and they bind to a RING-finger subunit via a conserved cullin domain.

RING finger
A small protein domain that binds two atoms of zinc (consensus $CXXCX_{(9-39)}CX_{(1-3)}HX_{(2-3)}C/HXXCX_{(4-48)}CXXC$). Many RING-finger domains interact with ubiquitin-conjugating (E2) enzymes and have ubiquitin ligase (E3) activity.

TPR domain
(Tetratrico peptide repeat domain). A 34-amino-acid sequence repeat, clusters of which fold into a helical structure and mediate protein–protein interactions.

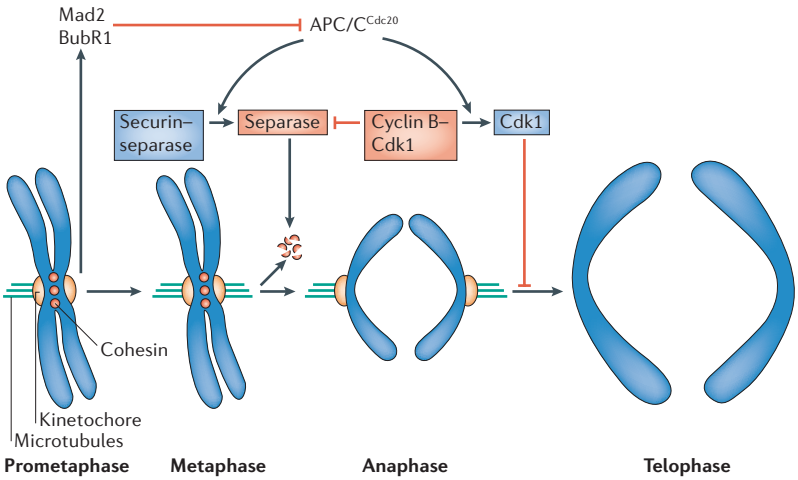


Figure 2 | **Regulation of anaphase and mitotic exit by APC/C^{Cdc20}.** During prometaphase, spindle-assembly-checkpoint proteins such as Mad2 and BubR1 are activated at kinetochores that are not (or not fully) attached with microtubules (indicated in green). Activated Mad2 and BubR1 inhibit the capability of anaphase promoting complex/cyclosome^{Cdc20} (APC/C^{Cdc20}) to ubiquitylate securin and cyclin B and thereby prevent anaphase and mitotic exit. In metaphase, when all kinetochores are attached to microtubules, APC/C^{Cdc20} ubiquitylates securin and cyclin B and thereby activates the protease separase and inactivates the cyclin-dependent kinase-1 (Cdk1). Separase then cleaves cohesin complexes (shown as red circles) that are holding sister chromatids together and thereby initiates sister-chromatid separation. Cdk1 inactivation leads to the dephosphorylation of Cdk1 substrates by protein phosphatases, and thereby enables exit from mitosis. In vertebrates, CDK1 inactivation also contributes to separase activation.

these enzymes, propeller structures that are similar to the ones found in APC/C co-activators and 'jelly-roll fold' domains that are structurally almost identical to Doc1 (REFS 45,46) collaborate in substrate binding and catalysis[49].

Essential functions of APC/C in mitosis

The capability of APC/C to selectively recognize its substrates at the correct time is essential for several key events in mitosis, the initiation of anaphase, exit from mitosis and the preparation for the next round of DNA replication.

Cyclin proteolysis. APC/C was initially discovered as a ubiquitin ligase that is essential for cyclin destruction in mitosis[25,50,51]. This process is initiated in metaphase[52] (that is, when all of the chromosomes have been attached to both poles of the mitotic spindle) and it is essential for Cdk1 inactivation and subsequent exit from mitosis (FIG. 2; REF. 53). The initiation of cyclin proteolysis depends on the form of APC/C that is associated with Cdc20 (APC/C^{Cdc20}). Although APC/C^{Cdc20} can attach ubiquitin residues to a number of different lysine residues in cyclin B in an apparently non-selective manner[54], Cdk1, the binding partner of cyclin, is spared from this modification and from the subsequent fate that cyclin experiences in the proteolytic channel of the 26S proteasome. However, once stripped off of cyclin B, Cdk1 is predicted to undergo a conformational change that prevents both ATP hydrolysis and access of protein substrates to the active site, resulting in the complete inactivation of Cdk1 (REF. 55). This situation allows protein phosphatases to dephosphorylate Cdk1 substrates, which is an essential prerequisite for disassembly of the mitotic spindle, chromosome decondensation, reformation of a nuclear envelope and formation of a cytokinetic furrow.

Cdk inactivation by cyclin proteolysis also has more far-reaching consequences for the subsequent cell cycle. A period of low Cdk activity during telophase and G1 is essential for the formation of pre-replicative complexes (pre-RCs) on origins of replication, on which DNA polymerases initiate DNA synthesis in S phase. Cdk activity inhibits the assembly of these complexes, and DNA replication is therefore strictly dependent on previous progression through mitosis when Cdk inactivation is initiated by APC/C^{Cdc20} (reviewed in REF. 56). This indirect S-phase-promoting role of APC/C is also part of the mechanism that restricts DNA replication to once per cell cycle. Because S phase depends not only on a period of low Cdk activity during which pre-RCs are assembled, but also on a subsequent phase of elevated Cdk activity during which DNA synthesis is initiated, every origin of replication can only be 'fired' once per cell cycle.

In budding yeast, cyclin degradation is the only essential S-phase-promoting function of APC/C, but, in insects and vertebrates, DNA replication also depends on the APC/C-mediated degradation of geminin, a protein that inhibits incorporation of the replication factor CDT1 into pre-RCs and thereby prevents the proper assembly of these complexes[57–60].

Sister-chromatid separation. The most well known and possibly most important function of APC/C, and the one that has lent the 'APC' its name, is its role in promoting anaphase (FIG. 2). To allow chromosome segregation, the cohesion that holds sister chromatids together first has to be dissolved. In metaphase, APC/C^{Cdc20} initiates this process by ubiquitylating securin, a small protein that functions both as a co-chaperone and as an inhibitor of the protease separase. Once activated, separase cleaves the Scc1 subunit of cohesin, a complex that holds sister chromatids together, and this cleavage therefore dissolves cohesion between sister chromatids (reviewed in REF. 61). Although securin destruction is essential for the activation of separase, budding yeast, cultured human cells and even mice can live without securin[62–65]. The implication is that there must be securin-independent mechanisms that control separase activity. One such mechanism might be the phosphorylation of separase and the subsequent stoichiometric association with Cdk1–cyclin B, which is sufficient for separase inhibition in *X. laevis* egg extracts[66,67]. APC/C^{Cdc20} might therefore contribute to separase activation by ubiquitylating both securin and cyclin B.

Life without APC/C. The genetic inactivation of APC/C has caused lethality in all species in which it has been investigated so far, ranging from fungi to mouse (REF. 68 and references therein). Because the expression of D-box mutants of securin and cyclin that cannot be recognized

Table 2 | **APC/C inhibitors**

Inhibitor	Species	Proposed to inhibit	Proposed mechanism	References
CDK1	Sc, Sp, Hs	APC/C^{CDH1} in S and G2 phase	CDH1 phosphorylation, causing dissociation from APC/C, and in Sc also nuclear export	72,76–79,154
Cdk1	Xl	APC/C^{Cdc20} in prometaphase (SAC)	Cdc20 phosphorylation, causing interaction with Mad2	155,156
SCF	Hs	APC/C^{CDH1} in S phase	CDH1 ubiquitylation/degradation	94
APC/C^{CDH1}	Sc, Hs	APC/C^{CDC20} in anaphase	CDC20 ubiquitylation/degradation	82–85
MAD2	Sc, Hs	APC/C^{CDC20} in prometaphase (SAC)	Inhibition of CDC20-substrate release	101–104,157
MAD2B*	Xl, Hs	APC/C^{CDH1} (and APC/C^{CDC20}?)	Inhibition of CDH1-substrate release	157,158
BUBR1	Hs	APC/C^{CDC20} in prometaphase (SAC)	Sequestration of CDC20	105–107
BUB1	Hs	APC/C^{CDC20} in prometaphase (SAC)	CDC20 phosphorylation	159
MAP kinase	Xl	APC/C^{Cdc20} in prometaphase (SAC)	Cdc20 phosphorylation	160
EMI1	Xl, Hs	APC/C^{CDC20} in prophase, APC/C^{CDH1} in S and G2 phase	Competitive inhibition of substrate binding to CDC20 and CDH1	90,91,118
RCA1	Dm	APC/C^{CDH1} in S and G2 phase	Unknown	88
XErp1/EMI2	Xl, Hs	APC/C^{CDC20} in meiosis II (CSF)	Unknown	139–141
Mes1	Sp	APC/C^{Cdc20} during meiosis I exit	Competitive inhibition of substrate binding to Cdc20	130
Mnd2	Sc	APC/C^{Ama1} in meiosis I	Unknown	126,127
RASSF1A	Hs	APC/C^{CDC20} in mitosis	CDC20 binding	161
Xnf7	Xl	APC/C^{Cdc20} in mitosis	Unknown, the E3 activity of Xnf7 is required	162
RAE1–NUP98	Mm	APC/C^{CDH1} in prometaphase (SAC)	Unknown, associates with APC/C^{CDH1}	163
Apoptin	CAV	APC/C^{CDC20} and APC/C^{CDH1}	APC1 binding, APC/C dissociation	164
Unknown	HCMV	APC/C^{CDH1} in G0 phase	Inhibition of the CDH1–APC/C interaction	165
E4orf4	HAV	APC/C^{CDC20}	Recruitment of PP2A to APC/C when E4orf4 is overexpressed in Sc	166

* MAD2B is also known as MAD2L2. APC/C, anaphase promoting complex/cyclosome; CAV, chicken anaemia virus; Cdk1/CDK1, cyclin-dependent kinase-1; Dm, *Drosophila melanogaster*; E3, ubiquitin ligase; EMI, early mitotic inhibitor; HAV, human adenovirus; HCMV, human cytomegalovirus; Hs, *Homo sapiens*; MAP, mitogen-activated protein; Mm, *Mus musculus*; Mnd2, meiotic nuclear division protein-2; RAE1, Rab escort protein-1; RCA1, regulator of cyclin A-1; SAC, spindle-assembly checkpoint; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; Xl, *Xenopus laevis*; Xnf7, *Xenopus* nuclear factor-7.

by APC/C inhibits chromosome segregation and mitotic exit, respectively (reviewed in REF. 1), the role of APC/C in destroying securin and mitotic cyclins was generally assumed to be responsible for this lethality. However, many other proteins are also degraded in an APC/C-dependent manner (Supplementary information S1 (table)), and it is therefore possible that the stabilization of these proteins would also inhibit cell proliferation in APC/C mutants. This remains a possibility for most species, and in particular for multicellular organisms in which, for example, the degradation of geminin is required for DNA replication. However, recent studies indicate that the only essential functions of APC/C in budding yeast are indeed the degradation of securin and B-type cyclins. If the need to destroy these proteins is eliminated through genetic manipulation, yeast cells can proliferate in the absence of otherwise essential APC/C subunits[69] or its Cdc20 co-activator[70]. This is a remarkable result because it shows that the inactivation of other yeast APC/C substrates is either not essential, or that degradation is only one of several mechanisms that can inactivate these proteins.

This finding also has important practical implications because, for the first time, mutant forms of APC/C that lack essential subunits can now be generated and analysed[14,30,69]. Last, this finding lends support to the speculation that APC/C might have co-evolved either with Cdks to antagonize their activities, or with separase, to promote its activation in mitosis.

Regulation of APC/C in mitosis

Given that the inappropriate activation of APC/C could cause fatal errors in cell-cycle progression, APC/C-dependent degradation reactions are tightly controlled. Most of these mechanisms operate at the level of APC/C, often through APC/C-inhibiting proteins and enzymes (TABLE 2).

Activation of APC/C^{Cdc20} and APC/C^{Cdh1}. Like E2 enzymes, the co-activators Cdc20 and Cdh1 associate with APC/C only transiently, but in this case the interactions are tightly regulated, and the regulation of this association is one of the key mechanisms that determines when during the cell cycle APC/C is active (FIG. 3).

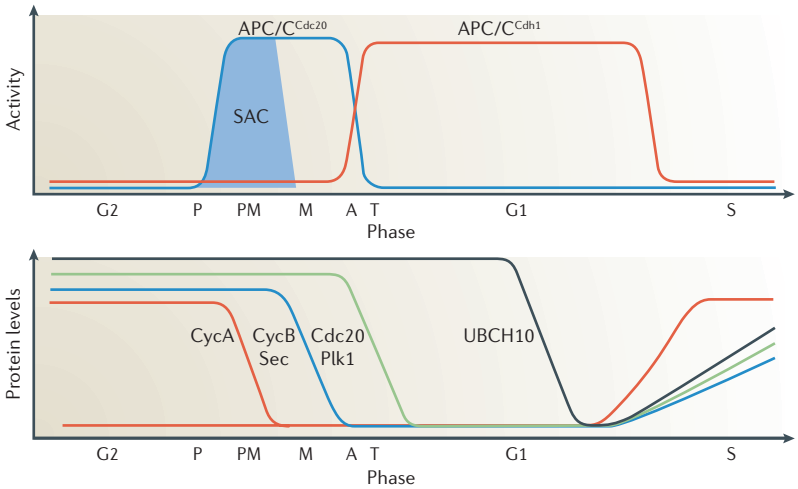


Figure 3 | **Activation of APC/C by Cdc20 and Cdh1 during the cell cycle.** Anaphase promoting complex/cyclosome^{Cdc20} (APC/C^{Cdc20}) is thought to be assembled in prophase (P) and initiates the degradation of cyclin A (CycA) already in prometaphase (PM). Proteolysis of cyclin B (CycB) and the separase inhibitor securin (Sec) also depends on APC/C^{Cdc20} but is delayed until metaphase (M) by the spindle-assembly checkpoint (SAC). During anaphase (A) and telophase (T), APC^{Cdh1} is activated, contributes to the degradation of securin and cyclin B, and mediates the destruction of additional substrates such as Polo-like kinase-1 (Plk1) and Cdc20, which leads to the inactivation of APC/C^{Cdc20}. In G1 phase, APC/C^{Cdh1} mediates the destruction of the ubiquitin-conjugating (E2) enzyme UBCH10, and thereby allows for the accumulation of cyclin A, which contributes to the inactivation of APC/C^{Cdh1} at the transition from G1 to S phase.

Cdc20 is already transcribed and translated during S and G2 phase, but it can only associate efficiently with APC/C in mitosis when several subunits of APC/C have been phosphorylated by mitotic kinases such as Cdk1 and Plk1 (REFS 71–75). By contrast, Cdh1 is prevented from efficient interaction with APC/C as long as Cdh1 is phosphorylated by different Cdks during the S and G2 phase and in the early stages of mitosis[72,76–79]. As a result, APC/C^{Cdc20} is active early in mitosis, whereas Cdh1 can only activate APC/C once APC/C^{Cdc20} has decreased the Cdk1 activity by initiating cyclin destruction, and when subsequently phosphates have been removed from Cdh1 by protein phosphatases such as Cdc14 in yeast[80].

These opposing effects of phosphorylation on APC/C^{Cdc20} and APC/C^{Cdh1} result in the switch from a high to a low Cdk state that is required for exit from mitosis and subsequent DNA replication. High Cdk1 activity in mitosis leads to the assembly of APC/C^{Cdc20}, which initiates cyclin proteolysis and decreases Cdk1 activity. This drop in Cdk1 activity promotes the formation of APC/C^{Cdh1}, which then maintains cyclin instability in G1 and enables a new round of DNA replication by permitting the assembly of pre-RCs.

Inactivation of APC/C^{Cdc20} and APC/C^{Cdh1}. APC/C^{Cdc20} is already inactivated during mitotic exit[81] (FIG. 3), presumably because APC/C dephosphorylation leads to the disassembly of APC/C^{Cdc20}, and because Cdc20 itself is a substrate of APC/C^{Cdh1} (REFS 82–85). By contrast, APC/C^{Cdh1} is inactivated later at the G1–S transition[72,76–79]. This inactivation is essential for the accumulation of APC/C substrates such as cyclins that are required for the initiation of DNA replication and subsequent entry into mitosis.

In budding yeast, the inactivation of APC/C^{Cdh1} depends on S-phase Cdks[85] that phosphorylate Cdh1 and thereby prevent its interaction with APC/C. The major cyclin that activates S-phase Cdks is Clb5, a protein that is targeted for destruction in mitosis by APC/C^{Cdc20} (REF. 70) but that does not seem to be a substrate for APC/C^{Cdh1} (REFS 31,32). It is therefore possible that the inactivation of APC/C^{Cdc20} by APC/C^{Cdh1} allows the accumulation of Clb5 during G1 phase, which then eventually leads to the inactivation of APC/C^{Cdh1} at the G1–S transition.

A similar situation might exist in *D. melanogaster* in which the overexpression of cyclin E, an S-phase cyclin that is neither a substrate of APC/C^{Cdc20} nor APC/C^{Cdh1}, is sufficient to stabilize APC/C substrates[86]. This is consistent with the possibility that cyclin E–Cdk2 inactivates APC/C^{Cdh1} by phosphorylating Cdh1. However, a second mechanism is required in *D. melanogaster* cells to keep APC/C^{Cdh1} inactive during the G2 phase. This mechanism depends on regulator of cyclin A-1 (Rca1), a protein that allows the accumulation of cyclin A during the G2 phase by inhibiting APC/C^{Cdh1} (REFS 87,88).

In vertebrates, the situation might be different (FIG. 4) because, in a purified system that is composed of human proteins, only cyclin A–CDK2 and not cyclin E–CDK2 was found to inhibit APC/C^{CDH1} (REF. 89), implying that the accumulation of cyclin A and not of cyclin E might be critical for the inactivation of APC/C^{CDH1}. However, cyclin A is itself a substrate of APC/C^{CDC20} and APC/C^{CDH1}, raising the question of how sufficient amounts of cyclin A can ever accumulate to inactivate APC/C^{CDH1}. Two mechanisms have recently been discovered that might be the solution to this problem (FIG. 4).

Vertebrate cells contain an orthologue of *D. melanogaster* RCA1, called early mitotic inhibitor-1 (EMI1), the expression of which is stimulated at the G1–S transition by the E2F transcription factor[90]. EMI1 inhibits APC/C^{CDH1} and can thereby allow the accumulation of APC/C substrates at the G1–S transition[90]. *In vitro*, EMI1 can competitively inhibit the binding of substrates to N-terminal fragments of CDC20 and CDH1 (REF. 91). However, it is not known whether this property explains the capability of EMI1 to inhibit APC/C^{CDH1} *in vivo*, because more recent evidence implies that substrates have to bind to the C-terminal WD40 domain of CDH1 to be ubiquitylated[14]. Although it is clear that EMI1 inhibits APC/C^{CDH1}, the precise mechanism of this inhibition is not yet fully understood.

A second model is based on the observations that cyclin A degradation critically depends on the levels of UBCH10 (REF. 92), and that UBCH10 itself is degraded in an APC/C^{Cdh1}-dependent manner during the G1 phase[7,92,93]. It has therefore been proposed that APC/C^{Cdh1} initiates its own inactivation by ubiquitylating UBCH10, which would lead to the stabilization of cyclin A and to the subsequent inhibition of APC/C^{Cdh1} by cyclin A–Cdk2 (REF. 92). In human cells and fission yeast, Cdh1 levels are also reduced in the S phase when Cdh1 is phosphorylated[72,78,79] due to the ubiquitylation by ubiquitin ligases of the SCF family[94].

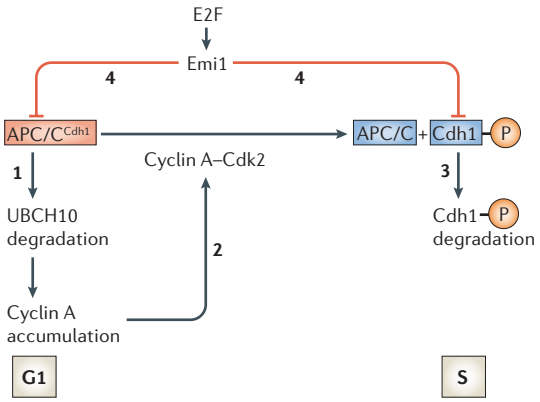


Figure 4 | **Inactivation of APC/C^{Cdh1} at the transition from G1 to S phase.** The inactivation of anaphase promoting complex/cyclosome^{Cdh1} (APC/C^{Cdh1}) at the end of G1 phase is important to allow the accumulation of proteins that are required for DNA replication and mitosis, such as cyclin A and cyclin B. Four different mechanisms have been proposed to contribute to this inactivation process in vertebrate cells. **1** | During the G1 phase, the APC/C-interacting ubiquitin-conjugating (E2) enzyme UBCH10 is itself degraded by APC/C^{Cdh1}. This process leads to the stabilization of those APC/C^{Cdh1} substrates that are ubiquitylated in a distributive manner, such as cyclin A[92,148]. **2** | Cyclin A activates cyclin-dependent kinase-2 (Cdk2), which in turn phosphorylates Cdh1 and thereby dissociates Cdh1 from APC/C[72,89]. **3** | Phosphorylated Cdh1 is ubiquitylated by SCF and thereby targeted for destruction by the 26S proteasome[94]. **4** | The transcription factor E2F activates the expression of early mitotic inhibitor-1 (Emi1), and Emi1 then inhibits the activity of APC/C^{Cdh1} (REF. 90). P, phosphate.

APC/C^{Cdc20} and the spindle-assembly checkpoint. The phosphorylation of APC/C subunits is already initiated at the beginning of mitosis in prophase, before spindle assembly has even started[75]. As APC/C phosphorylation promotes the binding of Cdc20, it is possible that APC/C^{Cdc20} is already assembled at this stage. Indeed, the destruction of several APC/C substrates such as cyclin A and Nek2A is initiated as soon as the nuclear envelope disintegrates at the transition from prophase to prometaphase (REFS 40,95–97 and references therein). If APC/C^{Cdc20} were able to initiate sister-chromatid separation and exit from mitosis already at this stage, there would not be enough time to bi-orient all the chromosomes on the mitotic spindle. To avoid this situation, the activity of APC/C^{Cdc20} is restrained by several mechanisms. The most important of these is the spindle-assembly checkpoint, which inhibits the capability of APC/C^{Cdc20} to initiate anaphase until all of the chromosomes have been bi-oriented (FIG. 2). Remarkably, the presence of a single kinetochore that is not attached to spindle microtubules is sufficient to delay anaphase[98,99], possibly by creating a diffusible signal that can inhibit APC/C^{Cdc20}. Furthermore, the spindle-assembly checkpoint can control APC/C^{Cdc20} in a substrate-specific manner, because it inhibits the capability of APC/C^{Cdc20} to ubiquitylate B-type cyclins and securin[52,81] without preventing the degradation of cyclin A and Nek2A[40,95–97].

Several proteins have been identified that are required for a functional spindle-assembly checkpoint and that are enriched on unattached kinetochores where checkpoint signalling is initiated (reviewed in REF. 100). Two of these proteins, called Mad2 and BubR1, have been shown to interact directly with APC/C^{Cdc20} *in vivo* and to inhibit its ubiquitylation activity *in vitro*[101–107]. Biochemical and structural studies have revealed that Mad2 has the remarkable ability to associate tightly, but in a mutually exclusive manner, with either Cdc20 or with another protein that is essential for checkpoint function, called Mad1 (REFS 108–110). When bound to Mad1, Mad2 is stably recruited to unattached kinetochores[108,111]. It has recently been proposed that this Mad1–Mad2 complex at kinetochores functions as a template for the assembly of Cdc20–Mad2 complexes[112]. According to this model, the Mad2 subunit of Mad1–Mad2 complexes would form dimers with diffusible Mad2 molecules and would somehow catalyse the tight association of diffusible Mad2 with Cdc20 (FIG. 5). It has been proposed that Mad2–Cdc20 complexes could then themselves function as templates for the formation of additional Cdc20–Mad2 complexes. Although speculative at the moment, this model is attractive because it could explain how the presumably weak checkpoint signal that is generated by a single unattached kinetochore could be amplified.

Although it is clear that Mad2 has an important and direct role in inhibiting Cdc20, it is not known whether Mad2 performs this function in isolation or as part of a larger protein complex. BubR1, the second checkpoint protein known to inhibit APC/C^{Cdc20}, and the related yeast protein Mad3, are part of such a complex[105–107,113]. This complex is present throughout the cell cycle and contains Cdc20, the checkpoint protein Bub3 and, according to some reports, also Mad2 (the complex that is composed of all four proteins is called mitotic checkpoint complex (MCC)[105]). Both MCC and recombinant, purified BubR1 are much more potent inhibitors of APC/C^{Cdc20} than purified Mad2 (REFS 105–107), but this observation could simply reflect the requirement for Mad1 in Mad2 activation.

For neither Mad2 nor BubR1 it is understood how they inhibit Cdc20. Mad2 can be found in association with APC/C^{Cdc20}, implying that Mad2 does not prevent binding of Cdc20 to APC/C[101,102,114,115]. For recombinant BubR1, it has been shown that its binding to Cdc20 can prevent the association of Cdc20 with APC/C[106,107]. However, it is not known whether this is the physiological mode of the function of BubR1, because activation of the spindle-assembly checkpoint also leads to association of BubR1 with APC/C[105,116,117], which is not what would be predicted if the primary function of BubR1 was to sequester Cdc20 away from APC/C.

Inhibition of APC/CCdc20 by Emi1. In *X. laevis* egg extracts and in purified systems, Emi1 cannot only inhibit APC/C^{Cdh1}, but also APC/C^{Cdc20} (REFS 91,118). By contrast, the *D. melanogaster* orthologue RCA1 seems to antagonize only APC/C^{CDH1} (REF. 88). At the beginning of mitosis, Emi1 itself is targeted for degradation by Plk1, which creates phospho-sites on Emi1 that are recognized by SCF$^{\beta TrCP}$ (REFS 119,120). It has been proposed

Checkpoint
A surveillance mechanism that delays progression through the cell cycle if processes such as DNA replication and spindle assembly have not been completed.

Kinetochore
A large proteinacous structure that assembles on centromeric DNA, binds the plus ends of microtubules and thereby connects chromosomes with spindle poles.

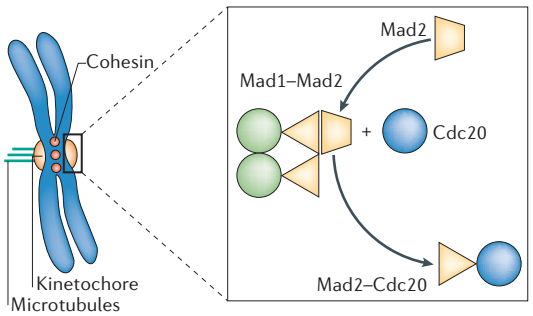


Figure 5 | **Activation of Mad2 at unattached kinetochores.** Mad2 is an inhibitor of anaphase promoting complex/cyclosome^{Cdc20} (APC/C^{Cdc20}) that is activated at kinetochores that are not (or not fully) attached to spindle microtubules. Mad2 exists in two different states, in an 'open' conformation (yellow rhomboid) in the cytosol, and in a 'closed' conformation (yellow triangle) that is recruited to kinetochores via the spindle-assembly-checkpoint protein Mad1 (green circle). The template model of Mad2 activation[112] proposes that the open Mad2 forms a conformational heterodimer with the closed Mad2 at kinetochores. This interaction somehow converts the open Mad2 molecule into a closed form that associates with Cdc20, analogously to how prion proteins propagate conformational changes through oligomerization. How Mad2 inhibits the capability of Cdc20 to activate APC/C remains unknown.

that Emi1 inhibits APC/C^{Cdc20} at the beginning of mitosis in prophase, at a time when the spindle-assembly checkpoint might not yet be active, until Emi1 itself is destroyed. This hypothesis could explain why cyclin A destruction is only initiated in prometaphase, although APC/C^{Cdc20} might already be activated by Cdk1 in prophase. The notion that Emi1 destruction is required for the timely activation of APC/C^{Cdc20} is supported by the observations that the degradation of cyclin A and other APC/C substrates is delayed in mouse cells that lack the SCF adaptor protein βTrCP, in which Emi1 cannot be degraded[121], and that cells in which non-degradable Emi1 mutants are expressed are delayed in prometaphase[122]. However, cells from which Plk1 has been depleted initiate cyclin A degradation with normal kinetics[123,124]. It remains to be seen whether Emi1 is targeted for destruction by other kinases or residual amounts of Plk1 in these cells, or whether Emi1 destruction is not essential for cyclin A proteolysis, which would be similar to the situation that is observed for RCA1 in *D. melanogaster*.

Regulation of APC/C in meiotic and endo cycles

In mitotic cell cycles, ploidy is maintained by strictly alternating DNA replication with chromosome segregation, but variations of this scheme are frequently used to generate cells with altered ploidy. In meiosis, two rounds of chromosome segregation without an intervening S phase lead to the formation of haploid germ cells, and in endoreduplication (endo) cycles, multiple rounds of S phase cause the generation of polyploid cells. In these situations, APC/C activity is regulated by additional mechanisms.

Inhibition of APC/C^{Ama1} by Cdk1 and Mnd2 in meiosis I. When budding yeast cells enter meiosis, a third co-activator, activator of meiotic APC/C protein-1 (Ama1), is expressed as well as Cdc20 and Cdh1 (REF. 125). APC/C^{Ama1} is required for sporulation and it also contributes to the degradation of the securin Pds1 and the cyclin Clb5 in anaphase of meiosis I[126]. Because Pds1 and Clb5 degradation leads to the loss of sister-chromatid cohesion and exit from meiosis, respectively, APC/C^{Ama1} is inhibited during the early stages of meiosis I by at least two mechanisms that prevent the precocious initiation of these events. During metaphase, Cdk1 contributes to the inhibition of Ama1, perhaps by directly phosphorylating Ama1. Accordingly, Cdk1 inactivation by APC/C^{Cdc20}-mediated cyclin destruction is required for the activation of APC/C^{Ama1} in anaphase I[126].

Furthermore, the inhibition of APC/C^{Ama1} during pre-meiotic S phase and prophase is strictly dependent on meiotic nuclear division protein-2 (Mnd2) (REFS 126,127), a protein that has only been identified in yeasts so far. Mnd2 is a stoichiometric subunit of APC/C both in mitotic and meiotic cells[128,129], although it is only essential for viability in the meiotic cells, in which Ama1 is expressed. Remarkably, Mnd2 only prevents the activation of APC/C by Ama1, but not the activation by Cdc20 or Cdh1 (REF. 126). How Mnd2 performs this function is unknown, as is the mechanism by which Mnd2 is inactivated at the onset of anaphase I so that APC/C^{Ama1} can become active.

Inhibition of APC/C^{Cdc20} by Mes1 in meiosis I–II. To ensure that meiosis I is followed by meiosis II, and not by S phase, cells must exit meiosis I without completely inactivating Cdks. Otherwise, pre-RCs could be assembled that would permit another round of DNA replication. In fission yeast, it has recently been shown that the small protein Mes1 functions as an inhibitor of APC/C^{Cdc20} and prevents the complete degradation of cyclins during exit from meiosis I[130]. Interestingly, Mes1 binds to the WD40 domain of Cdc20, as does the mitotic cyclin Cdc13, implying that Mes1 modulates APC/C activity by competing with cyclins for Cdc20 binding[130].

Inhibition of APC/C^{Cdc20} by CSF in meiosis II. In contrast to meiosis I, meiosis II is usually followed by another S phase, but in diploid organisms this round of DNA replication must not be initiated until two haploid germ cells have fused to form a zygote. Before fertilization, vertebrate eggs are therefore arrested in metaphase of meiosis II by an activity known as cytostatic factor (CSF)[131]. CSF inhibits APC/C[132] and thereby prevents anaphase and exit from meiosis II. The establishment, but not the maintenance, of CSF activity depends on cyclin E–Cdk2 and on a signalling pathway that involves the kinase Mos (reviewed in REF. 133). In *X. laevis* eggs, the spindle-assembly checkpoint proteins Bub1, Mad1 and Mad2 are also required for the establishment of CSF, but, surprisingly, only Mad1 is needed for its maintenance[134,135].

A second protein that was initially thought to be essential for CSF activity is Emi1 (REF. 136), but more recent studies have shown that Emi1 is unstable in meiosis II, as it is in mitotic cells, and it is therefore presumably not responsible for the inhibition of APC/C^{Cdc20} in CSF-arrested eggs[137,138]. However, a protein related to Emi1, known as XErp1 or Emi2, is stable during meiosis II until fertilization occurs and is required to maintain the CSF arrest[139–141]. Similar to Emi1, XErp1 is targeted for degradation by Plk1, but unlike Emi1, XErp1 can only be recognized by Plk1 once XErp1 has been phosphorylated by calmodulin-dependent kinase II[138,142]. This kinase is activated by a transient increase in intracellular calcium levels that occurs during fertilization, and the degradation of XErp1 and the subsequent activation of APC/C^{Cdc20} can therefore only occur once fertilization has occurred.

Role of APC/C in endo cycles. Endo cycles can lead to the formation of polytene chromosomes in which several sister chromatids remain connected, or these cycles can lead to the formation of polyploid cells. In *D. melanogaster*, ovarian nurse cells first generate polytene chromosomes but later separate their sister chromatids and thereby become polyploid. This transition from polyteny to polyploidy is affected by mutations in Morula, the *D. melanogaster* orthologue of the APC/C subunit Apc2 (REF. 143). In Morula mutants, nurse cells undergo several endo cycles, but then accumulate cyclin B, enter mitosis and remain arrested instead of transiting to the polyploid state. These observations imply that in some endo cycles APC/C activity is required to suppress entry into mitosis. Whether APC/C is constitutively active in endo cycles, or whether it has to be activated at the polyteny–polyploidy transition, remains unknown.

Substrate and spatial regulation of APC/C

APC/C activity is largely controlled by co-activators and inhibitors, but a number of observations indicate that, in addition, APC/C-mediated ubiquitylation reactions are regulated at the substrate level or by restraining these reactions to specific locations in the cell.

Regulation of APC/C substrates by phosphorylation. In most cases, APC/C seems to ubiquitylate its substrates independently of their post-translational modification state (reviewed in REF. 144). By contrast, SCF ubiquitin ligases can recognize many of their substrates only if they have been phosphorylated or hydroxylated (reviewed in REF. 24). However, it has recently been discovered that phosphorylation can protect substrates from APC/C. When human cells re-enter the cell cycle from quiescence, the replication factor CDC6 is phosphorylated by cyclin E–CDK2. The phosphorylated sites are located directly adjacent to the D-box of CDC6, and therefore prevent recognition of CDC6 by APC/C^{CDH1} (REF. 145). This mechanism allows CDC6 to accumulate before other APC/C substrates, so that CDC6 can initiate pre-RC formation before geminin and cyclin A would prevent this process. Similarly, substrate phosphorylation has been proposed to delay the APC/C-dependent degradation of Aurora-A until the end of mitosis[146].

Quiescence
The physiological state of cells that are not in the cell cycle.

Intrinsic regulation of APC/C^{Cdh1} by substrate ordering. Similar to CDC6, cyclin A accumulates earlier than other APC/C substrates at the G1–S transition[92], when cyclin A is thought to contribute to the inactivation of APC/C^{Cdh1} (REF. 89). This preferential stabilization of cyclin A as well as the phenomenon that Cdc20, Plk1 and Aurora-A are degraded in a sequential order during mitotic exit[147] have recently been attributed to kinetic differences in the ubiquitylation of these substrates[148]. According to this model, 'early' substrates are ubiquitylated in a processive manner; that is, substrates only require a single APC/C-binding event to obtain a ubiquitin chain. By contrast, 'late' substrates are modified in a distributive fashion; that is, these proteins repeatedly shuttle on and off the APC/C before a polyubiquitin chain has been assembled on them[148]. Distributive substrates would therefore be susceptible to de-ubiquitylation by de-ubiquitylating enzymes and to competition by more processive substrates, and these substrates can therefore be degraded later than processive substrates. How processively a protein is ubiquitylated is at least in part dependent on the identity of its D-box, and can therefore be an intrinsic property of substrates. Cyclin A is a distributive substrate, and this might explain why it cannot be degraded any more at the end of G1 when UBCH10 levels drop[148].

Spatial regulation of APC/C. In mitotic animal cells, a large fraction of APC/C is present in a soluble form in the cytoplasm, but APC/C and CDC20 can also be detected on different parts of the spindle apparatus. APC/C is enriched on unattached kinetochores[149], which raises the interesting possibility that APC/C might transiently interact with kinetochores to associate with inhibitory spindle-assembly-checkpoint proteins.

APC/C is also present on centrosomes and spindle microtubules[75,150], as is cyclin B[52]. In human cells, centrosomal cyclin B molecules are degraded before cytoplasmic cyclin B[52], and in syncytial *D. melanogaster* embryos, cyclin B is only degraded in close vicinity to mitotic spindles, whereas the bulk of cyclin B remains stable until the Cdh1 orthologue Fizzy-related is expressed later in development[151,152]. Interestingly, *D. melanogaster* mutants in which centrosomes dissociate from mitotic spindles can still degrade cyclin B on the detached centrosomes but no longer on the spindle[153]. It is therefore possible that APC/C^{CDC20} is locally activated at centrosomes by CDK1 and PLK1 (which are both enriched there), and that cyclin B and possibly other APC/C substrates have to move to this site to be ubiquitylated.

The discovery of APC/C: ten years on

During the decade since APC/C was discovered[25,50,51,150] we have learnt that there are more APC/C subunits, substrates and regulatory mechanisms than anyone could have predicted. Some of these groups might continue to grow in size, but the main future challenge will be to understand how all of these molecules and mechanisms actually work. Obtaining insight into how APC/C recognizes, ubiquitylates and discharges its substrates, and how

these steps are controlled, will require various approaches: quantitative *in vivo* imaging of the interactions between APC/C and its regulators and substrates in space and time; kinetic and quantitative biochemical analyses of the reactions that are mediated by APC/C; and solving the structures of APC/C-pathway components at both the atomic and the macromolecular levels by applying crystallography, NMR and high-resolution EM. These molecular approaches will have to be complemented by rigorous *in vivo* tests of our current ideas. Are all of the proposed inhibitors (TABLE 2) important for controlling APC/C activity under physiological conditions, and if not, which ones are? Reverse loss-of-function genetics will be essential to answer this important question.

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Competing interests statement
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